

## Effects of research diving on the physico-chemical profile of Bundera Sinkhole, an anchialine remiped habitat at Cape Range, Western Australia

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### Abstract

Bundera Sinkhole, in arid north-western Australia, contains a community of stygofauna, including remipedes and thaumatocypridid ostracods, its only known location in the eastern hemisphere. Water in the cave has a complex physico-chemical depth profile that is probably important to the functioning of this community. The effect of research diving on the physico-chemical profile was examined using, firstly, semi-closed circuit equipment (rebreathers) to minimise the physical and chemical disruption to the profile and, secondly, open circuit SCUBA to investigate the impacts of standard research diving operations. Both open and closed circuit diving had a measurable impact on the gross physico-chemical environment of the cave of blurring the interface between physico-chemical zones, but open circuit diving had a markedly more obvious impact. Importantly, diving extended the oxic zone downwards through a layer that previously contained a cascade of nitrogen species and a sulphidic layer in the vicinity of the thermo-halocline that is probably associated with chemoautotrophic production. The stratification in the redox environment was disrupted by both types of diving.

### 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 ecosystems often contain a diverse assemblage of relict species (Sket 1981, 1996); at least ten new families, and a new class (Remipedia) of crustaceans have been described from anchialine systems in recent years (GA Boxshall, Natural History Museum, London, pers. comm.).

Remipedes are typically found in communities consisting almost entirely of stygobiont crustaceans comprising thermosbaenaceans, hadziid amphipods, ostracods, cirrolanid isopods, mysids, and caridean shrimp. While the general structure of these anchialine communities is predictable from the Caribbean region, the Canary Islands and north-western Australia (*e.g.* Baltanas & Danielopol 1995; Yager 1981, 1987a,b, 1989, 1994; Holsinger 1989; Danielopol 1990; Yager *et al.* 1994; Yager & Humphreys 1996), the detail of each is regionally unique at the mid to lower taxonomic levels.

Although these faunistically distinct and diverse ecosystems may have persisted through geological eras (Danielopol *et al.* in press; Humphreys in press a), they appear vulnerable to anthropogenic impacts and need to be the focus of conservation assessment to protect their relictual fauna, often endemic at higher taxonomic levels

(class in the case of Remipedia). The reasons for the vulnerability of anchialine systems are several fold. The thin soil cover, typical of karst areas, provides little filtration of percolating fluids making them prone to ground-water contamination and the open conduit hydrological systems permit the rapid and distant spread of any introduced contaminants, be they nutrients or toxins. Furthermore, the flushing of groundwater will be exceptionally low in the arid Cape Range, and in the related anchialine ecosystems of Barrow Island (Humphreys in press b), making the residence time of contaminants long. The introduction of energy into subterranean systems changes the energy balance and the competitive abilities and permits epigeal organisms to displace hypogean organisms that are adapted to a low energy environment (Malard 1995). Hence, these ecosystems are sensitive to pollution (Iliffe *et al.* 1984; Notenboom *et al.* 1994), but paradoxically they may occur in widely variable and extreme physico-chemical conditions (Sket 1986; Humphreys 1999). Together these factors have led to anchialine systems becoming the subject of wide-spread conservation assessment (Sket 1981; Maciolek 1986; Brock *et al.* 1987; Ridgley & Chai 1990; Thomas *et al.* 1991, 1992; Iliffe 1992; Bailey-Brock & Brock 1993) and public interest (*vide* Waikoloa Anchialine Pond Preservation Area Trust Fund in Hawai'i; Brock *et al.* 1987). The red shrimps of the cosmopolitan family Atyidae, which contains many widely vicariant congeneric species (Holthuis 1986), that inhabit some anchialine pools are linked with mythology in some

countries (e.g. Fiji, Hawaii and the Philippines) and social taboos have facilitated their conservation (Choy 1987).

Anchialine systems in drowned sinkholes (cenotes) are typically highly stratified, having a freshwater layer overlying and separated from warmer seawater by a marked thermohalocline or density interface (pycnocline; Yager & Humphreys 1996; Humphreys in press a). The characteristic anchialine fauna mostly inhabits this region of seawater (Yager & Humphreys 1996) which is typically hypoxic (Yager *et al.* 1994; Yager & Humphreys 1996), and may occur beneath one or more layers of hydrogen sulphide (Humphreys 1999). The pycnocline may also be associated with dense growths of sulphur and nitrifying chemoautotrophic bacteria (Pohlman *et al.* in press; Humphreys 1999). As all known remiped habitats exhibit such marked stratification, and as remipeds have only been collected by cave divers from beneath a density interface, the maintenance of this stratification may be required for the functioning of these anchialine ecosystems.

Bundera Sinkhole (numbered 6C-28 in the Australian Karst Index) is the only deep anchialine system known in Australia and is the only continental anchialine system known in the southern hemisphere (see Iliffe in press; Fig 1; Humphreys, 1999). This narrow cave is the only known site for the crustacean class Remipedia (*Lasioneectes exleyi* Yager & Humphreys) in the southern hemisphere (Yager & Humphreys 1996) and the site lies outside conservation reserves. *Lasioneectes exleyi* is listed as endangered under both Western Australian and Commonwealth of Australia legislation. In addition, Bundera Sinkhole is the only known locality for *Danielopolina* sp nov Danielopol *et al.* (Ostracoda: Halocyprida: Thaumatoocyprididae), and is the only known eastern hemisphere location for that genus, and *Liagoceradocus branchialis* Bradbury & Williams (Crustacea: Hadziidae). A number of other stygal species are also found in the system; *Stygiocaris stylifera* Holthuis (Malacostraca: Decapoda: Natantia: Atyidae) and *Milyeringa veritas* Whitely (Perciformes: Eleotridae). The contiguous anchialine system also contains a number of other taxa with Tethyan disjunct distributions, including the genera *Haptolana* (Isopoda: Cirolanidae), *Halosbaena* (Thermosbaenacea), and *Ophisternon* (Pisces: Synbranchiformes), and gammarid amphipods (Humphreys 1993a,b,c; Barnard & Williams 1995; Bradbury & Williams 1996a,b, 1997a,b). Above the thermohalocline in the photic zone, this anchialine system also contains a number of non-stygal plants and animals; the algae *Rhizoclonium ?tortuosum* (Dillw) Kuetz (Chlorophyta: Cladophoraceae) and *Lamprothamnium papulosum* (Wallr) J Gr (Charophyta: Characeae), and the invertebrates ?Spionidae (Annelida: Polychaeta), *Halicyclops* sp nov (Copepoda: Cyclopidae), ostracods, *Iravadia* sp (Mollusca: Iravadiidae), water striders (Gerridae: Hemiptera) and *Kiefferulus intertinctus* Skuse (Chironomidae, Diptera).

Diving is necessary to study these anchialine caves but the effect of diving, both research and recreational, needs to be assessed. Diving in these stable anchialine caves tends to disrupt the physico-chemical stratification by finning and rise of exhalant air from SCUBA equipment; these effects are enhanced in deep narrow caves by the confined escape route and progressive expansion of exhalant air. This disruption of the physico-chemical

stratification is indicated by the reduction in visibility and by the loss of large bacterial colonies associated with the thermo-halocline. The disruption is likely to be more prolonged in systems where the flow of freshwater is limited by low recharge such as in arid northwestern Australia.

This study describes the geomorphological and hydrological context of Bundera Sinkhole, and documents the short-term effects of diving on the physico-chemical profile. We used rebreathing SCUBA to eliminate most of the bubbles from exhaust gases and thus reduce physical disturbance to the ecosystem and contamination through oxygenation or carboxylation. To examine disruption of stratification of the water column by open-circuit SCUBA, the final few dives in the sinkhole used open-circuit SCUBA gear or rebreathers set on open circuit. In addition, we examined the limited data that are available for the longer-term stability of the system.

## Methods

### Bundera Sinkhole

Bundera Sinkhole is located on the Cape Range peninsula in north-western Australia, 1.7 km inland from the Indian Ocean in the middle of a flat, 4.5 km wide coastal plain. The flooded sinkhole starts in the Bundera calcarenite (the type section) deposited on a wave-cut platform, the lower of a series cut into the Tulki Limestone of Miocene age in the Cape Range anticline (Wyrwoll *et al.* 1993). The water surface is little disturbed by wind as the water surface lies ca. 5 m below the lip of the sinkhole, the opening of which is quite small (ca. 10 x 20 m). The flooded sinkhole leads into a passage, inclined at ca. 30° from the horizontal, which extends about 70 m (Fig 1 of Humphreys, 1999) to a maximum depth penetrated by divers of 33 m. The main cave extends towards the west but there are minor easterly passages in line with a gorge entering the coastal plain 2.8 km to the east.

Characteristic of anchialine systems, the water level is influenced by marine tides of the area. Comparison of the predicted amplitude of coastal tides at Norwegian Bay (24 km south; Anon 1996) with the recorded tides from Bundera Sinkhole shows the amplitude in the sinkhole ( $0.61 \pm \text{sd } 0.24 \text{ m}$ ,  $n=17$ ) to be 16%  $\pm$  sd 0.05% of the ocean amplitude ( $0.09 \pm \text{sd } 0.04 \text{ m}$ ,  $n=17$ ). The semi-diurnal form of the ocean tidal curve is reflected in the sinkhole (Fig 1A) but the range of the diurnal tide changes little over the lunar cycle and the general water level increases towards spring tides and decreases around neap tides (Fig 1B) giving an overall range of ca. 0.35 m. It is not evident if the thermo-halocline moves vertically with the tides. 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 algal mats spread over much of the surface and greatly restrict light penetration.

### Climate

The climate of the Cape Range peninsula is arid and annual evaporation (3219 mm) greatly exceeds the sparse precipitation (median 280 mm). Mean daily temperature is ca. 27 °C but the mean monthly maximum temperature

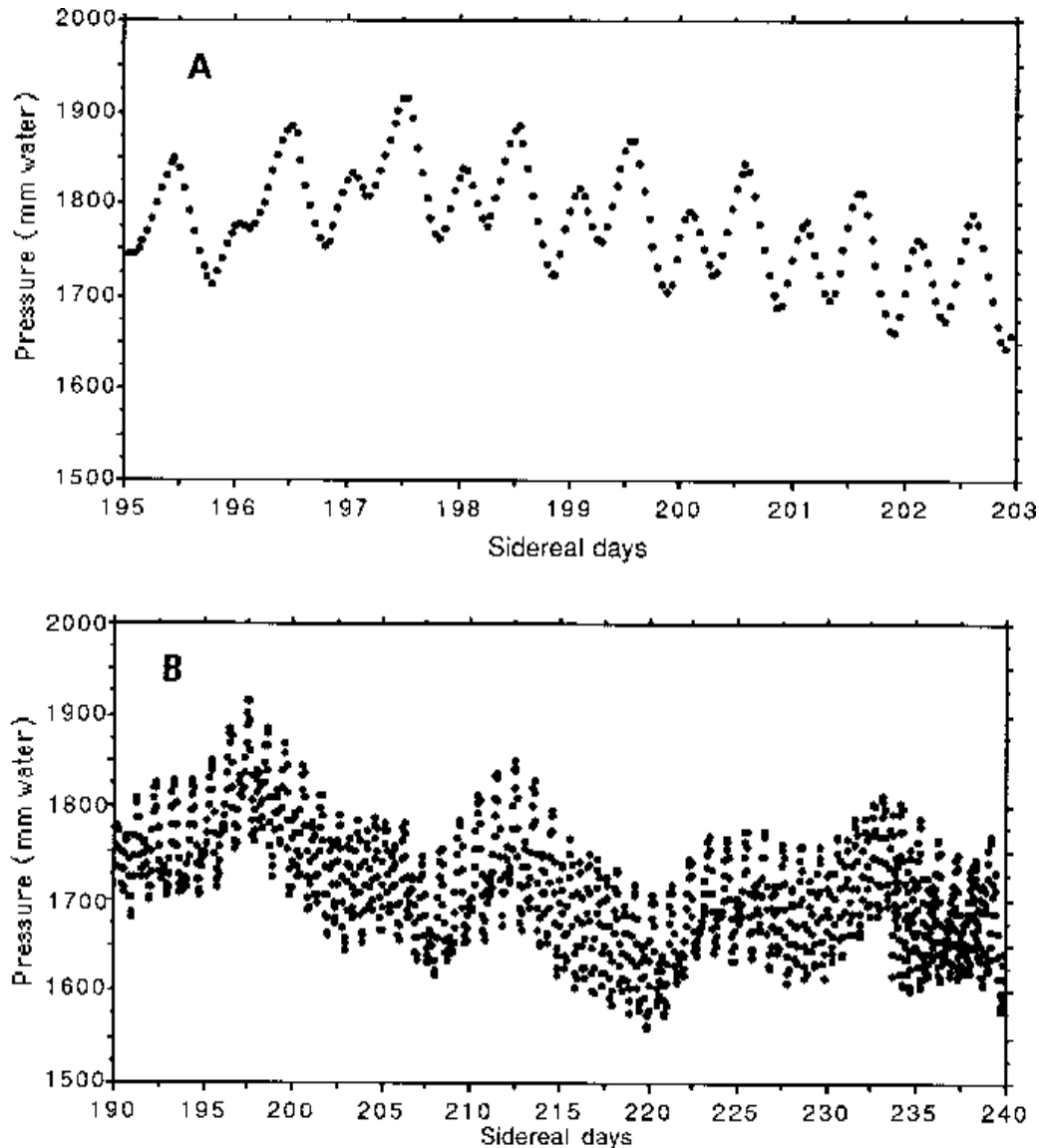


Figure 1. Tide recording in Bundera Sinkhole showing the semi-diurnal tides. A: 8 day period. B: 50 day period.

exceeds 35 °C for four months of the year. Relative humidity is low (mean at 9 am = 53%; at 3 pm = 33%) and the rainfall is sporadic, predominantly falling in large episodes of various meteorological origins (Beard 1975; Gentilli 1979). The infrequent large rainfall events flood the caves deeply, at mean intervals of 56 months (Humphreys *et al.* 1989), and are the major recharge episodes of the freshwater aquifers.

#### Regional ground-water

The general hydrogeological model for oceanic islands has been applied to the ground water of Cape Range peninsula (Allen 1993). Following the Ghyben-Herzberg principle, a fresh water lens overlies salt water so that locally a wedge of salt water intrudes under the fresh water contained in the limestone, and between which a zone of mixing occurs that broadens towards the coast. In the Exmouth area, on the north-eastern part of the Cape Range peninsula, the freshwater-seawater transition, at about five kilometres from the coast, is exceptionally far

inland (Martin 1990). This considerable inland extent is related to the high transmissivity of limestone, low groundwater flow resulting from the aridity of the Cape Range area, and lowering of the water table through water abstraction (Davidson 1995). In the Exmouth area, the inland limit to the salt-water interface appears to be controlled by the presence of solution cavities and channels below the water table (Martin 1990). Comprehensive chemical analysis of the water above and below the thermohalocline in Bundera Sinkhole was given in Yager & Humphreys (1996).

#### Diving in Bundera Sinkhole

The first recorded dive in the sinkhole was in 1991 to a depth of three metres in the narrow eastern extension of the cave (R Cooper, Perth, pers. comm.). The cave was first penetrated fully on 6 August 1993, specifically to search for the remipedes that had been predicted to occur in the area (Poore & Humphreys 1991; Yager & Humphreys 1996). Research diving conducted from 6-12

August 1993, 19-21 March 1994, 24-26 May 1995, 21-24 September 1997 was partly reported by Yager & Humphreys (1996). We know of only two other dives on this site, by sports divers on a single occasion in 1998.

The diving gear employed was typical of that used for cave diving, with redundancy. The Dräger "Atlantis" rebreathers used in 1997 were modified to have the required redundancy. These are a semi-closed circuit design in which the exhalant air is passed through soda lime to remove carbon dioxide, and then recirculated. Only a small amount of gas escapes into the water during use, the amount depending on the flow rate which is pre-set by the diver to suit the diving depth and the work rate. The rebreathers were modified by the addition of bubble diffusers to minimise large, rapidly rising, bubbles. The gas release rate was reduced by fine adjustment to the initial gas introduction rate and the gas mix. This resulted in an almost negligible off-gassing at the operating depth, extended the bottom time, and reduced the overt disturbance of the water column.

In 1997, the total dive time was 16.7 hours comprising 17 person-dives in seven dive sessions, mostly to the full depth of the cave (33 m). Twelve person-dives totalling 11.8 hours were made using rebreather equipment, followed by five totalling 4.9 hours using either the rebreathers on open circuit or standard SCUBA gear.

On our first entry to the cave in 1993, and our first dive in 1997, the full extent of the cave was recorded using a video camera (Sony Hi8), and additional video recording was taken in 1995.

### Sondes

In 1997, two 44 mm diameter sondes (Datasonde3 Multiparameter Water Quality Datalogger DS3, Hydrolab Corporation, Austin, Texas) were used singly or together in untethered mode to record each parameter every 1 to 5 seconds throughout each dive. On the first and last dives, a sonde was pushed (sensors foremost) by the lead diver to record the undisturbed water column. At other times, to enable other tasks to be performed by the divers, the sondes were strapped upside down to the diver's breathing equipment so that the sensors were at the front of the diver. The data were transferred to a computer at the end of each dive. The sondes were fitted with sensors to measure depth (m), temperature (°C), dissolved oxygen (DO, mg L<sup>-1</sup>), redox (mV), pH, and salinity (mg L<sup>-1</sup> TDS). 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<sup>-1</sup> 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).

### Tidal data

The tidal cycle in the sinkhole was recorded using a datalogger fitted with a pressure transducer (Model 392 logger; Dataflow Systems, Noosaville, Queensland); the mean value of two readings was recorded every 30 minutes. Readings were taken in the sinkhole for four months in 1996.

### Disruption of physico-chemical profiles

Several methods were used to detect changes to the water column brought about by diving: 1) observations of divers and from video records; 2) examination of the depth profiles of various physico-chemical parameters; and 3) statistical analysis of specific points in profiles. The latter included change in depth at which a given value of a parameter was reached, change in value of parameter at a given depth, and changes between open and closed circuit diving. The differences between open and closed circuit diving, morning and afternoon diving, and progressively over time, were analysed using Mann-Whitney U test, two factor ANOVA, and linear regression.

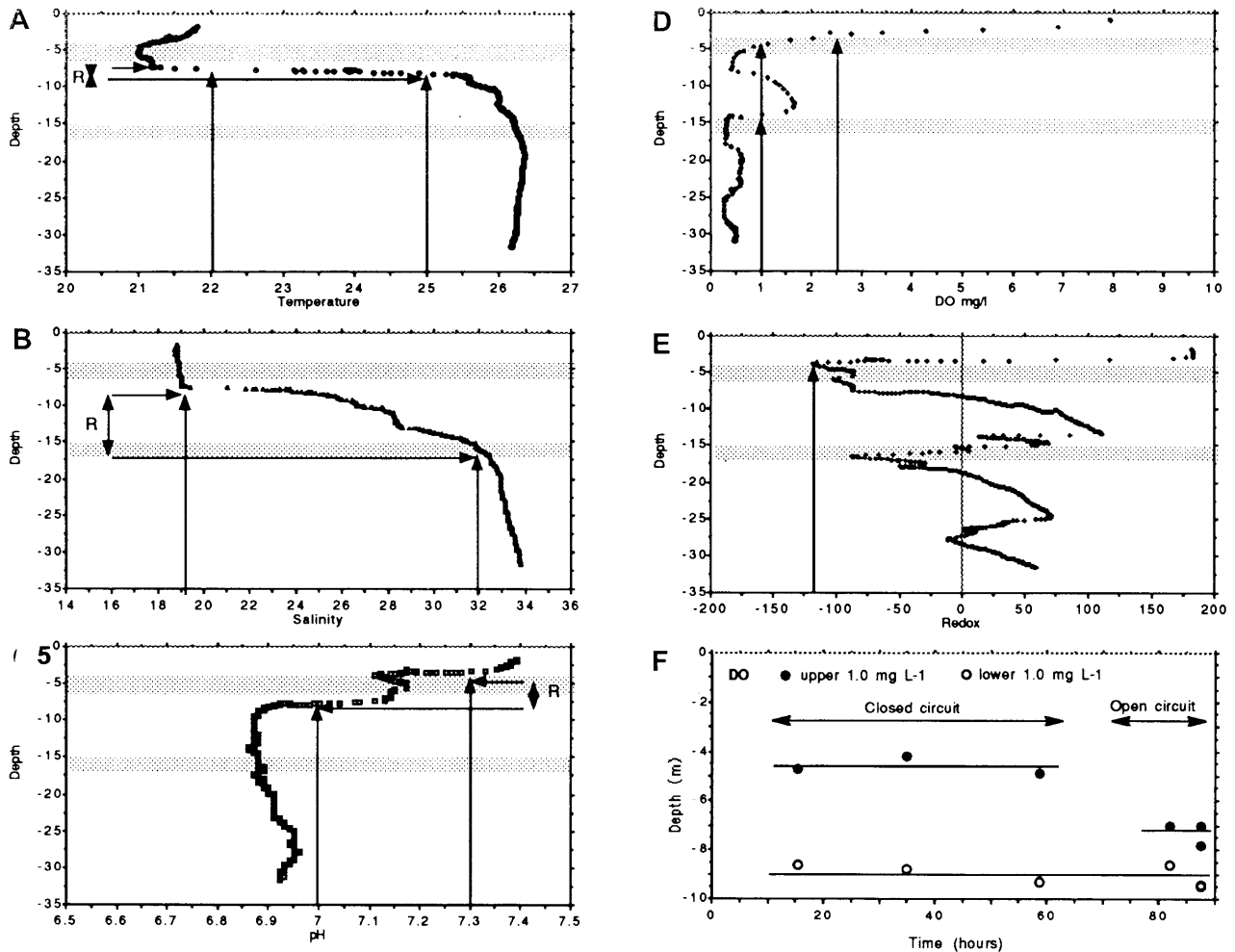
Depth is recorded below the water surface (bws), rather than from a fixed location in the cave, to allow for tidal movement of the thermo-halocline.

## Results and Discussion

The marked thermo-halocline of Bundera Sinkhole was present in all three years for which profile data are available, but the details of the stratification seem to vary between years as does the physical appearance of the water. In 1993, distinct thermoclines were reported by divers at depths of 8 m and 13 m (about the levels of the hydrogen sulphide layers in 1997; Fig 2A), but their magnitude is unknown. Wispy suspensions, probably bacterial colonies, were seen in the water column at a depth of about 14 m, just below the cave entrance restriction. In 1994, the suspensions were barely visible and they were not present in 1995 when a distinct tannic layer occurred at a depth of 22 m. A strong smell of hydrogen sulphide was encountered in all years in the vicinity of the density interface. In 1997, a marked refractive layer at about 8 m depth was observed at the start of the thermohalocline.

Surface water in the sinkhole is thick with algae and below which, on the initial dive in 1993, there was a zone of large insubstantial globular structures (probably bacterial monofilms) together with a zone of large chain-like filamentous structures (probably colonial sulphur bacteria) that are consistent in form with those ascribed to the *Beggiatoa-Thiothrix* group common in anchialine caves containing remipedes in the Caribbean region (Yager 1991). Similar structures ('sulphuretas') have been reported from otherwise afaunated caves in the Nullarbor, southern Australia (J James, Sydney University, pers. comm.). The massive bacterial colonies seen in mid-water on first entry in 1993 have not subsequently been seen to be as well developed. The reason is unknown, but could be the result of diving, rainfall or ground-water flow. However, filamentous and globular colonies of bacterial colonies were collected from the walls and floor of the cave in 1997.

Over a sequence of SCUBA dives in 1993, water below the thermo-halocline initially had high clarity but visibility decreased markedly over five dives until it was necessary to follow the guide-line to exit the cave. By contrast, in 1997 visibility remained high and the guide-line was not necessary to exit the cave despite considerably more diving activity with rebreathers. Some



**Figure 2.** Vertical profiles with depth of various physico-chemical parameters in Bundera Sinkhole in 1997, statistical test were conducted on the values of the parameters shown by the single-headed arrows and the range of the parameters (R) shown by double-headed arrows: A: temperature (°C), where the grey bands denote the zones of elevated hydrogen sulphide concentration (see Humphreys 1999; Fig 2F). Descent rate was  $0.05 \text{ m s}^{-1}$  (sd 0.028,  $n=21$ ). B: salinity ( $\text{g L}^{-1}$  TDS). C: pH. D: dissolved oxygen concentration ( $\text{DO mg L}^{-1}$ ). E: oxidation reduction (redox) potential (mV). F: location (m below water surface) of the upper and lower DO minima in relation to the periods of closed and open circuit diving.

of this difference between 1993 and 1997 may have resulted from the earlier dislodgment of sediments by bubbling from open-circuit SCUBA gear.

### Physico-chemical profile

The standing physico-chemical environment in Bundera Sinkhole has been reported in detail, including the nitrogen species profile, the polyphasic distribution of hydrogen sulphide and stable isotope distribution amongst the fauna (Humphreys 1999). Here only those parameters recorded by the sonde are considered as they were repeatedly recorded over a period of four days.

The water column had a strong physico-chemical stratification, with the different parameters exhibiting different types of stratification. These initial conditions as recorded during the first dive of the series are given here without further comment. There was a sharp thermocline with the temperature increasing with depth in the cave (Fig 2A) and a halocline not concordant with the thermocline (Fig 2B) with the upper brackish water overlying water close to seawater salinity. The pH

declined from greater than 7.5 at the surface to *ca.* 6.9 at *ca.* 8 m at the bottom of the thermocline (Fig 2C). pH was lower in the upper water during the afternoon than in the morning, the pH 7.0 contour being 0.6 m deeper in the afternoon (8.6 m) than in the morning (8.0 m;  $P=0.021$ ; Table 1), probably reflecting the photosynthetic activity in the photic zone; it was the only parameter showing a significant effect of time of day. The DO profile was polyphasic rapidly falling 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 2D). The redox profile was also polyphasic (Fig 2E), ranging from -125 mV to +175 mV, and loosely concordant with both the DO profile (Fig 2F) and the upper two minima with elevated levels of hydrogen sulphide (Humphreys 1999).

### Microbiotic environment

At high water in the sinkhole, the attached algae are submerged and the water appears clear, while at low water the algae spread over the water surface and impede

**Table 1**

The depths at which values of physico-chemical parameters are reached in Bundera Sinkhole for rebreathing diving (Closed) and subsequent open circuit or SCUBA diving (Open). Statistics are one-tailed Mann-Whitney U test.

	Closed	Open	n <sub>1</sub> , n <sub>2</sub>	U	P
Temperature 22 °C	-7.3 m	-6.7 m*	6,3	18	<0.025
Temperature 25 °C	-8.3 m	-8.7 m*	6,3	16.5	<0.05
Temperature range	1.1 m	2.0 m*	6,3	18	<0.025
Salinity 19 g L <sup>-1</sup>	-6.1 m	-4.5 m	6,3	18	<0.025
Salinity 32 g L <sup>-1</sup>	-15.9 m	-15.1 m*	6,3	17.5	<0.025
Salinity 19-32	-9.9 m	-10.6 m*	6,3	15.5	<0.1
DO 1.0 mg L <sup>-1</sup>	-4.6 m	-7.3 m*	3,3	9	0.05
DO 1.0 mg L <sup>-1</sup>	-8.9 m	-9.2 m*	3,3	6.5	ns
DO 2.5 mg	3.6 m	4.6 m*	3,3	9	0.05
pH 7.3	5.1 m	-4.3 m*	6,3	13.5	ns
pH 7.0	-8.2 m	-8.3 m*	6,3	9.5	ns
pH 7.3-7.0	-3.0 m	-4.7 m	5,2	10	0.05

\* no significant regression of variable on time if the open circuit diving is excluded, indicating minor effect of closed circuit diving.

light penetration. Hence, the penetration of light into the deep algal layer will vary with both solar (diurnal) and lunar cycles, the latter having both a semi-diurnal and 14-day periodicity. This variation in light intensity will have some impact on the photic environment of the photosynthetic algae and bacteria and the resultant balance of photosynthesis and respiration in the upper photic zone of the cave. However, the marked thermocline will partially isolate the lower cave environment from these effects, which, nonetheless, deserve further examination. Humphreys (1999) provides evidence for the presence of a complex microbiotic environment in Bundera Sinkhole, near the thermohalocline, involving dense growths of sulphur and nitrifying chemoautotrophic bacteria, similar to that reported for comparable system in Quintana Roo, Mexico (Pohlman *et al.* in press).

### Effects of diving

The gross form of most of the physico-chemical profiles changed little during successive dives with rebreathers, over four days in 1997, although there was a tendency for the sharp stratification at the initial thermohalocline to become less distinct with the interface thickening slightly. These changes through time are

examined in more detail to determine in what manner the profiles change, and whether there is a significant difference between changes for closed (rebreathers) and open (SCUBA) circuit diving equipment (Table 1).

**Temperature.** The position of the 22 °C isotherm moved upwards (7.3 m to 6.7 m) while that of the 25 °C isotherm moved downwards (8.3 m to 8.7 m bws; Table 1) resulting in the zone of water between 22 °C and 25 °C increasing in thickness from 1.1 to 2.0 m (Table 1), thus making the thermocline less distinct.

**Salinity.** The position of the 19 g L<sup>-1</sup> and 32 g L<sup>-1</sup> haloclines both moved upwards, from 6.1 m to 4.5 m bws and from 15.9 to 15.1 m bws respectively, so that the position rather than the thickness of this salinity band changed (Table 1).

**Dissolved oxygen.** The position of the upper 1.0 mg L<sup>-1</sup> DO isocline moved downwards, being at 4.6 m bws during the closed circuits dives and falling to 7.3 m bws after the open circuit dives (Table 1), while the lower 1.0 mg L<sup>-1</sup> DO isocline did not change over time, remaining at ca. 9 m bws). There is an apparent step effect here with a major change in level associated with open circuit diving (after 65 hours; Fig 2F). Similarly the position of the upper 2.5 mg L<sup>-1</sup> DO isocline moved downwards from 3.6 m to 4.6 m bws (Table 1).

The location of the lower 1.0 mg L<sup>-1</sup> layer did not change with treatment (Table 1), suggesting that the cause of the surface mixing is replacement of oxygenated water downwards rather than the exhalant air itself oxygenating the water i.e. a result of turbulence rather than a direct reoxygenation. The movement of the upper 1.0 mg L<sup>-1</sup> DO isocline to deeper water is especially significant because the more highly oxygenated water is intruding right through the upper hydrogen sulphide layer (Fig 2) which, from the evidence of the redox profiles (Fig 3A), was lost by the last dive. This intrusion of oxygenated water into this normally stable anoxic zone is likely to be of significance as this is the zone where the chemoautotrophic production is probably occurring (Humphreys 1999) and where the massive (presumed sulphur) bacteria colonies were disrupted after the first visit.

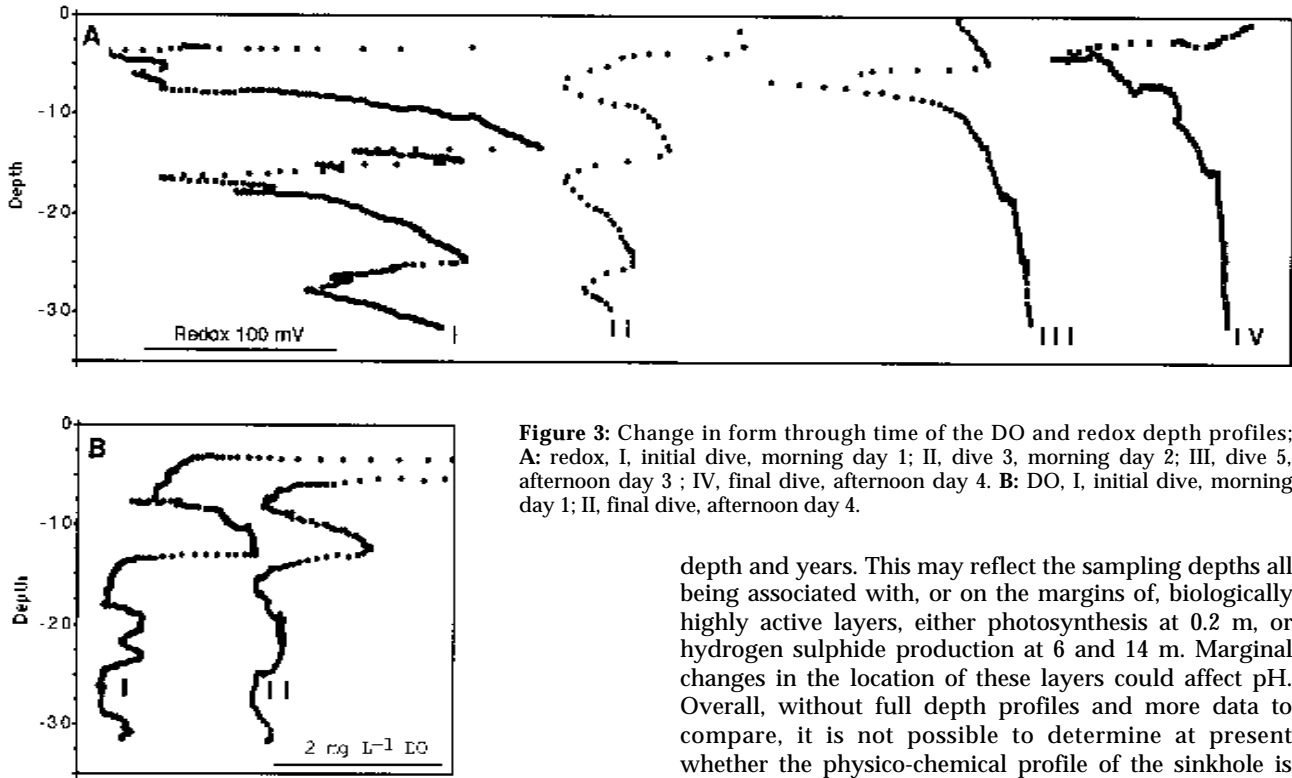
The general form of the DO depth profile changes quite subtly through time with some structure having been lost from the profile, especially in the lower sector, by the end of the field work (Fig 3B).

**pH.** The location of neither pH 7.0 nor pH 7.3 changed significantly with time or with treatment (P = 0.92 and P = 0.26 respectively) but the location of pH 7.0 and pH 7.3

**Table 2**

Between year comparison of temperature, salinity and pH at three depths in Bundera Sinkhole. The data are derived from three different sondes and from water chemistry data (see methods of Yager & Humphreys 1996). WC denotes water samples collected in bottles by divers.

Depth m	Temperature			Salinity				pH			
	1994 sonde	1995 sonde	1997 sonde	1994 WC	1994 sonde	1995 sonde	1997 sonde	1994 WC	1994 sonde	1995 sonde	1997 sonde
0.2	28	22.9	22.7	20	20.2	20	18.5	8.08	-	8.1	7.89
6	25.3	22.8	21.2	20	23.1	20	18.7	7.44	-	8.05	7.30
14	26.2	26.5	26.2	26.5	31.8	26.5	30.2	7.27	-	7.6	6.88



**Figure 3:** Change in form through time of the DO and redox depth profiles; **A:** redox, I, initial dive, morning day 1; II, dive 3, morning day 2; III, dive 5, afternoon day 3; IV, final dive, afternoon day 4. **B:** DO, I, initial dive, morning day 1; II, final dive, afternoon day 4.

diverged during the work with the range being significantly greater ( $P = 0.014$ ) in the open circuit period (Table 1).

**Redox.** The redox profile was initially strongly polyphasic (Fig 3A) but both its general form and magnitude changes markedly through time, with most of the structure having been lost from the profile, especially in the lower sector, by the end of the field work. Overall, the minimum redox value recorded each dive increased through time ( $F_{s,1.7} = 9.63$ ,  $P = 0.017$ ). This effect is not associated with time of day or state of the tide as dives were conducted in the morning and afternoon (and thus also at different tidal states) over four successive days (caption to Fig 3).

**Changes between years**

Complete depth profiles are available only for the 1997 sampling, but some data can be compared from three specific depths for 1994, 1995 and 1997 (Table 2). The high temperatures of 28 °C and 25.3 °C at the surface and 6 m in 1994 result from this record having been made in the hotter season (March), while the remaining readings were made in the cooler seasons. Hence, the thermal stratification in the water column of Bundera Sinkhole is much less marked in the warmer seasons. However, at a depth of 14 m the water temperature is effectively constant between years.

The salinity at the surface and at a depth of 6 m is remarkably constant between years given that it should be influenced by local rainfall. However, the deep salinity varied by 20%, but this variation could be attributed to quite modest errors in the depth of sampling (especially the water bottle sample, in 1994) given the steep salinity gradient at this depth. pH values are variable between

depth and years. This may reflect the sampling depths all being associated with, or on the margins of, biologically highly active layers, either photosynthesis at 0.2 m, or hydrogen sulphide production at 6 and 14 m. Marginal changes in the location of these layers could affect pH. Overall, without full depth profiles and more data to compare, it is not possible to determine at present whether the physico-chemical profile of the sinkhole is stable over time.

Both the distribution and abundance of the fauna differ between years, although little systematic sampling has been conducted (Table 3). This is especially noticeable in the main targets of the fauna sampling (*Lasionectes*

**Table 3**

Numbers of specimens taken or seen during the research dives as an indication of the changes to the biota in Bundera Sinkhole. + denotes presence, - denotes absence in samples. The location of the samples with respect to the thermohalocline is indicated.

	Below thermo-halocline	1993	1994	1995	1997
<b>Below only</b>					
<i>Lasionectes</i>	+	3	1	3	2
<i>Danielopolina</i>	+	1	-	41	1 <sup>2</sup>
<i>Liagoceradocus</i>	+	-	-	17	4 <sup>2</sup>
Polychaeta	+	-	1	3	-
<b>Above and below</b>					
<i>Milyeringa</i>	+	-	+ <sup>3</sup>	+	+ <sup>1</sup>
<i>Stygiocaris</i>	+	2	1	-	6
Other ostracods	-	+	+	+	+
Copepods	-	+	+	+	-
<b>Above only</b>					
Diptera	-	15	2	-	-
Mollusca	-	+	+	+	+
Other ostracods	-	+	+	+	+
Copepods	-	+	+	+	-

<sup>1</sup> Numerous *Milyeringa* counted by diver (see Humphreys 1999: Fig 3B). <sup>2</sup>A much greater effort was made to sample fauna in 1997 than in 1995 and resulted in the finding of several new taxa, so the apparent change in numbers is probably real. <sup>3</sup>Numerous *Milyeringa* counted at the bottom of the sinkhole from video recording taken by diver.

Table 4

Comparison of various physico-chemical parameter values from a number of anchialine caves. The range of values recorded is given. Some DO values are given in percent saturation (denoted %). Many of the data are plotted as profiles in Iliffe in press.

Location	Depth m	Temp. ° C	Salinity g L <sup>-1</sup>	pH	DO mg L <sup>-1</sup>	REDOX mV	Reference
Bundera	32	21.1-26.4	18.5-34	6.88-7.4	0.2-6.9	-120 to +115	this paper
Bundera	21	22.8-26.7	20-27	7.6-8.1	1-3	-	this paper
Cueva de los Carboneros, Cuba	28	25.4-29.9	0-36.5	7-8.2	0.1-7.98	-273 to +97	Yager 1994
Church Cave, Bermuda	20	16-20	22-36	-	50-100%	-	Iliffe <i>et al.</i> 1984
Government Quarry, Bermuda	20	17-21	18-36	0-90%	-	-	Iliffe <i>et al.</i> 1984
Mayan Blue Quintana Roo, Mexico	22	25.2-26.0	3-34	6.6-7.2	0.9	1.65	Pohlman <i>et al.</i> , in press
Movile Cave, Romania	-	20.9	-	7.29	-	-341	Sarbu in press.
Wonderland Cave, Bermuda	10	21.7-23.1	24-36	7.34-7.67	3.8-6.8	-	Iliffe <i>et al.</i> in press.
Cueva de los Carboneros, Cuba	31	25-30.5	3-37	7.1-8.15	0.1-6.2	-	Yager 1994

*exleyi*, *Danielopolina* sp and *Liagoceradocus branchialis*) and in the conspicuous fauna, namely the blind fish *Milyeringa veritas*. Some species have only ever been recorded below the thermo-halocline (e.g. *L. exleyi*, *Danielopolina* sp and *L. branchialis*), while others are recorded throughout the water column but the vertical distribution of which differs markedly between years (*M. veritas*). In 1994, there was a large concentration of the fish at the extreme depth of the cave, while they were entirely lacking from this area in 1997 when they were at concentrations just below the thermocline (ca. 10 m) and at ca. 20 m (Humphreys 1999; Fig 3B), between the H<sub>2</sub>S layers (Fig 2). This change in location of the principle predator (Humphreys & Feinberg 1995) suggests that the system is not stable.

The consequences of disruption to the physico-chemical stratification of the water column in the sinkhole are unknown, but it is pertinent to mention that remipedes globally have only ever been sampled below a thermo-halocline, always by cave divers in anchialine systems (Yager & Humphreys 1996). Hence, it is reasonable to expect that the loss of the thermo-halocline would be detrimental to the community. Secondly, breakdown of the physico-chemical stratification is likely to lead to the disruption of the biogeochemical processes associated with it.

#### Comparison with other areas

Most anchialine systems have a freshwater lens overlying sea water (Iliffe in press). This 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 similar to Bundera Sinkhole in its salinity profile (Iliffe in press; Fig 3).

A summary of published physico-chemical profiles of anchialine systems (Table 4) indicates that Bundera Sinkhole is the deepest of these and probably the smallest. In Bundera Sinkhole there is a polymodal DO depth profile, unlike any other DO depth profile, each of which exhibits a unimodal DO depth profile. By inference, this suggests that the other systems probably have a single H<sub>2</sub>S layer.

The physico-chemical environment in the Bundera Sinkhole is very complex (Humphreys 1999) 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 clearly disrupts the vertical stratification of the physico-chemical environment in Bundera Sinkhole and this may affect the unique anchialine community inhabiting the cave. As some of the fauna associated with such anchialine systems have only ever been collected by divers (e.g. remipedes) then diver access is essential for biological work. However, diving should be restricted and, where possible, conducted using rebreathing equipment. There is a lack of basic data on the gross physico-chemical environment in the sinkhole on groundwater movement, temporal changes in the profile resulting from the effects of episodic rainfall on surface input, as well as groundwater flow. A diver-free profiling system would be ideal to establish the long term changes in the sinkhole, 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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