PROGRAM

TWESDAY 17 NOVEMBER 2009

17:00	Registration	Hotel Palas Atenea
19:00	Wellcome reception offered by Palma City Council	Bellver Castle

WEDNESDAY 18 NOVEMBER 2009

08:30		Registration & Poster hanging
09:00	Damià Jaume	Opening
09:10	Iliffe	Historical and ecological perspectives of anchialine ecosystems
Geology, Hydrology and Hydrogeochemistry *— Chair: Carol Wicks*		
10:00	Mylroie & Mylroie	Void development on carbonate coasts: creation of anchialine habitats
10:20	Ginés, Ginés, Fornós, Gràcia & Merino	The littoral endokarst of Mallorca Island (Western Mediterranean)
10:40	Aubrecht & Schlögl	Jurassic neptunian dikes - Insight to fossil submarine cave biotas
11:00	Beddows	Primary and secondary stratifications as a control on mass and energy fluxes within the Yucatan Peninsula anchialine aquifer system
11:20	**Refreshments**	
11:40	Price, Stalker, Smith & Savabi	The geochemistry of nutrients (N, P, C) in seawater mixing zones in carbonate aquifers
Habitats and water quality *— Chair: René Price*		
12:00	Cuculić, Cukrov, Kwokal & Jalžić	Distribution of Hg, Cd, Pb, Cu and Zn in water columns and sediments of two anchialine caves in Mljet National Park - Croatia
12:20	Žic, Cukrov, Cuculić, Kwokal & Jalžić	Iodine and nutrient speciation in anchialine cave waters (Bjejajka Cave, East Adriatic Coast)
12:40	Basterretxea, Garcés, Tovar-Sánchez, Massanet & Alvarez	Response of coastal phytoplankton to submarine groundwater discharges: Role in the eutrophication of coastal areas
13:00	Bishop & Iliffe	Ecological physiology of anchialine organisms: A comparison between three cave systems
13:20	**Lunch**	

WEDNESDAY 18 NOVEMBER 2009

15:40	Mejía-Ortiz, López-Mejía, Hartnoll, Baldari & Sbordoni	Progressive adaptation of crustacean eyes from freshwater and anchialine caves
Microbiology and Ecosystem processes *— Chair: Damià Jaume*		
16:00	Brumsack & Beck	Research Group "BioGeoChemistry of Tidal Flats": A multidisciplinary process study in the NW German Wadden Sea
16:20	Pholman	Chemoautotrophy in anchialine caves: Reflections on the past and thoughts for the future
16:40	**Refreshments**	
17:00	Pakes, Wrighton, Thrash, Santis, Anderson, Iliffe & Coates	Anchialine cave microbiology: a multi-layered approach
17:20	Gonzalez, Brinkmeyer & Iliffe	Novel bacterial diversity in an anchialine blue hole from Abaco Island, Bahamas
17:40	van Hengstum & Scott	The control of hydrogeology and nutrient supply on foraminifera in Green Bay Cave System, Bermuda: the anchialine to submarine cave environmental transition
18:00	Talamante	Organic matter origin and diets: building the food web
18:20	Closure	

THURSDAY 19 NOVEMBER 2009

09:00	**Field excursion to Coves del Drac and Cova Genovesa (Manacor)**	Assemble at Hotel Palas Atenea
	Bring with you:	Camera, walking shoes, torch (flash light)
13:30	**Lunch**	Restaurant *Es Cruce* (road Palma-Manacor)
17:00	**Poster Session & Refreshments**	Hotel Palas Atenea
Poster	Bilandžija, Jalžić, Cukrov & Cukrov	Anchialine caves in Croatian karst area
Poster	Cuculić, Cukrov, Kwokal & Jalžić	Distribution of total and dissolved Cd, Pb, Cu and Zn in water columns and sediments of two anchialine caves in Mljet National Park, Croatia
Poster	Cukrov, Kwokal, Cuculić, Omanović & Jalžić	Ecotoxic metal concentrations in sediment from Croatian anchialine caves
Poster	Jalžić, Bilandžija, Cukrov & Cukrov	History of anchialine cave research in Croatia
Poster	Kwokal, Cuculić, Cukrov & Jalžić	Mercury distribution in water column and sediment of two anchialine caves in Mljet National Park, Croatia
Poster	Manconi	On a benthic community from a subterranean estuary in a karstic coastal cave (eastern Sardinia) with notes on Porifera

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Poster	Mejía-Ortiz	Diversity patterns of cave crustaceans in Mexico
18:20	Closure	

FRIDAY 20 NOVEMBER 2009

Evolutionary history, Historical biogeography and Molecular phylogenetics *— Chair: Tom Iliffe*		
09:00	Boxshall	Copepods and anchialine caves
10:00	Messana, Filippelli & Baratti	Microevolutionary processes in stygobitic isopods from the Western Mediterranean basin
10:20	Koenemann	The Tethys enigma – Optimization of DNA sequence data in paleogeographic analyses
10:40	Humphreys, Page, Stevens & Foster	Scale of movement in anchialine systems: progress in the eastern Indian Ocean
11:00	Boxshall & Jaume	Anchialine cave crustaceans: from ancient relicts to new colonists
11:20	**Refreshments**	
Chair: Geoff Boxshall		
11:40	Longley & Holsinger	Relict anchialine faunas of the Edwards Aquifer, Balcones Fault Zone and associated areas in south-central Texas and northern Mexico
12:00	Martínez & Wilkens	Origin and ecology of the anchialine fauna of Lanzarote (Canary Islands)
12:20	Botello, Iliffe, Juan, Pons, Alvarez & Jaume	Phylogeny of the genus *Typhlatya* (Crustacea: Decapoda: Atyidae): speciation patterns and time of divergence
12:40	Bauzà-Ribot, Juan, Pons & Jaume	Microevolutionary processes in the subterranean amphipod crustacean *Metacrangonyx longipes* deduced from mitochondrial DNA data
13:00	Por	The Anchialine-Ophel Continuum Concept - The case of three sites in the Mediterranean space
13:20	**Lunch**	
Biodiversity and Conservation — Chair: Bill Humphreys		
15:40	Iliffe & Kvitek	Search for Bermuda's deep water caves
16:00	Becking & de Voogd	Anchialine lakes in Indonesia
16:20	Cukrov, Jalžić, Bilandžija & Cukrov	Research history and anchialine caves characteristics in Croatia
16:40	**Refreshments**	

FRIDAY 20 NOVEMBER 2009

17:00	Merino, Mulet, Mulet, Croix & Gràcia	Cova des Pas de Vallgornera, a unique littoral cave
17:20	Sintes, Martínez-Taberner, Moyà, Ramón & Herndl	Structure and function of the microbial food web in the absence of light
17:40	Carol Wicks	Summation
18:00	Closure	
20:30	**CONFERENCE DINNER**	Restaurant *Sa Cranca* (2 min walk from Hotel Palas Atenea)

ABSTRACTS

Abstracts for posters and oral presentations are listed together in alphabetical order by first author

Oral presentation

Jurassic neptunian dykes – insight to fossil submarine cave biotas

Roman Aubrecht and Ján Schlögl

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Caves are rarely preserved in fossil record. Subterranean spaces in their final stage of evolution usually collapse and are recognized as collapse breccias. Neptunian dykes and sills, originally representing fractures and clefts in the sea bottom, are usually best preserved representatives of the submarine cave environments. Most of them were filled with younger sediment which prevented them from collapse. The sedimentary filling is mostly marine, therefore they provide the best insight to fossil submarine cave environments.

Two types of biota occur in neptunian dykes: 1. Allochthonous, open marine biota, washed into the clefts from the sea bottom, 2. Autochthonous cave biota which inhabited the subterranean spaces. Studies of Middle to Late Jurassic (Bathonian to Tithonian) neptunian dykes, sills and collapse breccias in the Pieniny Klippen Belt (Western Carpathians) brought some examples of the cave-dwelling fauna, e.g. cave-dwelling ostracods, serpulid reefs, ahermatypic corals, bivalves, brachiopods and small ammonites.

The studied dykes contain autochthonous fauna of ostracods *Pokornyopsis feifeli* (Triebel). The fissure fillings are of Bathonian to Oxfordian age connected with the Ammonitico Rosso type limestones, related to a global sea-level rise. *Pokornyopsis feifeli* were exclusively found in these submarine caves and were never mentioned from the above mentioned open-marine formations. On the contrary, in the non-Tethyan Germanic Jurassic *Pokornyopsis feifeli* (Triebel) and *P. bettenstaedti* (Bartenstein) were found in claystones deposited under water depth of 100-200 m. However, these occurrences are relatively rare. Therefore, Middle Jurassic was the time when this originally deep-marine fauna started to inhabit cryptic environments. *Pokornyopsis* ostracods are forerunners of the Recent anchialine ostracod faunas, namely of the genus *Danielopolina*, as most of the recent species of this genus live in anchialine caves; only one species inhabits the deep-water environment.

Late Jurassic (Tithonian) submarine cavities (possibly also representing openings of larger neptunian dykes) were found, containing cavity-dwelling community representing a succession of mostly solitary coelobite organisms, dominated by scleractinian corals and small-sized serpulids during the initial recruitment stage, and by serpulids during the following recruitment stages. These bioconstructors were accompanied with other suspension feeders: thecideidine brachiopods, oysters, bryozoans, sponges, crinoids and sessile foraminifers. The boundary between the first and the second recruitment stage represents an interval of aggregates growth interruption, when a thin sheet of cyclostome bryozoans developed. Corals and serpulids *Neovermilia* and *Vermiliopsis* are primary bioconstructors, all other associated organisms profited from the free spaces between the serpulid tubes. Except the first recruitment stage, the rest of the succession seems to be physically controlled by the gradual infilling of cavities. The aggregates were already bioeroded, mineralized and encrusted during their growth.

Larger serpulid tubes often contain numerous small-sized serpulid tubes attached on their internal surfaces. Two possibilities can explain this fenomenon: 1. The tubes represents serpulid larvae with a special recruitment pattern. Possible causes of such a larval behaviour probably involve several physical, biological or chemical factors. 2. The tubes represents a new unknown micromorph serpulid taxon. Such micromorph serpulids are well known from the actual marine habitats.

Another Late Jurassic (Early/Middle Oxfordian) dyke contained rich fauna of small-sized ammonites represented not only by Tethyan ammonites, but also by rare representatives of the Boreal paleobioprovince (e.g. *Cardioceras* sp.). This stratigraphic interval is normally lacking in the Pieniny Klippen Belt, thus the dykes infillings are the only "windowns" to the life in that period. There are three hypotheses concerning the small size of the ammonites collected from the dykes: 1. They were juvenile, small specimens, 2. They were adult specimens, the small size of which was caused by adaptation to the limited cave space, 3. They represented size-selected (allochthonous) cenosis of shells washed down from the open-marine environment. Our research still did not indicate which of these three possibilities is valid. Similar accumulation of small ammonites in the neptunian dykes were also described from Sicily.

The examples of *Pokornyopsis* ostracods and *Cardioceras* ammonites both represent Boreal open-marine fauna. However, in the Tethyan zone to which also the Pieniny Klippen Belt belongs they were found almost exclusively in the cryptic environments (*Pokornyopsis* even appeared as a good indicator of fissure habitat in this zone which was successfully utilized several times). The Jurassic submarine fissures and cavities evidently served as cryptic habitats for the Boreal microfauna as well as traps for the benthic and necto-benthic assemblages otherwise not unregistred in the fossil record due to stratigraphic hiatuses.

Oral presentation

Response of coastal phytoplankton to submarine groundwater discharges: role in the eutrophication of coastal areas

Gotzon Basterretxea[1], Esther Garcés[2], Antonio Tovar-Sánchez[1], Ana Massanet[1] and Itziar Alvarez[1]

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This work reports the results of a study of submarine groundwater discharge (SGD) to coastal waters of Mallorca and Cabrera Islands and its effects on nearshore plankton communities. Submarine groundwater discharges (SGD) to the coastal ocean are considered to be a major conduit for inputs of nutrients and other substances. The importance of SGD in delivering freshwater and dissolved substances to the coastal ocean is particularly significant in arid and semiarid regions, which comprise about 1/3 of the world's watersheds. At these sites, continuous groundwater outflow along the coast may be of greater ecological significance than surface runoff. Even modest SGD can impact receiving coastal waters, as concentrations of nutrients (especially compounds of nitrogen and/or phosphorous), carbon, trace metals and other elements in groundwater may be several orders of magnitude higher than in seawater, particularly when the marine environment is oligotrophic.

The delivery of anthropogenically enriched groundwater has the potential to significantly impact the coastal ecosystems of Mediterranean islands, which are typically oligotrophic, leading to increased growth, biomass algae and primary production; changes in the balance of organisms; and water quality degradation. Small Mediterranean islands are also vulnerable to SGD, because infiltration is important and riverine discharge is low in their carbonate catchments. In the last decades, changes in agricultural practices and intense tourism have lead to groundwater enrichment with fertilizers and wastewater. For example, in the vicinity of Palma, nutrient concentrations in the aquifer have increased from less than 20 mg/L to more that 150 mg/L in 30 years. This is attributed to the combined effects of changes in land use, intensive fertilization and wastewater reuse for irrigation.

Depending on geological and hydraulic characteristics, the influence of groundwater as a source of nutrients can range from regional scales to highly localized hotspots. Yet, most of the environmental problems derived for diffuse groundwater discharges in the Balearic Islands are local and generally related to the presence of semi-enclosed coastal areas where seawater renewal is low. Indeed the combination of increased nutrient availability and low water renewal, constitute a new and unique environment for which several phytoplankton species with harmful effects may become dominant.

In the present work we chemically characterize the components that are diffusely supplied to the coastal waters by SGD. Two areas have been extensively sampled: (1) the embayed beaches o Mallorca Island and (2) Cabrera National Park. The former is a populated island impacted by agricultural and tourism activities. Contrarily, Cabrera, is

a protected area, with low human pressure in which groundwater nutrient levels should be close to pristine.

Groundwater represents a vector for terrestrial elements in both systems, however, de magnitude and geochemical composition of discharged water substantially varies. The concentrations of dissolved inorganic nitrogen (DIN) in coastal groundwater and nearshore seawater, which is directly influenced by discharge, were found to be higher than those in offshore seawater. DIN was markedly enhanced along the coast; however, this was not the case for PO_4 concentrations, which do not present significant nearshore enhancement. N:P ratios in shelf waters were close to Redfield (i.e 17.8), but increased above 20 in the coast. Furthermore, our results reveal general relationships between microalgal biomass and SGD of nutrients along the coast.

We also present experimental results of coastal communities incubated 'in situ' with different concentrations of groundwater. In the experimental additions of SGD, chlorophyll *a*/phytoplankton experienced significantly increase growth rates relative to control treatment. The results suggest that SGD is an important source of nutrients enhancing plankton growth and could influence the productivity and biogeochemial cycling of oligotrophic waters.

Oral presentation

Microevolutionary processes in the subterranean amphipod crustacean *Metacrangonyx longipes* deduced from mitochondrial DNA data

Mª del Mar Bauzà-Ribot[1], *Carlos Juan*[1], *Joan Pons*[2] *and Damià Jaume*[2]

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Metacrangonyx is a phylogenetically enigmatic taxon of thalassoid subterranean amphipods with a bizarre geographic distribution. The 18 currently recognised species are distributed through groundwaters of Hispaniola Island (Caribbean), Fuerteventura (Canary Islands), Morocco, Algeria, the Balearic Islands, Elba Island (Italy), and the Middle East (Sinai Peninsula and Dead Sea depression). Because the dispersal capacities of these organisms are limited (they lack dispersive, swimming larvae and are tied to inland groundwater habitats), the amphi-Atlantic distribution of the genus has been related to vicariance by plate tectonics and to peripatric speciation, the latter associated to coastline regression episodes. In fact, marine regressions could be considered as special vicariance events over the evolutionary history of thalassoid subterranean lineages.

The study of the tempo and pattern of invasion of subterranean waters by this genus has been initiated through the study of mitochondrial DNA markers *Cox1* and *16S* and the nuclear *H3* in 13 populations of the endemic Balearic species *M. longipes*, a common inhabitant of anchialine waters of Mallorca and Menorca, but which has penetrated also deep inland on Mallorca.

Although the standing variation for the loci considered was rather low within localities, the occurrence of a significant degree of divergence (up to 9.2%) and of spatial structuring between populations was observed. The analyses pointed out that there is no gene flow even between subterranean systems placed a few kilometers apart. Moreover, there was no significant correlation between geographical and genetic distances, suggesting that populations are better grouped according to sea level fluctuations. Thus, it is likely that amphipods from caves placed on the same paleo-shoreline are closely related genetically since they were isolated sinchronously by the same sea regression episode.

ML and Bayesian phylogenetic analyses using the three markers rendered the same overall topology, providing evidence for the occurrence of at least six distinct lineages/phylogroups, which are consistent with the palaeogeography of the Balearics. Approximate dating of the divergence found among the different lineages suggests that marine regressions associated to the Messinian Salinity Crisis and the Pleistocene glacial/interglacial cycles have been the main modulators of the evolutionary relationships among Balearic populations of *M. longipes*.

Oral presentation

Anchialine lakes in Indonesia

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Anchialine lakes are unique marine habitats that could provide new insights into complex ecological and evolutionary processes in the marine realm. This rather scarce and relatively unknown ecosystem comprises of land-locked Holocene water bodies with inhabitants of marine descent. These lakes represent a combination of multiple heterogeneous environments with varying degrees of isolation from the open sea. As a result of the relative isolation from the open sea, the anchialine lakes are expected to harbor isolated populations, endemic subspecies, and new species. The marine flora and fauna of these lakes are, however, sparsely documented. Pilot surveys in anchialine lakes in East Kalimantan, Indonesia, showed that sponges were one of the most dominant taxa in terms of biomass and diversity. The objectives of the present study were to locate and document unexplored anchialine lakes in Indonesia and to record the sponge species within these lakes. We subsequently compared the lakes composition to that of coastal mangroves adjacent to the lakes in order to establish true lake-endemics. Over 400 sponge specimens were collected from anchialine lakes and mangrove systems located in East Kalimantan and West Papua in Indonesia, belonging to at least 40 genera and 25 families. In this presentation we will discuss our preliminary results that show some analogies to island systems in terms of species-area relationships, high endemicity, and genetic isolation of populations.

Oral presentation

Primary and secondary stratifications as a control on mass and energy fluxes within the Yucatan Peninsula anchialine aquifer system

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Coastal aquifers and their anchialine ecosystems include a lens of meteoric water, commonly called the fresh water lens, which floats on top of higher density saline waters of marine origin. The whole of the lowland Yucatan Peninsula of Mexico hosts an uninterrupted anchialine aquifer system within a carbonate platform. The aquifer is accessible via thousands of sinkholes, locally called cenotes, and cave diving exploration concentrated along the Caribbean coast has documented 900 km of sub-horizontal cave systems to date. The cenotes in the interior of the peninsula including those of the Ring of Cenotes are 100+m deep blind pits, and therefore are associated with matrix and fracture flow through the aquifer. In contrast, the cenotes of the Caribbean coast are collapsed sections of shallow horizontal cave ceiling, and thus are part of the interconnected conduit networks extending inland from the coast.

Multiparameter probes are effective at characterizing even small scale (0.10-0.01 m) water column structures based on specific electrical conductivity (~salinity), temperature, as well as pH and dissolved oxygen. Using a dataset of almost 200 water column profiles, the Yucatan anchialine aquifer system includes: 1. Fresh cap layer, 2. The bulk of the fresh water lens, 3. A fresh-saline mixing zone commonly with internal secondary haloclines, 4. A shallow saline zone of 1-5 m thickness and slightly dilute compared to marine water, 5. A deeper saline zone of full marine salinity waters.

The site geomorphology is a primary control on the localized water column characteristics with particular influence exerted by the depth levels of the conduit ceilings, depth of breakdown piles relative to fresh-saline mixing zone depth, and depth of the floors of the conduits. The maximum thickness of the fresh cap layer coincides with the depth of the conduit ceiling depth. The horizontal turbulent flow through caves integrates waters from the base of the fresh cap layer, forming a visually striking contrast between the clear conduit waters and the fresh cap layer rich in organic acids leached from the forest liter. The fresh-saline mixing zone does not vary systematically in thickness with distance over 1-100 km from the coastline, but instead is locally thickened near break-down blocks spanning the mixing zone as saline water is turbulently mixed into the base of the fresh water lens. Closely spaced water column profiles along conduits also reveal the reestablishment of the unperturbed thinner mixing zone within 10's of meters downstream. The depth of the density interface separating the shallow slightly dilute marine salinity waters from the deep full marine salinity zone coincides with the floor level of the uppermost tier of conduits.

The combined results of the water column characterizations and observations of flow using quantitative traces, instrumental point velocity measurements, and long term flow monitoring, demonstrate the effective decoupling of the groundwater circulation between each of these identified vertically stacked sub-stratifications within the Yucatan anchialine aquifer system. Within the fresh cap layer, water flux is principally via the matrix and fractures as shown by the distinct thermal regime, the long lived

coloration, and marked algal blooms feeding on the higher nutrient concentrations in these effectively trapped and stagnant waters. In contrast, the tidally modulated fresh water circulation through the conduit networks is typically at rates of 0.5 – 2.5 km/day, with peak velocities in some sites exceeding 10 km/day. The fresh water lens flows coastward via the conduits, with only short and rare reversals. In the shallow saline zone, the circulation is of the same velocity range as the overlying fresh water, but commonly with full-reversals including periods of sustained inland saline flow that occur all while the overlying fresh water flows coastward. Direct flow measurements supported by temperature as a non-conservative natural tracer has revealed an efficient shuttling of marine water via the conduit networks to a distance of 8+ km inland. At the saline-saline interface, a further saline circulation cell is identified where water of marine salinity continuously flows inland, presumably to discharge on the opposing coast.

The significant density gradient across the fresh-saline mixing zone completely decouples the circulation of the fresh and saline water masses, indicating the negligible role of entrainment in coastal conduit aquifer circulation. While the fresh-fresh and saline-saline mixing zones are 1/10 to 1/100 that of the principal fresh-saline mixing zone, these secondary density interfaces nonetheless are environmentally significant and effective at decoupling the fluxes between the water masses. Flow within the Yucatan anchialine aquifer is therefore dominated by horizontal flow vectors, with mixing between water layers localized in areas of perturbation. It is indicated that water column characterization is a necessary foundation for ecological studies in anchialine systems as a basis for the conceptualization of where nutrient and energy fluxes are possible, and also identification of subtle vertically stacked ecosystem portioning.

Poster presentation

Anchialine caves in Croatian karst area

Helena Bilandžija[1], Branko Jalžić[1,2], Marijana Cukrov[1] and Neven Cukrov[3]

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The karst area covers more than 50 % of the Croatia. The whole Croatian coast of the Adriatic Sea is a karst region characterized by a porous limestone and semi-porous dolomites with some flysch, an impervious thin-layered sedimentary rock, as hydraulic barriers.

Over 9000 caves are known, many of which are located on the islands or along the coastline. Up till now only 64 anchialine caves are partially explored and/or described in the literature. All of the anchialine objects in Croatia are situated near the shoreline (less than 100 meters away) and most of them have a pit-like entrance. They are mostly small and not interesting for speleologists; the longest one is 245 m long cave Medvjeđa špilja and the deepest is Jama u Podstražišću pit with 45 m deep dry part and over 50 m deep water layers. Tides are more or less notable in all anchialine caves and in several caves a stream of fresh water flows over brackish and marine layers. Most of the caves don't have clear connection to the sea but a few, like cave Medova buža, have an open pathway.

There are few very specific anchialine caves in Croatia. For instance, the cave Rudnik kod Medveje is of artificial origin. This cave is a former borehole which was drilled in search for drinkable water. The caves Sumporača velika and Sumporača mala are anchialine caves with elevated concentrations of sulfur. Water and mud from these caves were used for medical purposes during the past. Cave Orljak is unique anchialine cave at the Adriatic coast because it is connected to the estuarine (brackish) water.

Some of the anchialine objects are interesting from a paleontological point of view like Vrtare Male Pit. This is one of the greatest finding sites of Pleistocene fauna, where the remnants of an elephant, horse, rhinoceros, lion, cave bear, wolf, deer, and a lot of micromammals and birds were excavated.

Anchialine caves are inhabited by phylogenetically and biogeographically interesting animal taxa. Deep-sea sponges are known from several localities. 21 various animal taxa was described from 7 anchialine caves: Veštar, Jama iznad Vrulja, Jama Bač II, Živa voda, Supurina, Jama na Badiji, and Šipun. The most remarkable is the cave Šipun, it is a type of locality with 14 animal taxa, 8 troglobionts, 2 troglophiles, and 4 stygobionts.

Intensive tourism, pollution, and rapid urbanization are major causes for endangerment of anchialine habitats. All speleological objects as well as all subterranean fauna are strictly protected by the Croatian laws, however, active protection is still missing.

Oral presentation

Ecological physiology of anchialine organisms: A comparison between three cave systems

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We examined the ecological physiology of organisms inhabiting three different anchialine environments consisting of caves on five islands in the Bahamas, three caves in the Yucatan of Mexico, and the Túnel de la Atlántida, Lanzarote, Canary Islands. The objective of this study was to look at how metabolism, including oxygen consumption and enzyme activities, varies by cave system and the unique physical environment each system presents.

The Bahamian and Yucatan systems, like many anchialine environments, have suboxic and anoxic regions. The Túnel de la Atlántida system differs from the Bahamian and Yucatan anchialine caves by an abundance of dissolved oxygen. During our monitoring period, dissolved oxygen in the Túnel de la Atlántida did not fall below oxic levels (8.0 – 2.0 ml O_2/L). In Bahamian and Yucatan systems, permanent salinity stratification in the cave water column and the presence of a hydrogen sulfide layer prevents the circulation of oxygen as well as nutrients in the cave systems. In these environments, oxygen levels fall to suboxic (0.2 – 0.0 ml O_2/L) and even anoxic (0.0 ml O_2/L) levels at the hydrogen sulfide layers. Because Lanzarote is a relatively dry island, lacking significant fresh groundwater, there is neither water column stratification nor a hydrogen sulfide layer in the Túnel de la Atlántida. Additionally, the 2 m tide range provides for the pumping of large volumes of oxygenated sea water into the cave with each tidal cycle. As a result of this tidal exchange, plankton is swept in from the open ocean and terrestrial organic matter enters from the inland portion of the lava tube. In the sunlit portions of the Jameos del Agua lagoon, there is also photosynthetic primary production and bacterial chemoautotrophic primary production occurs within the cave.

Metabolism was determined by directly measuring oxygen consumption and indirectly, through the examination of activities of key metabolic enzymes. Citrate synthase (CS) located at the beginning of the Kreb's citric acid cycle, is an indicator of an organism's maximum aerobic potential. Lactate dehydrogenase (LDH) is the terminal enzyme in glycolysis that contributes to both aerobic and anaerobic metabolic pathways. It serves as an indicator of glycolytic potential. In facultative anaerobes, malate dehydrogenase (MDH) plays a dual role. In the presence of oxygen, phosphoenolpyruvate (PEP) is converted to pyruvate and via the Krebs citric acid cycle is oxidized completely to CO_2 and H_2O. In anaerobic conditions, PEP is carboxylated to oxaloacetate which is further reduced to malate by malate dehydrogenase (Hochachka *et al.* 1973). Examination of these enzyme activities provides insight into how anchialine organisms address the physiological challenges presented by their environment.

Metabolic cave adaptation was evident in organisms from all three environments. All stygobiontic organisms examined had lower metabolic rates than rates observed for similar epigean organisms. When compared to organisms from anchialine limestone

cave systems in the Bahamas, the Lanzarote invertebrate oxygen consumption rates were significantly greater. Since many caves have their own endemic fauna, it is difficult to compare organisms between caves. However, the anchialine caves in the Bahamas, Yucatan and the Túnel de la Atlántida all have the amphipod *Spelaeonicippe* (Family Pardaliscidae). *Spelaeonicippe* found in all three systems were compared based upon mass with *Spelaeonicippe buchi* from Lanzarote being significantly larger than *S. provo* from the Bahamas. Additionally, Hippolytid shrimp, *Barbouria* spp., thought to be a recent colonizer of cave systems, can be found in both the Bahamas and the Yucatan. Respiration rates of *Barbouria* were lower than reported values for epigean Hippolytid shrimp yet greater than rates observed in truly cave- adapted species. Enzymes of the *Barbouria* were anaerobically poised with LDH activities exceeding those of CS in all organisms, however; unlike other anchialine organisms, MDH activities were lower than LDH activities, indicating less adaptation to a reduced oxygen environment and supporting a more recent introduction to the anchialine environment.

Although all three systems are anchialine environments, significant differences in the physiology of the stygobiontic invertebrates exist between the systems based upon the morphology of the cave and the resulting oxygen content and/ or potential food supply. Organisms from the Túnel de la Atlántida had a greater metabolic rate and individuals reached a larger size. Time since colonization and degree of cave adaptation also play a role in the ecological physiology of anchialine organisms.

Oral presentation

Phylogeny of the genus *Typhlatya* (Crustacea: Decapoda: Atyidae): speciation patterns and time of divergence

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The shrimp genus *Typhlatya* includes 17 species inhabiting groundwater, mainly anchialine, habitats scattered west-to-east from the Galapagos to the Mediterranean. Its extremely disjunt distribution pattern has prompted considerable biogeographical debate and controversy since the genus is strictly subterranean and has never been reported from normal marine environments. The aim of this work was to study the pattern of diversification in several *Typhlatya* species by inferring their phylogenetic relationships, and to estimate the time of divergence between species. We have gathered nucleotide sequences of the mitochondrial genes 16S, COI, Cytb and the nuclear H3 and 18S genes from ten out of the 17 currently recognised species of the genus. According to our genetic analyses, *Typhlatya* does not constitute a monophyletic group as it currently stands, the east Pacific *T. galapagensis* representing a separate lineage closely related to *Antecaridina*. In addition, *T. kakuki* —a presumed endemic to Acklins Island in the Bahamas- results to be genetically identical to *T. garciai* from Caicos, and should be considered only as a population of the latter species with a completely regressed cornea. Our results lend support and strengthen previous studies of other authors suggesting the inclusion of the Australian genus *Stygiocaris* into *Typhlatya*. Within this new *Typhlatya* concept, two lineages appear clearly supported, one including the former *Stygiocaris* (three species, one of them not yet formally described); the other, embracing the rest of *Typhlatya* except *T.* cf. *monae* from the Dominican Republic, which remains unresolved in the cladogram. The topology of the second clade shows a clear geographic (amphi-Atlantic) projection, with a lineage comprising the two Mediterranean species *vs.* a [Yucatan+Bahamas+Mid-Atlantic Ridge] clade. The Yucatan clade appears as clearly monophyletic, as it is the [Bahamas+Bermuda+ Ascension] clade. Our results are congruent with an origin for the two Mid-Atlantic Ridge species in the northern Caribbean area, which lend support to previous statements on the potential for overseas dispersal of Atyid larvae and the role played by the Gulf Stream in the colonization of Bermuda by some members of its anchialine fauna. Regarding the species of Ascension and Bermuda islands (*T. rogersi* and *T. iliffei*), their position at terminal branches of the cladogram is not congruent with previous hypotheses relating the origin of the Atlantic anchialine fauna at the deep-sea Mid-Atlantic Ridge. Divergence times estimated using the average 2.3% rate of evolution in mitochondrial genes are congruent with a vicariant scenario for the diversification of the genus related to the fragmentation of the Tethys ocean.

Oral presentation

Copepods and anchialine caves

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Free-living copepods can be both abundant and diverse in anchialine cave systems. Families such as the Epacteriscidae and Speleophriidae are commonly regarded as part of the typical anchialine faunal suite, and families such as the Ridgewayiidae could claim a similar status. Other, smaller families including the Speleoithonidae and Boholindae are known only from anchialine habitats. Updated phylogenies for the larger families are presented here and compared. Of particular interest are the presence and position on the trees of clusters of congeneric species. Wider comparisons are made with other anchialine crustaceans in an attempt to identify any general patterns of speciation. In this review patterns are identified in two dimensions (i.e. geographical distributions) and in 3-D, in relation to tectonic events that change zoogeographic distributions through geological time. The fourth dimension - depth - impacts primarily through fluctuations in sea-level through time, which result in regressions and strandings. Patterns of adaptive radiation are analysed in an attempt to understand the key factors shaping the evolutionary histories of copepods in anchialine caves.

Oral presentation

Anchialine cave crustaceans: from ancient relicts to new colonists

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The exploration of anchialine systems over the past three decades has revealed an astonishing diversity of metazoan species, especially in the Crustacea. The exciting discovery phase revealed a crustacean fauna of such novelty that it initially defied classification. Early studies recognised several new higher taxa, including a new class, the Remipedia, and new orders and suborders, such as the Platycopioida and Mictacea, in addition to numerous new families. The classification and/or the composition of many of these taxa are still unstable and we will explore the factors generating this instability. Where classification systems are more stable, many anchialine crustaceans tend to occupy relatively basal positions in their respective lineages, despite the obvious specialisations that adapt them to life in such an extreme ecosystem. So should we view anchialine crustaceans as relicts?

The zoogeography of anchialine crustaceans is another remarkable attribute – with many genera exhibiting extreme disjunct distribution patterns. As exploration progressed, comparative faunistic studies at widely separate localities revealed remarkable similarities, and led to the development of the concept of the 'typical anchialine faunal suite' consisting of, for example, a remipede, a thermosbaenacean, a *Danielopolina*, an epacterisicid copepod, etc. Narrative explanations of the origins and history of anchialine crustacean faunas have emerged, from which it has been inferred that the typical faunal suite is ancient and that its modern distribution patterns have been largely shaped by tectonics and extinctions. How well are such explanations supported? We will review the available evidence base, and will explore ways in which these explanations can be tested.

Oral presentation

Research Group “BioGeoChemistry of Tidal Flats”: A multidisciplinary process study in the NW German Wadden Sea

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The research group “BioGeoChemistry of Tidal Flats” was established in 2001 by the German Science Foundation (DFG) to perform an in-depth study of physical, chemical, and microbiological processes in sediments, pore waters, the water column, and water column particulates of a backbarrier system on tidal, seasonal and annual time scales. The goal of this project, which is funded until 2009, is to gain a fundamental understanding of the budget of dissolved and particulate compounds within the tidal flat system and its impact on the southern North Sea.

In this contribution we will focus on the link between organic matter and nutrient cycling within sandy tidal flat sediments and the nutrient and trace metal budget of the water column. The main drivers of biogeochemical reactions are plankton blooms in spring and summer. These biological events are reflected by annual nutrient dynamics in the backbarrier water column, which are monitored at high resolution (at least hourly) on a time series station located in the tidal inlet between the Islands Spiekeroog and Langeoog. This time series station is permanently operated and permits continuous measurements throughout the year. Thus, it provides on-line nutrient data (Si, Σnitrite/nitrate, phosphate) as well as physical parameters (T, S, water level, turbidity) and meteorology. Additionally, pressure/temperature sensors and pore water lances are deployed in the tidal flat sediments on a transect from the low water line to the top of the tidal flat to gain insight into processes driven by advection. Pore waters are retrieved "in situ" on a monthly basis down to depths of five meters.

From our current understanding, we can state that the biogeochemical reactions associated with organic matter degradation within the sedimentary column are fuelled by the seasonal incorporation of algal material into the porous sands. Degradation products, i.e., regenerated nutrients or redox-sensitive trace metals like manganese are enriched in pore waters and are partly re-circulated into the water column during low tide, when pore waters are draining from tidal flat margins. The backbarrier water column reflects this pore water seepage on tidal and seasonal scales.

Tidal flats may be regarded as a "bio-reactor", where particulate organic matter is degraded trough a suite of microbially mediated redox steps and associated nutrients are liberated and finally recirculated into the water column by discharging pore fluids.

Distribution of Hg, Cd, Pb, Cu and Zn in water columns and sediments of two anchialine caves in Mljet National Park, Croatia

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Distribution of Hg total, and Cd, Pb, Cu and Zn total and dissolved concentrations in water columns, and their total amounts in sediment columns of two anchialine caves Bjejajka and Lenga, in the remote Mljet National park in Croatian Southern Adriatic during four years period (2006 - 2009) was studied.

Water samples for Cu, Cd, Pb and Zn determination were collected in pre-cleaned high-density polyethylene bottles (HDPE) (1 l), while for Hg water was collected in 1 l borosilicate glass bottles. Sediment column samples for all metals were collected in hand-driven acrylic corers, both by speleo scuba-diver. Cu, Cd, Pb and Zn measurements were performed by differential pulse anodic stripping voltammetry (DPASV). Obtained trace metals concentrations are presented as dissolved and total fractions. Total concentrations were measured in acidified (pH < 2), unfiltered and UV irradiated samples, while dissolved concentrations were obtained from filtered (0.45 µm cellulose nitrate filter), acidified and UV irradiated samples. Hg measurements were performed by cold vapour atomic absorption spectrometry (CVAAS). Mercury concentrations found in unfiltered, acidified and UV irradiated samples are presented as total (Hg_{tot}). Additionally, temperature, salinity, pH and dissolved oxygen concentration were determined directly in water columns of both anchialine caves. Salinity increased constantly with depth in the range from 3 ‰ at the surface to 38 ‰ in bottom water layer, showing water column stratification.

In general, concentrations of all metals in water columns of both caves were significantly higher in comparison to metals amount found in seawater samples taken in near vicinity (~ 100 m). Moreover, total Cu (up to 28 µg l^{-1}) and Zn (up to 10 µg l^{-1}) in Lenga cave water column were found to be considerably higher comparing to Bjejajka cave (Cu < 5 µg l^{-1} and Zn < 4.5 µg l^{-1}), while Pb exhibited similar abundance in both caves. Most intriguingly, mercury and cadmium concentrations in water column of Bjejajka cave were greatly elevated in comparison to their amounts found in water column of Lenga cave. Substantial concentrations of total Hg (920 ng l^{-1} at 7 m) and Cd (300 ng l^{-1} at 9 m) were found in water column of Bjejajka cave in January 2009, while in Lenga cave water total Hg and Cd did not exceed 15 and 42 ng l^{-1}, respectively. In surface seawater samples collected in the vicinity of the Bjejajka cave (~ 100 m), quite low total concentrations of 0.5 ng l^{-1} Hg, 6 ng l^{-1} Cd, 33 ng l^{-1} Pb, 220 ng l^{-1} Cu and 190 ng l^{-1} Zn were detected. In Bjejajka cave surface sediment samples, mercury concentrations were up to 3.3 mg kg^{-1} and cadmium up to 1.34 mg kg^{-1} dry weight. On the other hand, in Lenga cave sediment, Hg and Cd concentrations were considerably smaller, 0.8 mg kg^{-1} and 0.7 mg kg^{-1} dry weight, respectively. Namely, small colony of bats seasonally inhabits Bjejajka cave. Concentrations of mercury and cadmium in guano of these mammals were found to be 0.31 and 1.8 mg kg^{-1} wet weight,

respectively. These values are in the range of Hg concentrations in sediment, and above the range of Cd concentrations usually reported in surface sediments. Unlike Bjejajka, bats were not spotted in Lenga cave, which is in accordance to its considerably lesser Hg and Cd concentrations. Most probably, bats were the main natural source of mercury and cadmium elevated concentrations in water column and sediment of Bjejajka cave. Higher Zn and Pb content in surface sediment in Lenga cave were detected, comparing to those found in Bjejajka cave sediment (Lenga cave: Zn up to 700 mg kg^{-1}, Pb up to 65 mg kg^{-1} dry weight; Bjejajka cave: Zn up to 160 mg kg^{-1}, Pb up to 18.5 mg kg^{-1} dry weight). All metals concentrations in Bjejajka cave increased with sediment column depth.

This has been the first report ever on Cu, Cd, Pb and Zn both concentrations, total and dissolved in water column of the anchialine caves. Also, mercury speciation in water columns was assessed. Five different mercury species were characterized: total, reactive, monomethylmercury, total gaseous mercury and labile ionic mercury. Presented results express specific metals behaviour, particularly mercury and cadmium in aquatic system of Bjejajka and Lenga anchialine caves, specific natural objects in Mljet NP.

Oral presentation

Research history and anchialine caves characteristics in Croatia

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The karst area covers more than 50 % of the Croatia and over 9000 caves are known in that area. Up till now only 64 anchialine caves are partially explored and/or described in the literature and our research group has information's about another 50. During history anchialine caves were very important because they were valuable source of drinkable water. The first information on an anchialine cave in Croatia was written by the Croatian geologist Josip Poljak in 1920. He described the cave "Urinjska špilja" near Rijeka, drow it's topographical map, and reported that cave water was brackish. In 1950's Stanko Karaman studied faunistically anchialine waters around Dubrovnik, including the cave Šipun in Cavtat. Since his first visit of the cave "Šipun" (1958), during 1960's, till 1980's significant research of anchialine caves along the Adriatic coast was conducted by Boris Sket from Ljubljana (Slovenia). Faunistically and ecologically explored have been more than 30 anchialine caves along the entire coast but mainly at the National Park Kornati Islands, beside the "Šipun" cave. This resulted in the later confirmed ecological scheme of the anchialine water bodies: salinity stratification, local oxygene depletion, and faunistic stratification by biotic exclusion. The first described snorkel dive in an anchialine cave was made by Bruno Puharić, in the "Urinjska špilja" cave in 1963. Few years after that, Rupert Riedel (Wien), investigated some submarine caves near Rovinj city and on the Krk Island. An important contribution to subsequent explorations was made by the geographer Ivo Baučić who in 1962 completed the list of caves on the Adriatic coast and islands. In the period between 1968 and 1975 geological and paleontological research of anchialine caves were made by Srećko Božičević and Mirko Malez, mainly on Lošinj, Cres and Rab Islands. Tonči Rađa from Split investigated anchialine caves during 90's and at the beginning of this century, sampling their fauna. Recent research of some anchialine caves was done on Hvar and Krk Islands by the Natural History Museum of Rijeka and the University of Zagreb.

Research became more intense after 2005 due to Branko Jalžić, who on the suggestion of Frano Kršinić from the Institute for Oceanography and Fisheries, started researching of anchialine caves along Croatian coast of the Adriatic Sea. In that period came into existence a multidisciplinary group interested in anchialine cave research. Extensive interdisciplinary field work and new data on water, sediment, and biota of anchialine cave environment was done by members of the Croatian Biospeleological Society, Croatian Natural History Museum and Ruđer Bošković Institute. From this associate research, F. Kršinić described new genera and species of copepod crustaceans and V. Žic describe distribution of iodide and iodate in anchialine cave-waters.

In Croatia anchialine caves are mostly small and not interesting for speleological research. The longest one is 245 m long cave "Medvjeđa špilja" and the deepest is "Jama u Podstražišću" pit with 45 m deep dry part and over 50 m deep water column.

Most of the caves don't have clear connection to the sea, but tides are more or less notable in all anchialine caves and in several caves occasionally a stream of fresh water flows over brackish and marine layers. Same caves, like cave "Medova buža", have an open pathway to sea.

There are few very specific anchialine caves in Croatia. For instance, the cave "Rudnik kod Medveje" is of artificial origin. This cave is a former borehole which was drilled in search for drinkable water. The caves "Sumporača velika" and "Sumporača mala" are anchialine caves with elevated concentrations of sulfur. Water and mud from these caves were used for medical purposes during the past. Cave Orljak is unique anchialine cave at the Adriatic coast because it is connected to the estuarine (brackish) water and have low trace metal concentration. However, same caves like "Bjejajka" and "Lenga" have naturally elevated trace metals concentrations in water and sediment.

Some of the anchialine objects are interesting from a paleontological point of view like "Vrtare Male" Pit. This is one of the greatest finding sites of Pleistocene fauna, where the remnants of an elephant, horse, rhinoceros, lion, cave bear, wolf, deer, and a lot of micromammals and birds were excavated.

Anchialine caves are inhabited by phylogenetically and biogeographically interesting animal taxa. Deep-sea sponges are known from several localities. 21 various animal taxa was described from 7 anchialine caves: "Veštar", "Jama iznad Vrulja", "Jama Bač II", "Živa voda", "Supurina", "Jama na Badiji", and "Šipun". The most remarkable is the cave "Šipun", it is a type of locality with 14 animal taxa, 8 troglobionts, 2 troglophiles, and 4 stygobionts.

Underground habitats and species are extremely vulnerable and threatened by external influence. Because of that all speleological objects, as well as all subterranean fauna is strictly protected by the Croatian laws.

Poster presentation

Ecotoxic metal concentrations in sediment from Croatian anchialine caves

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A fundamental ecological aspect of ecotoxic metals is their toxicity for living organisms and the lack of biodegradability. Once introduced into the aquatic environment, ecotoxic metals are redistributed throughout the water column, deposited or accumulated in sediments and consumed by biota. Anchialine caves are mostly small and isolated, hence, very sensitive ecosystems. Therefore, it is very important to know ecotoxic metal concentrations, because elevated metal concentrations in these systems are often considered as indicators of anthropogenic influence. In all aquatic ecosystems, sediments act as ultimate sinks for ecotoxic metals. However, sediments are not only a sink, but may act as a source of metals in the aquatic environment. In order to evaluate the metal pollution in sediments, it is very important to be able to distinguish between the natural trace element record derived from sedimentation and records that may reflect the anthropogenic impact. For this is important to know present concentrations in as many as possible anchialine caves sediments.

As a whole Croatian Adriatic coast is a karst region, there are situated numerous anchialine caves. Sediment samples were collected from 6 anchialine caves (Jama kod Komune, Urinjska špilja, Vrtare Male, Sumporna špilja mala, Sumporna špilja velika and Šipun) in the period between 2005 and 2007. Surface sediment samples were collected by scuba diving using hand-made and hand-driven Plexiglas cores.

Sediments were wet sieved using Sieve shakers AS 200 Digit with 0.063 mm standard Retsch sieves (Haan, Germany) and the fine fraction (<63 μm) was separated for future analysis. Chemical analyses were performed by ICP, ICP/MS and for Hg determination by cold vapour atomic absorption spectroscopy. The sample material was digested with aqua regia digestion (0.5 ml H_2O, 0.6 ml concentrated HNO_3 and 1.8 ml concentrated HCl).

Chemical contents were obtained for 63 elements and in this paper Hg, Cr, Mn, Co, Ni, Cu, Zn, As, Ag, Cd and Pb concentrations were evaluated.

Total metal concentrations in anchialine caves sediments varied for Hg (0.15 to 1.06 mg/kg), for Cr (29 to 234 mg/kg), Mn (215 to 684 mg/kg), Co (6.9 to 18.2 mg/kg), Ni (57 to 225 mg/kg), Cu (25 to 77 mg/kg), Zn (41 to 133 mg/kg), As (9.9 to 41.6 mg/kg), Ag (0.07 to 0.40 mg/kg), Cd (0.23 to 0.61 mg/kg) and Pb (14 to 95 mg/kg).

In order to evaluate possible toxicity, sediments were compared with Sediment Quality Guidelines from National Oceanic and Atmospheric Administration, USA (1999). ERM (effect range low) and ERM (effect range median) were calculated for 9 ecotoxic metals (Hg, Cd, Pb, Cu, Zn, Cr, Ni, As and Ag). ERL guideline represents concentrations below which effect were rarely observed, while ERM guideline represents concentrations above which toxic effect frequently occur.

The concentration levels of mercury were seriously elevated (above ERM guidelines) in Šipun and Urinjska špilja caves, and elevated (above ERL guidelines) in all other researched caves, except Vrtare Male cave. Chromium concentrations levels were elevated (>ERL) in four caves. The concentrations levels of nickel were seriously elevated (>ERM) in all researched caves. Copper concentrations level were elevated (>ERL) in all caves except Urinska špilja cave. Arsenic concentrations levels were elevated (>ERL) in all caves. The concentrations level of zinc, silver and cadmium were below ERL guidelines. Similarly, lead concentrations levels were below ERL guidelines except in Šipun cave, where are above ERM guidelines.

It is important to understand that these guidelines (ERM & ERL) were not derived as toxicity thresholds. That is, there is no assurance that there will be a total lack of toxicity when metal concentrations are less than the ERL values. Similarly, there is no assurance that samples in which ERM values are exceeded will be toxic. Toxicity, or a lack thereof, must be confirmed with empirical data from toxicity tests.

NOAA (1999). National Oceanic and Atmospheric Administration, Sediment Quality Guidelines developed for the National Status and Trends Program. (http://archive.orr.noaa.gov/cpr/sediment/SPQ.pdf)

Oral presentation

The littoral endokarst of Mallorca Island (Western Mediterranean)

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Mallorca Island shows extensive limestone areas all along its coastal fringe. Specifically, the karst developed in the Upper Miocene carbonate rocks of eastern and southern coasts of the island, the so called Migjorn region, is renowned by its spectacular and well-decorated littoral caves –like Coves del Drac, in Manacor municipality– early explored at the end of 19th century by Edouard A. Martel. Over the years, the large amount of morphological and topographical data collected by cavers seem to confirm that the most typical caves, within the Migjorn karst region, consist basically of one or more collapse chambers connected in an apparently random pattern and partially drowned by the postglacial rise of the water table. Today increasing knowledge on the coastal cave systems is supported on new cave diver explorations and detailed underwater surveying. This new data suggests that previous assumptions about the patterns of the caves developing in this eogenetic karst must to be updated in order to embrace the information provided specially by cave diver mapping. Regarding cave-patterns, several major evidences should be outlined: the connectivity between neighbouring caves becomes greater than expected and, at the same time, extensive collapse features also characterize the main underwater segments of these coastal caves; furthermore some long joint-guided passages are occasionally found below the water table.

Regarding its morphogenesis, the endokarst developed in the Upper Miocene carbonates of Migjorn is much more heterogeneous and complex than postulated some decades ago. The observations carried out in some extensive cave-systems show a strong lithological control in the configuration of the resultant caves. In short, a very sharp dichotomy exists between the passages and chambers formed in the reef front facies, where abundant corals are present, and those galleries excavated in the lagoon facies, which have a very lower permeability. In the former the big breakdown chambers are dominant, whereas in the latter the solutional passages with a strong structural control are the ruling tendency. In general terms, the speleogenesis and morphological evolution of the Migjorn's endokarst were controlled by sea-level fluctuations along the Quaternary, including the presence of conspicuous Phreatic Overgrowths on Speleothems (POS) that record Mediterranean sea-stands.

Finally, it is worth to be mentioned the recent explorations of new extensions in Cova des Pas de Vallgornera (Llucmajor municipality), with a current development longer than 63,000 m, which have supplied new insights regarding the morphogenesis of such outstanding site from the Migjorn region. This exceptional cave seems to contain evidences of a complex speleogenesis that embraces, besides the coastal mixing zone karstification, a noticeable meteoric water recharge together with a possible basal recharge of hypogenic origin. In this respect, solutional rising flow features are abundant in the inner galleries, being presumably related to the geothermal phenomena recognized in the area.

Oral presentation

Novel bacterial diversity in an anchialine blue hole from Abaco Island, Bahamas

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Cherokee Road Extension Blue Hole, located in a pine forest on Abaco Island, Bahamas, is a dynamic anchialine ecosystem with micro and macro biota including of a several cm thick bacterial mat covering cave walls below the halocline, as well as nerillid worms and three types of crustaceans: stygiomysids, ostracods, and copepods. While numerous investigations have been conducted on the stygobitic macro fauna of anchialine caves, the extraordinary microbial communities of these habitats are by and large unstudied. Inland blue holes in the Bahamas are characterized by a high degree of vertical stratification and well delineated pycnocline. They are capable of sustaining numerous microhabitats, each with their own potentially unique microbial interactions. Identification and characterization of anchialine microbial diversity will permit "bottom-up" designs of the subterranean aquatic food webs. In order to accurately conduct a culture-independent phylogenetic survey of an anchialine blue hole, environmental DNA was extracted from samples of a rust colored bacterial mat collected from the cave walls in anoxic, fully marine waters at 40 meters depth. Environmental isolates were cloned and sequenced, revealing close affiliations to Deltaproteobacteria, Chloroflexi, and several new candidate divisions associated with extreme environments such as the deep sea, volcanic eruptions, and thermal springs. Analysis of hydrologic data from Abaco's inland blue holes shows that water temperatures found at depth below the halocline are significantly cooler (0.82-1.92°C) than the mean annual air temperature (25.5°C). A possible explanation for this cooler cave water would be the presence of active circulation of deep ocean water within the Bahamas carbonate platform, a process referred to an endo upwelling. Such a deepwater circulation model may help explain the presence of deepwater bacterial assemblages found within inland blue holes.

Oral presentation

Scale of movement in anchialine systems: progress in the eastern Indian Ocean

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Epicontinental and isolated seamount anchialine faunas each contain a different but predictable, often at the genus level, assemblage of higher taxa, the former characterised especially by remipedes (Remipedia), the latter by procaridid shrimps (Procarididae), both types occurring in the eastern Indian Ocean. Christmas Island is an isolated seamount that rises from abyssal depths of the Indian Ocean and is the only exposed member of a series of volcanos that formed in the mid-Cretaceous. Recently, the thaumatocypridid ostracod of the genus *Danielopolina,* a typical element of the epicontinental anchialine fauna, has been described from Christmas Island. The occurrence of *D.* (*Danielopolina*) *baltanasi* on Christmas Island represents the first such mixing of epicontinental and oceanic seamount anchialine faunas, and, remarkably, is placed in a different subgenus to the epicontinental species of the Indian Ocean, *D.* (*Humphreysella*) *kornickeri*, and from the only known abyssal species, *D.* (*Humphreysella*) *carolynae* from mid-Atlantic deep water (Humphreys, Kornicker & Danielopol, 2009. *Crustaceana,* 82: 1177-1203). This suggests that one element at least of epicontinental anchialine waters is capable of oceanic dispersal.

The eastern Indian Ocean hosts the most vicariant example of an otherwise amphi-Atlantic epicontinental anchialine assemblages (remiped type), but it remains the least studied. The coastline bordering the North West Shelf, Western Australia, is shallow and would have been laterally displaced by >100 km during Pleistocene sea level changes of about 140 m. Concomitant lateral and vertical displacement of the anchialine fringe across a shelf penetrated by shallow anticlines of Tertiary orogenic carbonates (such as Cape Range and Barrow Island) provided opportunities for isolation of anchialine lineages by stranding inland, or by fragmentation of the anchialine fringe by intruding land. Research to find surface sister taxa of anchialine species, the fish *Milyeringa veritas* Whitley, 1945 (Eleotridae), and the shrimps *Stygiocaris* spp. (Decapoda: Atyidae) has revealed both the ancient origins of these lineages and their diversification along the former coastal plain, now comprising peninsula, continental coast and islands. Both genera are more speciose than presently accepted and within species there is significant fine scale population differentiation that is, in places, congruent between genera and suggesting common isolation events. There are at least four species of shrimp and two fish within a distance of 250 km.

Oral presentation

Historical and ecological perspectives of anchialine ecosystems

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Although several anchialine species such as the hippolytid shrimp *Barbouria cubensis* (Von Martens, 1872) and the galatheid crab *Munidopsis polymorpha* Koelbel, 1892 have been know for more than a century, it was not until the advent of modern cave diving technology that a full appreciation of the anchialine ecosystem has been possible. Even as recently as 1965, Vandel stated that "animals (from marine caves) have not usually undergone noticeable modification" and thus "marine caves . . . have but a slight interest to the biospeleologist". Recognition of the potential significance of the anchialine habitat began in 1966 when Riedl coined the term 'randhoëhlen' or marginal caves to describe coastal caves containing brackish to marine water and listed 16 troglobiont species. In 1973, Holthuis discovered unusual shrimp inhabiting brackish pools from various locations around the world and referred to this habitat as 'anchialine'. At the 1984 Bermuda Marine Cave Symposium, the definition of 'anchialine' was broadened to include tidal, sea level pools within caves and the submerged passages connecting them to the sea.

Anchialine caves occur in karstic and volcanic terrains, primarily on islands in the tropics, but also along continental margins such at the Yucatan (Mexico) and Cape Range (Australia) Peninsulas where the hydrology is 'island-like' with a freshwater lens floating on underlying seawater. Locally, they are referred to as 'cenotes' in the Yucatan, 'blue holes' in Belize and the Bahamas, 'grietas' in the Galapagos, and 'jameos' in the Canary Islands. While some anchialine caves such as the 180 km long Sistema Ox Bel Ha in Yucatan are extensive horizontal systems, others such as Dean's Blue Hole in the Bahamas with a depth of 202 m are vertical shafts. Anchialine lava tubes include the 1.7 km long Jameos del Agua cave in the Canary Islands. Coastal tectonic faults that extend below sea level constitute another form of anchialine habitat. Such faults can be formed in volcanic rock (e.g., Galapagos Islands) or in limestone (e.g., Bahamas, Sinai Peninsula and Niue).

The water column in most anchialine caves is highly stratified, with large fluctuations in chemical and physical parameters occurring at one or more haloclines. Deep water in most anchialine caves approaches oceanic salinities. Due to the absence of light and photosynthetic oxygen production, dissolved oxygen levels are hypoxic to anoxic, with hydrogen sulfide layers occasionally occurring at the halocline. A pH minimum typically occurs at the halocline, possibly arising from microbial oxidation of particulate organic matter accumulating at the density interface.

Anchialine fauna primarily consists of crustaceans, although stygobitic fish, annelids, gastropods, sponges, and ciliates are also known. Among the anchialine Crustacea, the largest numbers of species are represented by amphipods, copepods, decapods, ostracodes, isopods, mysids and thermosbaenaceans, approximately in that order. A number of higher crustacean taxa are primarily or exclusively found in anchialine habitats including the class Remipedia; orders Thermosbaenacea, Misophrioida,

Mictacea and Bochusacea; and families Thaumatocyprididae (Ostracoda); Atlantasellidae (Isopoda); Stygiomysidae (Mysidacea); Procaridae, Agostocarididae and Macromaxillocarididae (Decapoda); and Epacteriscidae, Ridgewayiidae, Fosshageniidae, Speleoithonidae, and Novocriniidae (Copepoda).

Extraordinary patterns are evident when examining the biogeographic distribution of anchialine taxa. While several anchialine genera inhabit caves on opposite sides of the Atlantic or Pacific, others are found on opposite sides of the Earth. Such highly anomalous distributions suggest that these taxa relicts whose ancestors inhabited the Tethys Sea during the Mesozoic. The presence of anchialine fauna in the Mediterranean is also remarkable considering that this basin was completely dry for long periods during the Miocene early Pliocene. A rich and diverse anchialine fauna inhabits cave on mid ocean islands such as Bermuda that have never been close to a continent. Based on numbers of stygobitic species, the Bahamian archipelago appears to have been a possible center of origin for anchialine fauna.

Oral presentation

Search for Bermuda's deep water caves

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The mid Atlantic island of Bermuda harbors one of the richest and most diverse anchialine communities known from anywhere on Earth. However, all known anchialine caves in Bermuda (maximum depth – 26 m) were dry as recently as 18,000 ybp when glacial sea levels were ~130 m lower. Thus, alternate deeper habitats must have existed to shelter anchiane fauna for prolonged periods of lower sea level during the Pleistocene. In order to systematically search for such now deep water cave habitats, high resolution multibeam sonar and ROVs were used to map and explore the seafloor off Bermuda in 60-200 m depths along the outer shelf break edge of the submarine escarpment surrounding the Bermuda pedestal and two adjacent seamounts. Specific goals were to discover deep water cave and/or crevicular habitats and to characterize the nature, geological stratification and composition, and sea level history of the platform margin, in particular focusing on features directly relating to Pleistocene low sea stand events. During the survey, steep submarine cliffs, wave cut notches, terraces, landslides, sinkholes, and overhangs/caves were identified and explored. A second phase of the project to be conducted in 2010 will utilize closed circuit, mixed gas rebreather dives to place hydrographic instruments and collect geological and biological samples.

Poster presentation

History of anchialine cave research in Croatia

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The first information on an anchialine cave in Croatia was written by the Croatian geologist Josip Poljak. He described the cave Urinjska špilja near Rijeka, drow it's topographical map, and reported that cave water was brackish (Poljak, 1920). In 1950's Stanko Karaman (then in Dubrovnik) studied faunistically anchihaline waters around Dubrovnik, including the cave Šipun in Cavtat. Since his first visit of the cave Šipun (1958), during 1960's, till 1980's significant research of anchihaline caves along the Adriatic coast was conducted by Boris Sket from Ljubljana. Faunistically and ecologically explored have been more than 30 anchihaline caves along the entire coast but mainly at the Kornati Islands National Park, beside the Šipun cave. This resulted in the later confirmed ecological scheme (Sket 1986, 1996) of the anchihaline water bodies: salinity stratification, local oxygene depletion, and faunistic stratification by biotic exclusion. Technically, an adapted Cvetkov net and simple sampling bottles were mostly used, but also scuba diving samplin method.

The first described snorkel dive in an anchialine cave was made by Bruno Puharić, in the Urinjska špilja cave (Puharić, 1963). Rupert Riedel, (Wien) investigated some submarine caves near Rovinj andon the Krk Island (Riedel 1966). Later on, he and Ozretić also investigated coastal anchihaline caves, mainly near Rovinj (Riedl & Ozretić, 1969). An important contribution to subsequent explorations was made by the geographer Ivo Baučić who completed the list of caves on the Adriatic coast and islands (Baučić, 1962).

In the period between 1968 and 1975 geological and paleontological research of anchialine caves were made by Srećko Božičević and Mirko Malez (Malez, 1969), mainly on Lošinj, Cres and Rab Islands. Tonči Rađa from Split investigated anchialine caves during 90's and at the beginning of this century, sampling their fauna.

Research became more intense after 2005 due to Branko Jalžić, who on the suggestion of Frano Kršinić from the Institute for Oceanography and Fisheries, started researching of anchialine caves along Croatian coast of the Adriatic Sea. Extensive field work and interdisciplinary data on water, sediment, and biota of anchialine cave environment was done by members of the Croatian Biospeleological Society, Croatian Natural History Museum and Ruđer Bošković Institute (Žic et al. 2008). From this associate research, F. Kršinić described new genera and species of copepod crustaceans (Kršinić, 2005, 2005a, 2008). Recent research of some anchialine caves was done on Hvar and Krk Islands by the Natural History Museum of Rijeka (Arko-Pijevac et al., 2001) and the University of Zagreb (Gottstein et al., 2007; Novosel et al. 2007).

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Oral presentation

The Tethys enigma – Optimization of DNA sequence data in paleogeographic analyses

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Many groups of obligatory groundwater organisms have similar disjunct geographic distributions, both at regional and global scales. Stygobiontic taxa with large-scale disjunct distribution patterns are often considered as remnants of ancient faunas. For example, crustaceans of the ostracode genus *Danielopolina*, the thermosbaenacean *Halosbaena* and the remipede order Nectiopoda are known from cave systems on the Canary Islands, the Caribbean region and Western Australia, thus showing similar distribution patterns. However, the biogeographic history of each group is unique and often quite complex, presumably involving different evolutionary scenarios with (re)-colonization events at different times.

Hypotheses that propose a Meso- or even Paleozoic origin for stygobionts with disjunct distributions have been traditionally based on comparative morphology and biogeography. In recent years, analyses of DNA sequence data have become an increasingly powerful tool to evaluate such hypotheses by estimating molecular divergence times. However, despite the growing number of publications in this field, the currently used methodical standard is far from optimal. Apart from taxon sampling and the choice of markers, the optimization of multiple sequence alignments is a critical, but frequently neglected factor in phylogenetic and phylogeographic analyses.

A recently completed analysis of 88 arthropod taxa showed that alignment optimization of ribosomal markers based on secondary structure information can have a radical impact on phylogenetic reconstruction. In this study, a sensitivity analysis was performed to assess the effects of different strategies of sequence alignment, alignment masking, nucleotide coding, and model settings. The results demonstrated that secondary structure alignment of the ribosomal genes 16S and 18S rDNA clearly outperformed commonly used algorithms for multiple sequence alignment (i.e., algorithms that do not consider secondary structure information). Moreover, alignment optimization had a more crucial impact on the resulting phylogeny than any of the model settings investigated.

Poster presentation

Mercury distribution in water column and sediment of two anchialine caves in Mljet National Park, Croatia

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Mercury distribution in water columns and sediment columns of two stratified anchialine caves Bjejajka and Lenga in the remote Mljet National park in Croatian Southern Adriatic during four years period (2006 - 2009) was studied. Also, temperature, salinity, pH and dissolved oxygen concentration of the stratified water column in both anchialine caves were obtained. Salinity constantly increased with depth in the range from 3 ‰ at the surface to 38 ‰ in bottom water. Water samples for mercury analysis were collected in 1 l borosilicate glass bottles, while sediment columns were collected in hand-driven acrylic corers, both by speleo scuba diver. Measurements were performed by cold vapour atomic absorption spectrometry (CVAAS). Mercury concentrations found in unfiltered samples are presented as total (Hg_{tot}).

Extremely high concentration of total Hg (920 ng l^{-1}) in the middle layer (7 m) of the water column of Bjejajka cave were found in January 2009., comparing to low 5 ng l^{-1} in the surface water sample of the cave, which is 2 order of magnitude difference. Moreover, in the vicinity of the Bjejajka cave (about 100 m), in the seawater sample taken at the surface, only 0.5 ng l^{-1} of total mercury was detected. In surface sediment samples in Bjejajka cave, mercury concentrations were up to 3.3 mg kg^{-1} dry weight. On the other hand, in Lenga cave, Hg water and sediment concentrations were drastically smaller (mostly below 7 ng l^{-1} and 0.8 mg kg^{-1} dry weight, respectively). Seasonally, small colony of bats inhabits Bjejajka cave. Concentration of total Hg in guano of these mammals was found to be 0.31 mg kg^{-1} wet weight, which is in the range of total Hg concentrations usually reported in sediment. This natural factor was the source of mercury high concentrations in water column and sediment in Bjejajka cave. Unlike Bjejajka, bats were not spotted in Lenga cave, which is in accordance to its considerably lesser Hg concentrations.

This is the first report on mercury distribution in anchialine cave water and sediment. Also, mercury speciation in water columns was assessed. Five different mercury species were characterized: total, reactive, monomethylmercury, total gaseous mercury and labile ionic mercury. Presented results suggest complex behaviour of mercury species investigated in water column and sediment of Bjejajka and Lenga anchialine caves.

Oral presentation

Relict anchialine faunas of the Edwards Aquifer, Balcones Fault Zone and associated areas in south-central Texas and northern Mexico

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Our knowledge of the origin and geological characteristics of the subterranean Edwards Aquifer and associated systems have led to a better understanding of how a remarkably diverse assemblage of marine relicts, consisting largely of crustaceans, came to occupy present-day groundwater aquifers in southern Texas and northern Mexico. The invasion and colonization of groundwater systems in the study area by putative ancestors of the contemporary fauna probably occurred during recession of marine waters from the North American continent in the Late Cretaceous. Extensive karst terrains and possibly intermittent deposits of coarse sediments bordering on the western end of the Tethyan Sea would have provided anchialine conditions throughout much of what is now southern Texas and northern Mexico. Anchialine habitats in turn provided numerous habitats for the invasion of a marine/brackish water fauna consisting largely of amphipods (Artesiidae, Bogidiellidae, Hadziidae, Ingolfiellidae, and Sebidae); isopods (Cirolanidae) and thermosbaenaceans (Monodellidae), many of which are similar to the modern taxa in these groups.

Conditions in the study area in the Late Cretaceous probably resembled closely those seen today in the partially submerged karsts of the Bahamas, Yucatan Peninsula, and on or around many other islands in the Caribbean and in other regions where karst terrains are exposed to marine inundations and/or transgressions. Stranding, the phenomenon of marine/brackish water animals being literally “left behind” in caves when marine water recedes and is slowly replaced by freshwater is postulated to be the most significant factor in the transition of marine/brackish water organisms into freshwater forms. The organisms slowly adapt to living in limnic habitats as saline waters recede. In addition to the marine relict crustacean fauna in southern Texas and northern Mexico, there is presently a distinctly different subterranean amphipod fauna also living in many of these groundwater habitats. This fauna, consisting largely of members of the subterranean genus *Stygobromus* (Crangonyctidae), is believed to be of freshwater origin and to have invaded the Edwards Aquifer groundwater habitats in comparatively recent times. The fact that some parts of this aquifer, such as in the San Marcos area, contain as many as 10 different stygobiotic amphipod species, suggests that extreme ecological heterogeneity and habitat compartmentalization exist in these subterranean groundwater biotopes.

Poster presentation

On a benthic community from a subterranean estuary in a karstic coastal cave (eastern Sardinia) with notes on Porifera

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Recent faunistic surveys in eastern Sardinia karst resulted in the discovery of a benthic community peculiar for a Mediterranean coastal cave. The Bue Marino Cave is characterised by the presence of a subterranean river flowing along a necklace of large subaerial shallow water pools up to the cave entrance. Salt content ranges from pure freshwater (up to *ca*. 800 m from the entrance) to salinity values up to 32 ‰ in the intermediate zone, where usually freshwater and saltwater mixing occurs. The cave, well known for its diversified and peculiar endemic fauna (mainly Arthropoda) from the terrestrial and freshwater habitats, was unexplored until now for sessile benthos. The census highlighted, in the transition between the freshwater and the marine water zone, some hundreds metres from the entrance, the presence of a conspicuous sessile filter-feeders community. Porifera (2 taxa), Polychaeta (2 taxa), Bivalvia (1 taxon) and Tunicata (1 taxon) were found. Preliminary results suggest they belong to typical estuarine/coastal lagoon ecosystems. As for sponges a detailed analysis on their taxonomic status is in progress.

Oral presentation

Origin and ecology of the anchialine fauna of Lanzarote (Canary Islands)

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The island of Lanzarote is one of the oldest in the Canarian Archipelago with an estimated age of 15.5 million years. Processes involved in its formation were complex and included several periods of volcanism, followed by subsequent erosive phases with less volcanic activity. The porosity of the volcanic materials and the scarcity of rainfall caused by the low altitude of the island, facilitate the infiltration of seawater into the inland and the presence of a continuous anchialine water mass along the coastline of the island.

The most remarkable anchialine habitat is formed by the La Corona lava tube, which originated some 20.000 years ago. It extends from the Monte Corona volcano extending over 6.1 km to the seashore and a further 1.4 km beneath the seabed, terminating blind 80 m below sea level. Seawater floods the last 2.1 km of the lava tube forming three sections by natural collapses and skylights: the Lagos cave, the Jameos del Agua and the Túnel de la Atlántida. The Lagos cave marks the most inland penetration of seawater into the lava tube. It extends ca. 700 m but only the seaward 400 m are partially flooded and divided into three so-called lakes. The Jameos del Agua contains a 50 m long, and up to 10 m deep, anchialine lagoon. Two large collapse entrances on either side of the Jameos lagoon and a small vent hole directly above it allows mostly indirect daylight to reach the water. In contrast, all other lakes within the lava tube are in total darkness. The Túnel de la Atlántida is the longest and most complex section of the flooded lava tube. Except for its entrance pool, this part is completely submerged and crosses the coastline, extending beneath the sea.

The La Corona lava tube is the most voluminous and the best studied anchialine habitat on Lanzarote. It harbours up to 77 species, with the highest diversity among crustaceans (31 species) and annelids (16 species). Additionally, thirteen species of molluscs, two nematodes, two cnidarians, and one species each of Priapula, Ctenophora, Echinodermata and Echiura, were collected. All these groups contain both endemic and offshore species, in different proportions. Whereas endemic species are dominant among crustaceans (90%), only 36% of the annelids are exclusively found in the lava tube. Except for the molluscs, with one endemic species, the other higher taxa lack endemic species: Priapula and Nematoda are known only to genus level, whereas Cnidaria are represented by two and Ctenophora, Echinodermata, and Echiura are represented by one offshore species each.

Two groups of species inhabit the marine groundwater of Lanzarote. The first comprises species endemic to Lanzarote all of which show eye and pigment reduction. They belong in part to ancient taxa exhibiting disjunct distribution on both sides of the Atlantic and in the Pacific, including Remipedia, Thermosbaenacea (*Halosbaena*), Gammaridea (*Liagoceradocus*) or Isopoda (*Curassathura*), which favours an ancient

Tethyan origin. However, some species of this group have deep-sea affinities like the annelid *Gesiella jameensis*, the galatheid *Munidopsis polymorpha*, the ostracod *Danielopolina wilkensi* or several genera of misophrioid copepods. The second group of inhabitants is represented by typically offshore species that are not endemic to Lanzarote, and comprise stygophilic, supratidal and accidental species. In the lava tube, the first group predominate at those habitats with very different conditions from those offshore, such as the water column, crevices of lava blocks and interstices of lapilli (pyroclastic particles). Species of the second group are more abundant in illuminated areas, hypogean pools and in special habitats resembling those found offshore.

These patterns of colonization and adaptation leading to the evolution of the unique fauna at Lanzarote emphasises anchialine systems as excellent models for ecological studies. It enhances the case for integrated global studies of anchialine systems to understand pattern and process and the origins and evolution of these enigmatic ecosystems to the benefit of management and to ensure their conservation.

Poster presentation

Diversity patterns of cave crustaceans in Mexico

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In Mexico there are seven karst provinces around the country: Sierra Madre Occidental; Edwards Plateau; Sierra Madre Oriental; Neovolcanic Plateau; Sierra Madre del Sur; Chiapas karst system; and Yucatán Peninsula. Among aquatic animals, the crustaceans are in the taxonomic group best represented in underwater systems. In Mexico, there are almost 200 crustacean species that inhabit fresh, brackish and marine subterranean waters. In this study, species, genera and family richness was analyzed by state and karst provinces. Results show that in the analysis by state there are five groups: a) < 5 species: Durango, Hidalgo, Michoacán, Sonora, Tabasco and Puebla; b) 6-10 species: Nuevo León, Campeche, Guerrero and Tamaulipas; c) 11-20 species: Quintana Roo, Veracruz, Coahuila, Oaxaca; d) 21-30 species: Chiapas and San Luis Potosí and e) > 30 species: Yucatán. However, in the province analysis, the species richness is better represented in Yucatán Peninsula, Sierra Madre del Sur, and Sierra Madre Oriental. However, according to genus and family analyses, only the Yucatan Peninsula has a high diversity while the two other provinces with rich species counts have fewer families. The hotspots of cave crustacean diversity are located in anchialine and tropical caves, and few species have been reported in temperate caves from Sierra Madre Occidental and Edwards Plateau. In Yucatán Peninsula caves there are species from orders and classes that only are present in that area. The richness analysis showed that the abundance of caves, their location in tropical areas, and the heterogeneity of environments within the caves explain this diversity pattern.

Oral presentation

Progressive adaptation of crustacean eyes from freshwater and anchialine caves

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Reduction in structure and function of the eyes is a widely reported feature of cave crustaceans. Eye structure is here examined by Scanning Electronic Microscopy in various shrimps and crabs. These comprise carideans, *Agostocaris bozanici, Barbouria yanezi*, *Procaris mexicana and Calliasmata nohochi*, from anchialine systems in Cozumel Island; freshwater crabs, *Rodriguezia* spp., from Chiapas caves; six carideans, *Macrobrachium villalobosi*, *M. acherontium, M. sbordonii*; *Cryphiops luscus*; *C. perspicax*, *C. sbordonii* and *Creaseria morleyi*, three crayfishes, *Procambarus cavernicola*, *P. oaxacae reddelli*, and *Procambarus* spp., and one alpheid *Potamalpheops stygicola* from freshwater caves Oaxaca, Tabasco, Chiapas and Yucatán. All show eye reduction in varying degrees. However, anchialine shrimps have concurrently developed pores on the eyes with a putative chemosensory function. The significance of this development is considered in relation to the previously-described development of setae on the reduced eyes of freshwater cave crustaceans, with a presumed mechano-receptive function. Additionally, in some cave freshwater crabs, spines have developed on their eyes to extend mechano-receptive function. This variation in the sensory receptors developed on otherwise redundant eyes is considered in relation to the different pressures of the freshwater and anchialine cave environments: the chemical gradients within anchialine systems are presumed to be a major factor in the progressive adaptation of these crustaceans.

Oral presentation

Cova des Pas de Vallgornera: an exceptional coastal cave from Mallorca Island (Spain)

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The Cova des Pas de Vallgornera is located in the Llucmajor municipality, Mallorca island (Western Mediterranean), being the longest cave in the Balearic archipelago. Currently its surveyed length is over 63,000 metres. The cave is notable not only because its development, but also for the great deal of uncommon speleothems and solutional morphologies related, at least partially, to the freshwater-seawater mixing zone. Furthermore, there are extensive brackish water pools that cover many areas of the cave. It must be pointed out the special importance of the anchihaline ponds along with gours environments. The cave is under the protection of Conselleria de Medi Ambient, Govern de les Illes Balears (the Regional Environmental Authority) and was declared Site of Community Importance, within the Natura 2000 Network (European Council Directive 92/43/EEC). Access to the cave is restricted only to surveying and investigation tasks authorized by the Regional Authority.

Oral presentation

Microevolutionary processes in stygobitic isopods from the Western Mediterranean basin

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Although anchialine habitats are more frequent and biodiverse in tropical areas, they host some interesting and diversified subterranean fauna also in the Mediterranean region. Some stygobitic groups of isopods (i.e. Cirolanidae and Sphaeromatidae), constitute different examples of anchialine evolutionary history in this area.

The Cirolanidae, a predominant marine family with about 300 species, is one of the two families of Isopoda Flabellifera which include stygobitic elements (the other being Sphaeromatidae). These stygobionts are constituted by about 23 genera (with ten anchialine genera). These taxa represent an example of the thalassoid limnostygobitic fauna. They are characterized by high biodiversity in the Mediterranean area and represent a very interesting group for the research on evolutionary trends in subterranean ecosystems. The phylogeny of the Mediterranean species belonging to the genera inhabiting the basin identified some clades coherent with previous morphological studies. The application of a molecular clock outlines the evolutionary history of the various taxa, which likely took place within a time range between 120 to a few million years ago.

The family Stenasellidae with the genus *Stenasellus* represents an example of stygobitic fauna, with no evidences of intermediate forms or close epigean relatives. This genus has an interesting disjunct distribution occurring in the Far East, East Africa and in Europe (Sardinia, Corsica, Tuscany and in the Pyrenees grounwaters). Their limited dispersal ability has led to consider these taxa good palaeogeographic indicators. One of the characteristics of the family is the absence of close relatives in epigean environment, freshwater and marine. The recent discovery of some specimens of *Stenasellus* sp. in a marine cave in Sardinia opens a new scenario about the evolutionary history of Stenasellidae.

Oral presentation

Void development on carbonate coasts: creation of anchialine habitats

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Void development in carbonate coasts is primarily a result of dissolutional processes. However, because carbonate coasts are commonly an active depositional environment, oversteepening of bank margins with subsequent collapse can create talus and fracture voids of a non-dissolutional origin. Dissolutional processes and internal non-dissolutional collapse processes may subsequently overprint each other to create complex void structures. These complex voids can distort subsurface fresh-water flow regimes, and create confusion as to cause and effect in void development.

The carbonate coastal setting creates a unique speleogenetic environment which has been addressed by development of the Carbonate Island Karst Model, or CIKM. In the CIKM, the physical framework in which void development occurs along carbonate coasts contains four critical factors: 1) sea-level change; 2) sea water – fresh water mixing; 3) rock lithology; and 4) coastal complexity.

Sea-level change is dominated by glacioeustasy, which affects all carbonate coasts world-wide. Locally, tectonic activity may interact with glacioeustasy to create a more complex sea-level signature. Sea-level position controls the vertical location of the fresh-water lens, and hence the primary speleogenetic environment in coastal carbonates. All carbonate coasts exhibit contact between marine salt water and inland fresh water. This contact, either as a sharp halocline or a broader diffusion zone, creates a physical density interface as well as a potent geochemical mixing environment. Collection and oxidation of organics at the top and bottom of the lens drives dissolution; organic loading may create anoxic conditions in which bio-mediated processes enhance dissolution. The distal margin of the fresh-water lens creates a rapid flow regime that may control aspects of the dissolutional kinetics.

The carbonate lithology of many carbonate coasts, especially in the tropics and subtropics, is commonly eogenetic, such that the rocks are diagenetically immature and contain unaltered primary aspects of deposition, such as high porosity. Variations in original lithology, such as reef versus lagoonal versus eolian facies, can influence void development. The overall relationship of the carbonate units to non-carbonate units has an important impact on the nature of the dissolutional voids produced. *Simple carbonate islands* are entirely carbonate rock; *carbonate cover islands* are entirely autogenic recharge, but the lens is partitioned in the subsurface by non-carbonate rock; *composite islands* have exposed non-carbonate rock acting as allogenic recharge adjacent to carbonate rocks; and *complex carbonate islands* have faulting and interfingering of carbonate and non-carbonate rocks to create a very heterogeneous surface catchment and subsurface flow regime.

The largest voids found in small, simple carbonate islands are *flank margin caves*, which form in the distal margin of the fresh-water lens under the flank of the enclosing land mass. Smaller voids form farther inland along the top of the lens. In some island settings, such as the Bahamas, these inland voids form in lowland areas and commonly

express as collapse features in which specialty crops are grown, hence the name *banana holes*. In the vadose zone, *pit caves* form as fast-flow routes conducting water collected in the epikarst downward to the lens. On large islands, or on continental carbonate coasts, diffuse flow in the lens is inefficient and turbulent conduit flow develops. Where non-carbonate lithologies are present and perch fresh water, turbulent conduit flow may also develop.

Blue holes are defined as subsurface voids that are developed in carbonate banks and islands; are open to the earth's surface; contain tidally-influenced waters of fresh, marine, or mixed chemistry; extend below sea level for a majority of their depth; and may provide access to submerged cave passages. Blue holes are polygenetic, resulting from sea-level rise drowning pit caves and deep sink holes, from progradational collapse of deep-seated voids, and from bank margin fractures. Overprinting from numerous Quaternary sea-level changes make blue holes complex features.

Sea-level change in the Quaternary, primarily glacioeustatic, has created conditions where carbonate coasts have repeatedly migrated laterally in the 10s of km range, and the fresh-water lens position has migrated vertically over 100 m. The resulting dissolutional and non-dissolutional voids, and their interactive processes, represent the sum of numerous overprinting events. It is also important to recognize that during the Quaternary, sea level has been below its present elevation about 90% of the time, and that the current anchialine environments are both young and transient.

Oral presentation

Anchialine cave microbiology: a multi-layered approach

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This study aims to describe the geochemistry and microbial community structure of a vertical section of Cenote Crustacea, an anchialine cave in Quintana Roo, Mexico known for high densities of crustaceans. Several studies have suggested that anchialine systems support dynamic and potentially chemoautotrophic microbial communities, yet isolated bacteria exhibiting this metabolism have yet to be reported in the literature. This study used geochemical description of the water column and sediment of a section of Cenote Crustacea to inform the microbial habitat and infer biogeochemical processes at the time of sampling. Salinity-, temperature-, pH-, and dissolved oxygen- depth profiles were collected *in situ*. Water samples collected from above, in, and below the halocline as well as from the sediment were collected in triplicate for subsequent anion and organic acid concentration, as well as chemical oxygen demand determination. These analyses revealed that the marine environment in Cenote Crustacea could support anaerobic chemolithoautotrophs using sulfate as an electron acceptor. Furthermore, anaerobic enrichments inoculated with mat-containing cave sediment collected at the time of geochemical analyses and incubated without light have resulted in the isolation of nine sulfate-reducing chemolithoautotrophic bacteria and over 20 other strains. 16S rRNA gene sequencing of isolated chemolithotrophic bacteria has revealed members from the *Gammaproteobactera* and *Deltaproteobacteria*. In order to characterize microbial community differences between microenvironments of Cenote Crustacea, we extracted DNA and RNA from water above, in, and below the halocline as well as from mat-containing sediment. We then used oligonucleotide phylogenetic microarrays (PhyloChips) to explore differences in active and persistent members of the microbial community amongst and between these samples. It is our hope that the integration of geochemistry, microbial community structure, and future physiological studies on isolated bacteria will begin to elucidate the role of microorganisms in anchialine systems, such as Cenote Crustacea.

Oral presentation

Chemoautotrophy in anchialine caves: Reflections on the past and thoughts for the future

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The sharp interface at the halocline of anchialine caves is an ideal setting for numerous heterotrophic and chemoautotrophic microbial processes. While heterotrophy is dominant in most cave ecosystems, chemoautotrophic pathways in anchialine ecosystems have been implicated as sources of organic matter and energy that support higher trophic level fauna. However, the specific biogeochemical processes and microbes mediating the transformations are not well understood. Recent advances in marine organic geochemistry and microbiology offer unprecedented opportunities to delineate metabolic pathways and identify the associated microbes. I will reflect on previous studies that have suggested in-situ chemoautotrophy in anchialine and other cave ecosystems, review the basis for these interpretations and suggest approaches that might be considered for future investigations. I will draw upon techniques currently employed in marine systems; particularly deep-ocean cold seeps where chemoautotrophy is active. Knowledge obtained from the stable anchialine cave habitat has the potential to provide a better fundamental understanding of biogeochemical pathways in other globally relevant carbon cycles.

Oral presentation

The Anchialine-Ophel Continuum Concept - The case of three sites in the Mediterranean space

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Typhlocaris galilea, the blind prawn of Galilee, the largest subterranean invertebrate, measuring up to 7 cm in length, was discovered in the spring of Tabgha, near Lake Kinneret of Israel, in the Jordan Graben at - 220 m below sea level, one hundred years ago, in 1909, just 4 years after Racovitza's Cueva del Drach. *T. letheae* was found in a foothill spring of Gebel Ahdar on the Libyan sea shore and in 1923, a third species, *T. salentina* in the classical anchialine La Zinzulusa of Italy.

These prawns, form an ancestral palaemonoid family with no living representative in the open sea and their discontinuous distribution in the three widely distant circum-Mediterranean springs also left no doubt because of their large size. In the year that followed, the prawns, jointly with the very many subterranean species of marine descent, overwhelmingly crustaceans that were discovered, were considered to be stranded relics of Neogene anchialine environments of the Tethys ocean.

Recently, a new species *Typhlocaris ayyaloni* was discovered at Ayyalon in an accidentally accessed small lake of a deep inland aquifer in the coastal plain of Israel (Tsurnamal, 2008). At the contact with a confined aquifer, films of sulphide-oxidising bacteria provide the food for extremely numerous populations of various crustaceans. Based on this find, I proposed the existence of a subterranean continental chemosynthesis –based biome named Ophel (Por, 2007). The existence of this biome could also change our view of the anchialine biota, especially that in the Mediterranean.

First, to the above mentioned prawns, I shall add the thermosbaenaceans and cyclopoids which are associated with them at the three Mediterranean subterranean sites:

La Zinzulusa	**Ayyalon**	**Tabgha +Jordan valley**
Typhlocaris salentina	*T. ayyaloni*	*T. galilea*
Monodella stygicola	*Tethysbaena* n.sp[1]	*Tethysbaena relicta*
Metacyclops subdolus	*M.* aff. *subdolus* [2]	*M. subdolus auctorum*

[1.] H.P.Wagner in prep. ; [2.] D. Defaye & F.D. Por (*Crustaceana* in press)

The three taxa are convincingly part of the continuum of Ophel, the subterranean freshwater biome which is supported by chemosynthetic bioproduction. Its fauna is widespread in the interphases between the phreatic waters with the confined continental aquifers and surfaces centrifugally in springs, in the above cases, in an anchialine cave and in a deep tectonic rift.

I propose the following points for discussion:

▪ The anchialine environment offers a permanent bacterial chemosynthesis-based food resource in its chemocline. In the strange spectral crustacean-dominated world, it

offers a certain parallel to the open world estuaries, by offering a reliable food base in the oligotrophic tropical seas. Like the estuaries for the aquatic vertebrates, it played an important evolutionary role for its dominant taxon, the crustaceans.

- At present, the anchialine environments are inhabited by a mixture of two faunistic components: one stenohaline, of marine waters and another oligohaline-euryhaline, of continental subterranean waters, an extension of the Ophel biome.

- The marine component consists of species which might be of very old age and are not found in open surface waters, like the Remipedia, though some might be found in the deep sea like the ostracod *Danielopolina*. Some taxa, like the Anthuridae isopods seem to be recent evolutionary additions.

- The marine ancestry of the many of the Ophel taxa is probably very old and conjectural. The most typical major Ophel taxon is suborder Thermosbaenacea. A good exception though are the Cirolanidae isopods, where a whole series links the marine species, through a wealth of anchialine ones to Racovitza's typical Ophel inhabitant *Typhlocirolana* species several of which live also in the deep fossil aquifers of the Sahara and the Negev.

- The anchialine environments of the recent Mediterranean appear to have lost their marine component, during the Messinian salinity crisis and in the Pleistocene, jointly with the other tropical fauna. For instance no Cirolanidae or Remipedia have so far been recorded. Also the diversity of anchialine Calanoida is to my knowledge very low. Even at present, parts of the sea have a climate probably too dry to support abundant phreatic runoff, or else, open water salinities are seasonally often too variable.

- Probably for these reasons, the continental Ophel component in the anchialine waters of the Mediterranean is preponderant. I surmise that it re-colonized these environments when they became inhabitable again in post-Messinian times, coming from the inland aquifers.

Por, F.D. 2007. Ophel: a groundwater biome based on chemoautotrophic resources. The global significance of the Ayyalon cave finds, Israel. *Hydrobiologia* **592**:1-10.

Tsurnamal, M. 2008. A new species of the stygobiotic prawn blind prawn *Typhlocaris* Calman (Decapoda, Palaemonidae, Typhlocaridinae) from Israel. *Crustaceana* **81**:487-501.

Oral presentation

The geochemistry of nutrients (N, P, C) in seawater mixing zones in carbonate aquifers

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Geochemical reactions involving carbonate minerals and ion exchange have been known for some time to influence the concentrations of dissolved constituents in carbonate aquifers affected by seawater intrusion. Less well known are the effects of these reactions on nutrient concentrations and the availability of these nutrients in subterranean ecosystems and their adjacent surface water bodies. This presentation describes the occurrence of nutrients within the surface water and brackish groundwater along the carbonate coastline of south Florida, USA. In addition, the results of laboratory tests simulating the effects of seawater intrusion on a limestone block are also described.

Total nitrogen (TN), total organic carbon (TOC), and dissolved inorganic carbon (DIC) concentrations were found to increase with depth in the groundwater. Ammonium was the dominant inorganic form of nitrogen in the groundwater, indicative of a reducing environment. Phosphorus (P) concentrations in the groundwater were found to increase significantly with salinity, and were in excess of conservative mixing of both fresh surface water and seawater. Both total and dissolved concentrations of P in surface water, shallow groundwater and deep groundwater were found to represent mixtures of three water types: 1) fresh surface water; 2) seawater; and 3) average brackish groundwater.

The results of both laboratory experiments and geochemical modeling suggest that dissolution of calcium carbonate minerals was most responsible for the release of P in brackish groundwater at low salinities (<28 psu). At higher salinities, ion exchange reactions involving bicarbonate ions in the intruding seawater were responsible for desorbing phosphorus. The results of this research suggest that as seawater intrudes into a fresh coastal aquifer, excess phosphorus would be expected to be released into the brackish groundwater. This excess phosphorus was most pronounced in oligotrophic regions. In areas of significant anthropogenic inputs of both N and P in the fresh groundwater, then both N and P were found to decrease in the brackish groundwater with salinity.

Oral presentation

Structure and function of the microbial food web in the absence of light

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The structure and function of the microbial food web was analyzed in littoral cave lagoons of Mallorca (Spain) located at varying distance from the coastline. In these cave lagoons, the trophic food webs in the absence of light are hypothesized to be sustained by allochthonous organic matter, which enters the system through seepage, though chemoautotrophic processes can also occur. We compared the microplanktonic food web of these lagoons with that of other aquatic net heterotrophic ecosystems which receive both autochthonous and allochthonous organic matter, such as oligotrophic marine ecosystems, and other environments characterized by the absence of light, such as the deep sea. The upper part of the water column had a similar structure and function to that of the oligotrophic open sea, but with a lower abundance and biomass of organisms, with an average of 4.7×10^5 heterotrophic bacteria (HB) ml^{-1} and about 150 heterotrophic nanoflagellates (HNF) ml^{-1}. The deep zones were similar to the deep sea, with an average bacterial abundance of 2.9×10^5 HB ml^{-1} and 50 HNF ml^{-1} and bacterial production values as low as 0.03 µg C l^{-1} d^{-1}.

Oral presentation

Organic matter origin and diets: building the food web

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Food webs studies are scarce in anchialine systems and marine caves making comparisons difficult. Stable isotopes are a powerful tool and an informative starting point to elucidate the origin of organic matter and diets in anchialine ecosystems. Anchialine research is young, quite challenging and dangerous so the human error and systematic error are not negligible. Anchialine research must not forget precision and accuracy because it is crucial to quantify variations within samples. In my talk I analyze stable isotope variation within and among species, and between different areas, and examine the range of variation found in the sources of organic matter input into the anchialine systems. Results from an anchialine cave in Mexico show huge variations. Atyid shrimp show variations of $\delta^{13}C$ >15 ‰ and $\delta^{15}N$ >10 ‰, amphipods show variation of $\delta^{13}C$ 9 ‰ and $\delta^{15}N$ >10 ‰. Diet assessment using mixing equations, indicates that omnivory is a strategy in Remipedes even in a cave with high shrimp abundances.

Oral presentation

The control of hydrogeology and nutrient supply on foraminifera in Green Bay Cave System, Bermuda: the anchialine to submarine cave environmental transition

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Phreatic caves are challenging environments to establish organism-environmental feedback relationships, largely due to the combined limitations of minimal macrofanua abundance and capacity for scientific observation (i.e., necessity of SCUBA). Furthermore, there is no factual evidence describing how stygobitic ecosystems or phreatic caves respond during environmental change (e.g., sea-level rise). Foraminifera (unicellular, shelled protists) are established proxies in coastal environments because of their sensitivity to critical environmental parameters (e.g., salinity, oxygen, nutrient flux, etc.). Although fossil foraminifera in caves have been documented, the ecology of modern foraminiferal in phreatic caves is unknown. Here we document extensive populations of foraminifera living in Green Bay Cave, Bermuda, which are highly dependant upon nutrient sourcing, supply, and hydrogeology. Green Bay Cave is the ideal location to study modern cave foraminifera because it has two interconnected entrances—an anchialine cave (Cliff Pool sinkhole) and a submarine cave (Green Bay)—which enables documentation of the anchialine to submarine cave faunal transition. As a result of the local hydrogeology, saline ground water (>1 m deep, salinity 35.5 g l^{-1}) floods the entire cave, except a thin meteoric lens in the open water of Cliff Pool sinkhole (< 1 m deep, salinity ~24.5 g l^{-1}). To investigate the modern foraminifera, surface sediment samples (n = 75) were collected on SCUBA throughout the entire cave system, whereby ~300 individuals were enumerated in each sample to obtain statistical significant abundances. No endemic species of foraminifera were identified in the cave; rather they were an eclectic collection of foraminifera from across Bermudian coastal and shelf environments. Foraminifera were present in every sample, and ranged in density from ~50 to 2500 cm^{-3}, with a maximum of 6822 foraminifera cm^{-3}.

Foraminiferal populations were compared using Q-mode cluster analysis (unconstrained, Euclidean distance co-efficient), indicating that foraminifera in phreatic caves clearly discriminate the anchialine and submarine environments. Green Bay Cave contains five separate spatial assemblages of foraminiferal: (A1) Meteoric Lens, (A2) Anchialine Cave, (A3) Marine Cavern, (A4) Submarine Cave, and (A5) an Oligotrophic Marine Cave. Euryhaline foraminifera (i.e., *Helenina, Discorinopsis*) characterize the Meteoric Lens Assemblage (A1) found in the brackish meteoric lens of Cliff Pool sinkhole. Salinity is a dominant control on foraminifera in other paralic environments, therefore a unique foraminiferal assemblage living in the brackish water of Cliff Pool Sink Hole is expected. Foraminiferal diversity immediately increased in full marine conditions below the meteoric lens, and the foraminferal community changes to the Anchialine Cave Assemblage (A2). This assemblage is located in the cavern and cave passages that are proximal to Cliff Pool sinkhole and dominated by opportunists and detrivores (i.e., *Bolivina, Siphogenerina, Textularia earlandi*). Considering dissolved oxygen (> 2 ml l^{-1}) and salinity (35 g l^{-1}) are optimal for foraminiferal growth, the Anchialine Cave

Assemblage is likely controlled by terrestrial nutrient flux into the cave from Cliff Pool sinkhole. These two assemblages collectively comprise what is traditionally considered an anchialine cave environment.

The submarine cave environment in Green Bay is comprised of three separate assemblages, which generally reflect a decreasing influence of the open marine environment and nutrient reflux from tidal pumping. When first entering the cave from Green Bay (quiescent lagoon environment), the first ~20 m is characterized by the Marine Cavern Assemblage (A3), a community that is dominated by foraminifera that are also common to Green Bay lagoon (*Quinqueloculina*, *Ammonia beccarii*). Beyond ~25 m penetration into the submarine cave, the Submarine Cave Assemblage (A4) begins and is dominantly comprised of *Triloculina, Spirophthalmidium*, *Spirillina*, and *Patellina*. This assemblage indicates decreased cycling of nutrients from the open water into these areas of the cave. In the most distal cave areas where the Oligotrophic Assemblage (A5) is located, decreased overall diversity and increased abundance of *Spirophthalmidium* and *Spirillina* indicate the least amounts of nutrients from outside the cave environment are reaching these areas. No relationship exists between the linear distance of sampling station through the limestone bedrock to open water (Belmont & Lower Town Hill Formations). A sample that is <15 m linear distance to the Atlantic Ocean, but through the bedrock, is quite oligotrophic. This emphasizes the importance of a physical cave opening to facilitate phreatic cave fertilization by tidal pumping in Bermuda. The is corroborated by tintinnid abundances in Green Bay (planktonic, ciliate protists), which indicate the most nutrient-rich water columns are located more proximal to physical cave openings. Interestingly, in two cave areas near the sea, the modern Oligotrophic Assemblage is currently living on a coarse sand substrate comprised of fossil foraminifera shells; species that are reliant on photosynthetic symbionts (e.g., *Archaias, Amphistegina*). Because these cave passages currently terminate at massive collapsed limestone, the fossil foraminifera shells predate the historic collapse events to when the cave was physically open to the sea, and these areas were historically submarine caverns environments.

These results indicate that extensive populations of benthic foraminifera live in phreatic caves, and are intimately controlled by hydrogeological parameters (circulation, nutrient reflux, water mass division). Considering the detailed biogeography of foraminifera in a single cave system, we question the broader influence of hydrogeology on macroinvertebrate biogeography. Lastly, because foraminifera readily preserve in the sediment record, we can use subfossil cave foraminifera as environmental proxies to determine prior sea levels and evaluate the response of phreatic caves during long-term environmental change.

Oral presentation

Iodine and nutrient speciation in anchialine cave waters (Bjejajka Cave, East Adriatic Coast)

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In oxic seawater dissolved iodine is principally consisted of iodate and iodide, at a total concentration of ~450 nM. Although iodate is thermodynamically favoured form of inorganic iodine, iodide concentrations may be high, as is the case in surface waters of temperate shelf seas. Both phytoplankton and bacteria are proposed to mediate in iodate reduction to iodide, but the results upon the extent of phytoplankton impact on the iodine system at natural iodine concentration levels are even contradictory, while reaction mechanisms remain largely unknown. Similarly, the opposite oxidation process of iodate formation is still not clear, but several recent findings in specific marine and lymnic environments, including the one in seven anchialine caves along the Adriatic coast, seem to support the hypothesis that oxidation is possibly related to bacterial activity during nitrification. In addition, bacterial respiration is considered to participate in iodate reduction at halocline.

The study of inorganic iodine speciation in anchialine caves waters was, however, only focused to iodate and iodide depth distributions, leaving significant uncertainty upon the total iodine concentration (that with organo-iodine included). Although organo-iodine is typically low in open ocean waters, in productive estuarine and marine environments it is found to be present in high concentration. To obtain information whether concentrations of a third iodine form are substantial enough so that might affect the inorganic iodine system, and whether there is a parallel between iodine and nutrient speciation (in particular that of nitrogen), an additional study of iodine speciation in the water column of Bjejajka Cave was conducted in January and July 2009. Water samples were collected from various depths and were analyzed for iodate, iodide and total iodine. In addition, physico-chemical parameters (salinity, temperature, dissolved oxygen, pH and alkalinity) and nutrients (nitrate, nitrite, ammonium, phosphate and silicate) were measured at the same depths.

Bjejajka Cave is an anchialine object situated on the island of Mljet (East Adriatic Coast), approximately 100 m from the nearest shore. The cave is 22 m high and 40 m long, with water depth of 12 m. Below the main pool which is ~4 m deep, a narrow tunnel extends into the karst rock. During both surveys water column was well stratified, with slight seasonal temperature variations obtained only in surface layers and pronounced, but rather diffuse salinity gradient below the well mixed surface layer. Due to organic matter degradation and lack of photosynthesis, hypoxia developed in deeper layers of the pool (~2.5 m in January and ~3.5 m in July) and maintained in deep water. While dissolved oxygen and pH profiles were similar, alkalinity increased linearly with decreasing salinity. Nutrient profiles were similar during both seasons, with concentrations well above those found in surrounding seawater in July. The

highest concentrations of nitrite and ammonium (up to 0.17 μM and 3.66 μM, respectively) were measured at the bottom of the main pool, reflecting both heterotrophic and autotrophic bacterial activity. Nitrate, phosphate and silicate concentrations were significantly higher at surface (up to 117 μM, 2.95 μM and 78.2 μM, respectively) than in deep water (~7 μM, ~0.25 μM and ~33 μM, respectively). While correlation between silicate and salinity was linear (R^2=0.962, N=16), hyperbolic-like relationship was obtained with nitrate and phosphate, but linear between them (R^2=0.972, N=17). Beside fresh-water source from soil leaching, an additional source of nitrate and phosphate could have also been bat droppings, while the source of silicate in deep water is not yet fully understood.

Unlike nutrients, total inorganic iodine concentrations linearly increased with salinity (R^2=0.948, N=16), attaining ~450 nM in bottom water of higher salinity (*S*=35 ‰), which is close to concentration found in surrounding seawater that feeds the cave. High iodate (mean=435 nM) and low iodide (mean=16 nM) concentrations in these samples contrast inorganic iodine speciation in Adriatic surface seawater, where the ratio between iodate and iodide is significantly lower (~4). During both surveys iodide concentration peaked at the bottom of the main pool, but the concentrations in July were about three times lower than in January (60 nM *vs*. 170 nM). These findings indicate that iodide oxidation to iodate probably occurs both within the karst matrix and the water column itself. These are the depths where heterotrophic bacteria might have contributed to iodate reduction, while autotrophic bacterial assemblages to iodide oxidation, as is the case with nitrogen speciation. Like total inorganic iodine, total iodine concentrations generally increase with salinity, but with some higher scatter (R^2=0.851, N=16). However, while regression of the data for total inorganic iodine against salinity gives an intercept (± std. error) of 8 (± 18) nM, with total iodine data the intercept is 212 (± 21) nM. It is only in near the bottom that total iodine concentrations were only about 5% higher than total inorganic iodine concentrations, as is the case in surrounding seawater. Thus, a third iodine form, which is probably organic in nature, appears to be a fresh-water derived species. Its high stability would explain conservative behaviour of total inorganic iodine in the waters of Bjejajka Cave.