

**Multicellular life in brine: Nematodes in the Great Salt Lake.** J. Jung<sup>1</sup>, T. Loschko<sup>1</sup>, M. S. Werner<sup>1</sup>

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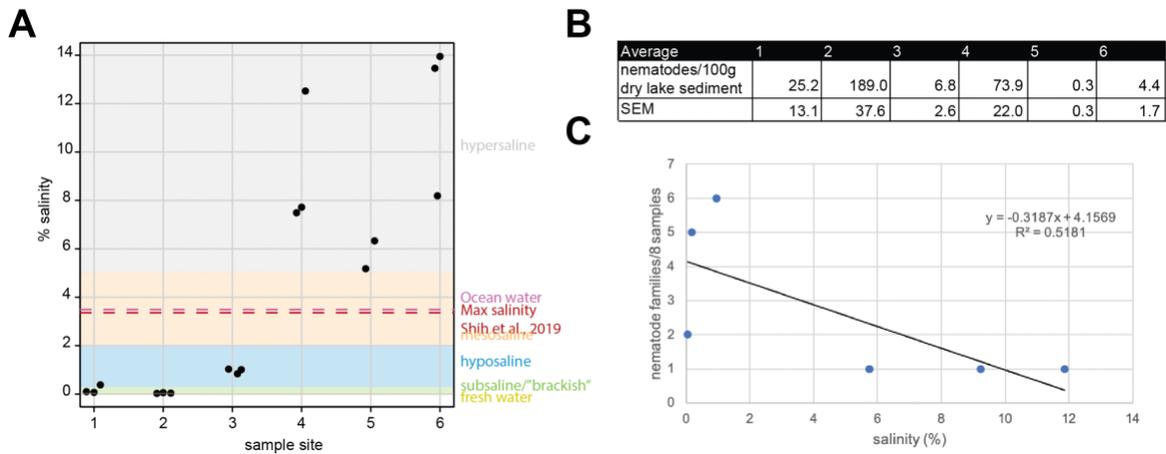
**Introduction:** Saline lakes (>3 g/L) are formed in terminal drainage basins when evaporation exceeds precipitation [1]. In hypersaline conditions (>50 g/L) the osmotic balance in plant tissue and animal blood or hemolymph is overwhelmed. Thus, geological, climatic, and anthropogenic forces that promote the formation of saline lakes can lead to mass extinctions of flora and fauna [2–4]. Remarkably however, small roundworms (nematodes) were recently found in Mono Lake, an arsenic-rich saline lake in California [5]. Nematodes are a remarkably successful and ancient phylum: they are the most abundant animals on Earth, they have been thawed (alive) from ~30,000-year-old ice, and can be found in the driest and coldest environments on our planet. Yet, the generality of these findings, i.e. the prevalence of nematodes in saline or hypersaline lakes, is currently unknown. More broadly, the adaptive potential of multicellular organisms to survive shifts to saline environments is poorly understood. Revealing these principles may also reveal the resiliency of multicellular organisms to drying lakes on Earth [6], and the possibility of complex life to exist in brines throughout our solar system [7,8].

One key to answering these questions is to identify biotic and chemical interactions between different trophic levels. Chemoautotrophs and photosynthetic bacteria can serve critical functions as primary producers in environments that are too harsh for plants [9]. In turn non-autotrophic bacteria, fungi and nematodes can promote ecosystem health by making nitrogen available to primary producers [10,11]. However, the evolution and stability of lake sediment ecosystems to rapidly changing salinities is unknown. To address these questions, we investigated whether nematodes or other microfauna inhabit the Great Salt Lake in Utah, a saline remnant of a massive ice-age freshwater lake.

**Results:** To date, only microbes, brine shrimp and brine flies are thought to inhabit the Great Salt Lake. We have acquired the first evidence that nematodes also reside there, including sites in brackish delta from freshwater inflows, to lake sediment approaching 16% salinity (Figure 1A-B). Notably, this is considerably higher than ocean water (3.5%) or recently sampled sediment from Mono Lake (0.01-3.4%)[5]. We also found a dramatic drop off in diversity with increased salinity (Figure 1C), consistent with specific adaptation to an extreme environment. Genotyping reveals that

nematodes from the most saline parts of the lake represent a new species of Monhysteridae – an ancient family of nematodes that is found in brackish waters. Finally, we have also found a strong correlation of nematode abundance with the presence of microbialites, benthic carbonate sediments formed by bacteria. Fossil evidence of microbialites date back to ~3.5 billion years ago and are among the earliest evidence of life on Earth [13]. We are currently genotyping nematode-associated microbes to assess whether they are grazing on the associated archaeal and prokaryotic bacteria that comprise microbialites. Collectively, these results demonstrate that nematodes commonly inhabit brine environments, that we haven't yet reached the limit of their tolerance to salinity, and that their ecology may be similar to that of an early earth ecosystem.

**References:** [1] Hammer U.T. (1986) *Saline Lake Ecosystems of the World*. [2] Weissflog L. et al. (2009) *Earth Sci.*, 425, 291–295. [3] Beutel M.W., et al. (2001) *Dordrecht*, 91–105. [4] Ermakhanov Z.K., et al. (2012) *Lakes Reserv.*, 17, 3–9. [5] Shih P.Y., et al. (2019) *Curr Biol.*, 29, 3339–3344. [6] Wurtsbaugh W.A., et al. (2017) *Nat Geosci.*, 10, 816–821. [7] Lauro S.E., et al. (2021) *Nature Astronomy*, 63–70. [8] Perl S.M., Baxter B.K. (2020) *Great Salt Lake Biology*, 487–514. [9] Jasser I. (2006) *Ecohydrology & Hydrobiology*, 69–77. [10] Martínez-Espinosa R.M., et al. (2020) *Int J Mol Sci.*, 21. [11] Ekschmitt K., et al. (1999) *Plant Soil*, 212: 45–61. [12] Oviatt C.G., Shroder J.F. (2016) *Lake Bonneville: A Scientific Update*. [13] Noffke, et al. (2013) *Astrobiology*, 12, 1103–1124.



**Figure 1:** (A) Salinity of sample sites from the Great Salt Lake. (B) Average abundance and standard error mean (SEM) of nematodes from different sample sites per 100g dry sediment. (C) Family diversity of nematodes plotted against salinity. Family taxa were identified by Sanger Sequencing 500 nucleotides of the small subunit ribosomal DNA (SSU).