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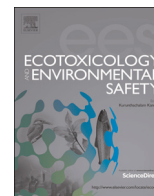
Coal fly ash effluent affects the distributions of *Brachionus calyciflorus* sibling species

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ABSTRACT

Fly ash, a coal combustion residue of thermal power plants and a source of multiple pollutants, has been recognized as an environmental hazard all over the world. Although it is known that fly ash effluent affects density, diversity and distribution of rotifers in drainage systems and receiving water bodies, the effect of fly ash effluent on the distributions of highly similar rotifer species remains unknown. In this study, the mtDNA COI genes of 90 individuals in *Brachionus calyciflorus* complex from Lake Hui (as a fly ash discharge water pond) and other two neighboring lakes (Lake Fengming and Lake Tingtang) were sequenced and analyzed, and the responses in selected life table demographic parameters (life expectancy at hatching, net reproductive rate, intrinsic rate of population increase and proportion of sexual offspring) of different rotifer populations to fly ash effluent were investigated. Overall, 72 mtDNA haplotypes were defined, and were split into two clades by the phylogenetic trees. The divergence of COI gene sequences between the two clades ranged from 11.8% to17.8%, indicating the occurrence of two sibling species (sibling species I and sibling species II). Sibling species I distributed in all the three lakes, showing strong capabilities for dispersal and colonization, which were supported by its higher level of gene flow (2.60–4.04) between the populations from Lake Hui and each of the other two lakes, longer life expectancy at hatching (101.6–148.2 h), and higher net reproductive rate (4.4–16.4 offspring/female) and intrinsic rate of population increase (0.60–0.98/d) when cultured in aerated tap water and fly ash effluent. Sibling species II distributed in both Lake Tingtang and Lake Fengming, showing that its dispersal existed between the two lakes. Considering that the distance between Lake Hui and Lake Fengming is shorter than that between Lake Tingtang and Lake Fengming, sibling species II is able to disperse at least from Lake Fengming to Lake Hui. The restricted distribution of sibling species II in Lake Hui might be attributed to its lower intrinsic rate of population increase (0.34–0.39/d) when cultured in aerated tap water and fiy ash effluent, which might be further lowered by the lower algal food level and quality in Lake Hui.

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1. Introduction

Aquatic habitats are adversely impacted by urbanization, deforestation, construction, irrigation (including dam construction), drainage of wetlands (particularly of freshwaters and peat swamps), and pollution (Dudgeon, 2008). Fly ash, a coal combustion residue of thermal power plants, has been recognized as an environmental hazard all over the world. Fly ash is a source of multiple pollutants (heavy metals i.e. As, Cr, Cd, Pb, Hg, Se, B etc. polycyclic aromatic hydrocarbons and radioactive elements),

contaminates both aquatic and terrestrial ecosystems (e.g., Pandey and Singh, 2010), and eventually causes serious health hazards to animals and people around coal fly ash basins and thermal power plants (e.g., Bryan et al., 2012; Dwivedia et al., 2012). As usual, fly ash is dumped in large land areas in slurry form known as fly ash dykes. In these dykes, water is removed from slurry and is collected into a manmade pond known as fly ash discharge water pond or fly ash lagoon. After fly ash has settled excess water is disposed of into nearby water body, such as a river. It is known that fly ash effluent affects density, diversity and distribution of plankton, aquatic invertebrates and benthic macro-invertebrate in drainage systems and receiving water bodies (e.g., Shrivastava and Shrivastava, 2012; Walia and Mehra, 1998).

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However, the effect of fly ash effluent on the distributions of highly similar rotifer species remains unknown.

Rotifera is a group of primary continental aquatic invertebrates, occurring in almost all types of water bodies (Segers, 2008). They produce diapausing resistant stages in their life cycle which allow them not only to survive in situ during adverse periods or drought but also to disperse their propagules in various ways, either abiotically (wind and water flow) and/or biotically (epi- and endozoochory) (Wallace et al., 2008). Due to their cosmopolitan distribution, shorter generation time, smaller body size (about 150 μm), rapid frequency of reproduction, professional endorsement of toxicological evaluations and feasibility of cultures in the laboratory, rotifers, especially *Brachionus calyciflorus* and *Brachionus plicatilis*, are frequently used for aquatic ecotoxicology (Snell and Janssen, 1995). In the past two decades, accumulating evidence has shown that many rotifer species, such as *B. plicatilis* and *B. calyciflorus*, traditionally categorized as cosmopolitan, are now recognized as cryptic species complexes (e.g., Ciros-Pérez et al., 2001; Derry et al., 2003; Gilbert and Walsh, 2005; Gómez et al., 1995, 2002a, 2002b; Li et al., 2008; Xiang et al., 2010). Toxicity tests have shown that four sibling species in *B. plicatilis* species complex displayed different sensitivities to Corexit 9500A®, propylene glycol and Macondo oil (Rico-Martínez et al., 2013), but the differences in the sensitivities in life table demographic parameters between sibling species in *B. plicatilis* or *B. calyciflorus* species complex to fly ash effluent remains unknown. Field based studies have indicated that the distributions of *B. plicatilis* sibling species are differentially influenced by a small number of environmental variables, primarily salinity (Gómez et al., 1995), such that different physiological tolerances confine siblings to different spatial and temporal distributions (Gómez et al., 1997). However, details of how underlying ecophysiological responses (e.g. survival, reproduction and population growth rate) and environmental tolerances differ among siblings are scarce (Lowe et al., 2007; Alcántrara-Rodríguez et al., 2012; Rico-Martínez et al., 2013). Such information is critical to understand the relative roles of environmental factors and sibling species interactions in determining distributions.

In this study, we investigated the sibling species composition in *B. calyciflorus* complex from three water bodies by analyzing fragments of the mitochondrial COI gene, compared the sensitivities in life table demographic parameters of siblings to fly ash effluent, and analyzed possible mechanisms involved in the effects of fly ash effluent on the distributions of siblings.

2. Materials and methods

2.1. Study sites

The three lakes, including Lake Hui, Lake Fengming and Lake Tingtang studied in this paper are all located in Wuhu city on the south bank of Yangtze River (between 119°21′ longitude and 31°20′ latitude) (Fig. S1, Supplementary materials).

Lake Hui is located in the north of Wuhu city, has an average depth of 6 m and a water surface area of 21.12 ha, and has been used as a fly ash discharge water pond for Wuhu thermal power station with 4×125 MW generating capacity since 2000. Lake Fengming is also located in the north of the city, has an average depth of 1.2 m and a water surface area of 40.27 ha, and is surrounded by several factories and farmlands. Lake Tingtang is located in the center of Wuhu city, has an average depth of 1.5 m and a water surface area of 13.47 ha, and is strongly subjected to recreational activities. Some parts of its basin are covered by submerged macrophytes.

Table 1

Summary information on the sampled populations of *Brachionus calyciflorus* species complex used in this study.

Sampling lake	Code	Sample size	pH	TDN (mg/L)	TDP (mg/L)	NH₃-N (mg/L)	Chl-*a* (μg/L)
Lake Hui	H	34	9.82	0.31	0.078	3.25	8.19
Lake Fengming	F	30	8.50	1.05	0.060	1.47	36.58
Lake Tingtang	T	26	7.82	0.8	0.015	1.89	32.76

Among the three lakes, Lake Hui had the highest pH value, and Al, As, B, Ca, Cr, K, Li, Mo, Se, Si and V concentrations, but the lowest Chl-*a* and total dissolved nitrogen (TDN) contents, and Mg and Ba concentrations. No obvious differences in other element contents were observed among the three lakes (Table 1; Table S1, Supplementary materials) (Zhang, 2010). According to the Chinese environmental quality standard for surface water (GB 3838-2002) (PRC EPA, 2002), the contents of As, B, Mo, Se and V in Lake Hui were 2, 4, 5, 8 and 6 times higher than the limited values in the surface water quality standard, respectively, and those of Sb in all the three lakes exceeded the limited value. The dominant phytoplankton species in Lake Hui, Lake Fengming and Lake Tingtang was *Melosira granulata var. angustissima*, *Macrocystis aeruginosa*, and *Pediastrum duplex* and *Scenedesmus dimorphus*, respectively.

2.2. Sample collection and clonal culture

Rotifers were collected from the three lakes between April and May 2009 during which *B. calyciflorus* in Lake Hui had the highest density across the year (Zhang, 2010). After collection, rotifer individuals were clonally cultured at 22 °C and fed on 1.0×10^6 cells/ml of *Scenedesmus obliquus* daily. For mass cultures, reconstituted hardwater (Gilbert, 1963) was used as the medium. The algae were grown in a semi-continuous culture using HB-4 medium (Li et al., 1959) renewed daily at 20%. Algae in exponential growth were centrifuged and resuspended in the rotifer medium. When they reached higher densities (200–300 ind./ml), approximately 100 animals were piped out from each clonal culture and filtered with a 30-μm plankton net, washed several times with double distilled water, centrifuged, and preserved in freezer until molecular processing. The rest animals in each clonal culture were maintained on 1.0×10^6 cells/ml of *S. obliquus* for life table experiment.

2.3. DNA extraction, COI sequencing and analysis

The 25 μL PCR premix was constructed as follows: $1 \times$ Buffer, 0.2 mM of each dNTP, 0.2 μM of each primer, 2.0 mM Mg²⁺, 2 μL template DNA and 2 U Taq Polymerase (Tiangen, China). The primers HCO2198 (5′TTAAACTTCAGGGTGACCAAAAAATCA3′) and LCO1490 (5′GGGTCAACAAATCATAAAGATATTGG3′) (Folmer et al., 1994) were synthesized by Nanjing Genscript Co., Ltd. (Nanjing, China). Amplifications were carried out using the following cycling conditions: predenaturation at 94 °C for 5 min; denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s and extension at 72 °C for 1 min, circulated for 30 times, and a final extension step of 10 min at 72 °C. After purified by a PCR Cleanup kit (Tiangen, China), the PCR products were ligated into PGM-T vector (Tiangen, China) and transformed into *Escherichia coli* cells using standard protocols. The insert fragments were sequenced using an ABI-PRISM 3730 automated sequencer in Nanjing Genscript Co., Ltd.

All the sequences were aligned using the Clustal X (1.8) software (Thompson et al., 1997) with default parameters and checked manually. A fragment of 667 bp was selected as target sequence. DNAStar computer package was used to test the percentage of