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Ciliates in chalk-stream habitats congregate in biodiversity hot spots

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Abstract

Free-living ciliates are a diverse group of microbial eukaryotes that inhabit aquatic environments. They have a vital role within the ‘microbial loop’, being consumers of microscopic prey such as bacteria, micro-algae, and flagellates, and representing a link between the microscopic and macroscopic components of aquatic food webs. This investigation describes the ciliate communities of four habitats located in the catchment of the River Frome, the major chalk-stream in southern Britain. The ciliate communities were characterised in terms of community assemblage, species abundance and size classes. The ciliate communities investigated proved to be highly diverse, yielding a total of 114 active species. An additional 15 ‘cryptic’ ciliate species were also uncovered. Heterogeneity in the ciliate communities was evident at multiple spatial scales, revealing hot spots of species richness, both within and between habitats. The ciliate communities of habitats with flowing water were composed of smaller ciliates compared to the still-water habitats examined.

Keywords: Ciliated protozoa; Cryptic biodiversity; Microbial eukaryotes; Microbial diversity; Mixotrophy; *Cyrtolophosis*

1. Introduction

Chalk-stream water courses originate from groundwater aquifers that arise from rain water percolating through chalk rock, a porous form of limestone (Berrie, 1992). They have distinct characteristics, such as high mineral content and clear calcareous waters with a pH of 7.4–8.0 that greatly influence their unique ecology (Berrie, 1992; Environment Agency, 2004). Chalk-stream habitats refer to the entire chalk-stream catchment, and not only to the main waterway (Environment Agency, 2004). Few studies have been published on protozoa and other microbial eukaryotes in chalk-stream environments (Gray, 1952; Baldock et al., 1983; Sleigh et al., 1992). The protozoan communities from such habitats can perhaps be expected to be distinct from those present in other freshwater habitats.

Sleigh et al. (1992) provided a summary of research carried out on biomass and production of protozoa in chalk-streams. These authors found that ciliates dominated total protozoan production, demonstrating their importance to secondary production. Baldock et al. (1983) reached the conclusion that protozoan abundance appeared to be negatively correlated with current velocity, with highest numbers recorded in slower waters.

The investigation presented here characterises the ciliate biodiversity of four ecologically different habitats located in the catchment of the River Frome, the major chalk-stream in southern Britain (UK).

2. Materials and methods

Samples were collected from several habitats located in the catchment of the River Frome, a chalk river about 60 km in length and a designated Site of Special Scientific Interest (Environment Agency, 2004), with a catchment of approximately 465 km^2, in Dorset, UK. Four sampling locations – sampling sites 1, 2, 3, and 4, respectively – were selected, covering a variety of ecological conditions. Three sampling locations were within 0.5 m-deep experimental channels (i.e. 15-m-long replicas of the chalk-stream river), connected to it and carrying the water that is diverted from the main river.

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The ecological conditions of the main river and those of the experimental channels are therefore the same. Sampling sites 1 and 2 were within one of the experimental channels that had a continuous flow of running water, with a bed composed of relatively fine sediment. Clusters of floating algae were present on the water surface of this channel. The first sampling site (site 1) was the thin layer of bed sediment in an exposed section of the channel that had no overlying macrophytes or floating algae. The top 1 cm^2 of superficial sediment (i.e. the layer corresponding to the water–sediment interphase, which is about 1 mm thick) was collected with a sterile pipette. Samples from the thick mat of floating algae (site 2) were also collected by trying to remove 1 cm^2 of the algal biomass directly into a sterile tube. Site 3 was a different experimental channel where water had been prevented from flowing. This channel had a thicker layer of bed sediment. Samples were collected from the top 1 cm^2 layer of water–sediment interphase. The fourth sampling location was a wet fen on the river's floodplain. This habitat was a relatively large, 0.5 m-deep, micro-aerobic body of still water surrounded by dense overhanging vegetation, resulting in reduced light penetration (Esteban et al., 2009a,b). Due to the inaccessibility of this pond, sediment samples had to be collected with a bottle sampler thrown from the shore and with a grab sampler attached to a long pole. Samples were transferred to sterile flasks that were left settling in the lab before removing the top 1 cm^2 of the sediment–water interphase.

Observations of live samples were carried out under light microscopy within 3 h after sampling. One ml subsamples of the original samples were transferred to Sedgewick Rafter counting chambers. Ten 1 ml subsamples from each sampling site were examined in total. The ciliates present in the ten 1 ml subsamples from each habitat were used to determine active ciliate species richness, abundances, community assemblages and cell sizes. These results were also used for the production of cumulative species richness curves, and for comparison between habitats. Simpson's (1949) diversity index was calculated for each habitat, and size–frequency distributions were constructed for each ciliate community. The latter enabled the identification of dominant ciliate size classes, and comparison of cell size distributions between communities. Total abundance of ciliated protozoa per species was calculated based on three countings for each sampling location. The taxonomic literature used for the identification of ciliate species was Foissner et al., (1992a,b, 1994, 1995), Kahl (1930–1935), Kreutz and Foissner (2006), and other specialised articles.

To investigate 'cryptic' ciliate diversity, a 50 ml subsample from each of the four sampling locations was transferred into sterile culture flasks that contained 1 ml of sterile soil extract medium to encourage growth of potential 'hidden' ciliate species, i.e. those that are either dormant or present in such low numbers in the samples that they pass undetected during examination of original subsamples (Esteban and Finlay, 2010). Each culture flask was observed under an inverted light microscope and ciliate species were recorded. The culture flasks were kept at room temperature and the ciliate species were monitored three days a week over four weeks.

3. Results

3.1. Ciliate species richness and community composition

A total of 114 ciliate species active at the time of sampling were observed in live samples of the River Frome catchment habitats during the three months of investigation. An additional 15 species, not observed in fresh samples, were subsequently recovered from the four habitats in the experiment designed to reveal the 'cryptic' ciliate species (Appendix A). This increased the total number of identified ciliates at the end of the study period, both active and cryptic, in this study to 129.

Fig. 1 shows the total active alpha-diversity of the four habitats and the cumulative species richness of ciliates over ten 1 ml subsamples from freshly collected samples. The pattern of the cumulative number of species for each habitat was different. The bed sediment of the flowing channel (site 1) was species-poor, yielding 23 species only (Fig. 1A). The progressive increase in the cumulative number of species of this community was relatively smooth and a plateau in the number of species was rapidly reached. The other chalk-stream habitats investigated were richer in species. The cluster of algae on the water surface of the flowing channel (site 2), located 2 m distant from the ciliate-poor sampling site 1, yielded 55 ciliate species (Fig. 1B). The non-flowing channel (site 3) yielded 33 species (Fig. 1C), and the wet fen 54 (site 4, Fig. 1D). Each habitat reached a plateau in the cumulative number of ciliate species before the tenth subsample was investigated, except for the wet fen, where the cumulative number of species still appeared to increase after ten subsamples.

Table 1 shows Simpson's diversity indices and their equitability values, calculated for each of the four habitats. Simpson's diversity index takes into account the number of species present and the equitability, i.e. the relative abundance of each species. The biodiversity represented by the indices generally follows expectations from the raw data and the cumulative species richness values. The flowing channel sediment (site 1) harboured the least diverse community (Table 1). The flowing channel floating algae (site 2) was the most diverse individual habitat, generating the highest equitability and Simpson's index value. All habitats had relatively low equitabilities.

The ciliate community composition of each chalk-stream habitat was completely unique compared to one another. Remarkably, one single species, *Coleps hirtus*, was common to all four habitats, and only few species were common to three of the four habitats. Most ciliate species observed were restricted to one or two habitats (Appendix A).

3.2. Rare ciliate species

Two rare ciliate species were observed during the investigation of ciliates from chalk-stream habitats. The rarely-found, lorica-building ciliate *Spirostomum semivirescens* (Esteban et al., 2009a,b) was common throughout the entire investigation in sediment samples from the wet fen (sampling site 4). Another rare species, *Cyrtolophosis major* (Fig. 5), was