

Filtering structures and particle size selection in coexisting Cladocera

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Summary. Five commonly co-occurring cladocerans, *Holopedium gibberum*, *Daphnia longispina*, *Ceriodaphnia quadrangula*, *Bosmina longispina*, *Diaphanosoma brachyurum* and the calanoid copepod *Eudiaptomus gracilis* were fed monodisperse fluorescent latex beads (0.5, 1, 5 μm) to reveal clearance rates and particle size selection. The results were compared to those obtained with labelled bacteria and yeast. All cladoceran species ingested the beads, and particle size selection varied from a strong predominance of the largest particles in *Holopedium*, to an almost non selective particle retention in *Diaphanosoma*. The results indicated a strong correlation between morphology of the filtering combs, measured by Scanning electron microscopy, and ability to retain the smallest particles.

Materials and methods

Animals were collected by vertical net hauls (45 μm) from epilimnion of three different brown-water lakes at different dates of summer 1984 and transferred to the lab in natural lake water. After 12 h pre-adaptation in aquaria at the lake temperature, they were gently transferred to 100 ml beakers dipped into the aquaria, thus avoiding air contact.

After 30 min adaptation, fluorescent monodisperse latex-beads (Fluoresbrites, Polysciences) of 0.5 μm , 1.0 μm and 5 μm were added to final concentrations of respectively $2.9\text{--}4.5 \cdot 10^6$, $1.1\text{--}2.0 \cdot 10^6$ and $0.45\text{--}1.0 \cdot 10^5$. After mixing by a glass-rod incubation lasted for 0, 2.5, 5, 7.5 and 10 min, then animals were killed with hot water (55–60° C). The fixed animals were filtered on a 45 μm gauze, washed and transferred to a counting tray with distilled water, and sorted according to length classes and species.

Varying with size and gut-filling (which could easily be distinguished in the dissection microscope), 5–15 animals of each group were placed in vials with distilled water and resolved for 5 min with ultra-sound (Gallenkamp, Soni-prep.). This gave an almost complete break-down of animal tissue. The suspended particles were filtered on a 0.2 μm polycarbonat filter and counted in a fluorescence microscope.

The cladocerans *Holopedium gibberum*, *Daphnia longispina*, *Ceriodaphnia quadrangula*, *Bosmina longispina*, and *Diaphanosoma brachyurum* were investigated, as well as the calanoid copepod *Eudiaptomus gracilis*, the cyclopoid copepod *Thermocyclops oithonoides* (both nauplii, copepodids and adults) and the rotifers *Keratella cochlearis* and *Kellicottia longispina*. Dry weights from lengths were estimated from Bottrell et al. (1976) and Larsson (1978).

To show whether feeding rate would influence particle selection, two experiments were performed, both with starved animals (12 h in aquaria with food concentration decreasing below 0.2 mg C/l) and prefed animals (12 h fed on yeast, 10–20 mg C/l).

In an additional experiment, *Holopedium*, *Daphnia*, *Ceriodaphnia* and *Eudiaptomus* were fed with ^{14}C -labelled bacteria (*E. coli* culture with cell sizes of $1.5 \times 1.0 \mu\text{m}$) and yeast (bakers yeast, diameter 5.0–5.5 μm). This was done simultaneously with one of the latex-bead experiments, using prefed animals from the same lake, treated in the same manner.

From all samples ethanol-preserved animals were critical-point dried, dissected, mounted on stubs and examined

The morphology and basic functions of the filtering apparatus of cladocerans are known since the early works of Storch (1924). The role of the morphology and filtering process in food size selection, especially concerning the lower size limit of retainable particles, have however first been discussed during the last few years (Korinek and Machacek 1979; Geller and Müller 1981; Gerritsen and Porter 1982; Gophen and Geller 1984).

Food particles smaller than 1 μm (e.g. mainly bacteria) may contribute significantly to the available food for freshwater zooplankton. To assess the ability of different species to utilize this food spectrum, both direct measurements of the filter combs (Korinek and Machacek 1979; Geller and Müller 1981) and clearance rates on bacteria (Gophen et al. 1974; Peterson et al. 1978) as well as artificial particles (Gliwicz 1969, 1977) have been performed. Although the ability to retain bacteria or small particles have been shown in a large number of filter-feeding zooplankton, the underlying mechanisms are so far poorly understood.

The present paper gives the results from a combination of these methods, where morphological characteristics of filtering combs and particle size selection were studied at coexisting species. This should also give information about competitive abilities according to the availability of potential food.

In the size selection experiments fluorescent, monodisperse latex beads (0.5, 1 and 5 μm) were used, and finally the testing and usefulness of this presented method is discussed.

by scanning electron microscopy. Interetae and setulae distances of several individuals of different sizes and from different populations were measured.

Results

Distances between setulae were rather constant within adults of each species, and no significant differences were found between populations from different lakes. Distance between setae showed considerable variations however, depending on site of measurements on the comb (Table 1).

Based on these morphological features, the species could be divided into three groups (Fig. 1), with *Holopedium gibberum* as the typical "macrofiltrator" (setulae distance 1.8–3.9 μm), *Bosmina longispina* as an intermediate (1.1–1.4 μm) and *Daphnia longispina*, *Ceriodaphnia quadrangula* and *Diaphanosoma brachyurum* as "microfiltrators" (<1.0 μm). *Daphnia* and *Ceriodaphnia* had almost identical intersetulae distance, while *Diaphanosoma* had by far the finest mesh-sizes (0.2–0.3 μm).

When exposed to the plastic beads, all cladoceran species showed an immediate uptake. Within seconds the food-groove was filled, and through 2.5–10 min, the gut was successively filled. Within all stages of copepods and rotifers, the beads were only occasionally recorded.

Clearance rates calculated for particles of different sizes showed considerable variation (Fig. 2–5). With exception of *Bosmina*, all species showed increased clearance rates with increased particle size, most pronounced in *Holopedium*, less in *Diaphanosoma*. In several of the experiments, however, *Holopedium* had almost identical clearance rates for the 0.5 and 1.0 μm beads.

Typically, clearance rates decreased with lower temperature (with exception of *Bosmina*) and in prefed animals. For all specimens, clearance rates for *E. coli* and yeast strongly resembled to clearance rates for the corresponding bead sizes (1.0 and 5 μm). *Eudiaptomus gracilis*, which, avoiding the plastic beads showed rather high clearance rates for yeast, whereas clearance rates for *E. coli* were almost zero.

Particle size selection (calculated from clearance rates) thus gave a strong preference for larger particles among *Holopedium*, intermediate for *Daphnia* and *Ceriodaphnia*, while *Diaphanosoma* only slightly discriminated between different sizes. *Bosmina* reacted in two different ways, with

a preference for large particles in summer, and small particles in autumn. Particle size selection was found to be independent on filtering rates for all investigated species.

Discussion

The plastic beads were consumed by all species of the investigated cladocerans. In all experiments, there was a linearly increasing uptake of beads within 10 min. Between 10–20% of the animals, regardless of species, did not feed on the beads. This might be dead or unhealthily individuals. Within cladocerans, where food uptake is linked closely to respiration, food selection is probably rather passive than active, at least on small particles (cf. Persson 1984), unless toxic and filamentous bluegreens are involved (Webster and Peters 1978). The parallel experiment with *E. coli* and yeast gave very similar clearance rates and selection values compared to the corresponding beads, indicating a similar response to this kind of "natural" food and beads.

The use of non-fluorescent latex beads or other kinds of artificial food have been used in feeding experiments with cladocerans (Burns 1968; Gliwicz 1969), copepods (Gliwicz 1969; Wilson 1973), ciliates (Fenchel 1980; Børsheim 1984) and marine filterfeeding larvae (Rassoulzadegan and Fenaux 1984). The use of fluorescent beads has several advantages (partly discussed by Børsheim 1984). They are highly visible, even inside animals, they are non-sticky and even the 5 μm beads stay in suspension for at least 15 min. They are thus useful not only in clearance and size-selection experiments, but might be used as tracer in natural food to study gut passage time, and for direct studies of feeding in living animals. Further, the percentage of non-feeding animals may easily be recognized, and irregular feeding intervals (which in fact frequently was observed among *Daphnia*) may be seen directly.

Concerning the copepods, the feeding of single particles coupled to chemosensory feeding (Friedman and Strickler 1975; Poulet and Marsot 1978) might explain the avoidance of beads by *Eudiaptomus* and *Thermocyclops*. Rotifers did also avoid the beads, although Børsheim (1984) reported some rotifers to ingest latex beads.

The presented experimental results give evidence of a strong relationship between mesh-size of the filtering combs and ability to retain small particles. Moreover, the intersetulae distance seems to be a more or less fixed property

Table 1. Dry weight, range of interetae and intersetulae distances and size-preference for different particles measured by the quotient of clearance rates on two different types of food: *Holopedium gibberum* (H.g.), *Daphnia longispina* (D.l.), *Ceriodaphnia quadrangula* (C.d.), *Bosmina longispina* (B.l.), *Diaphanosoma brachyurum* (D.b.) and *Eudiaptomus gracilis* (E.g.). Mean values for all experiments, standard deviation in brackets. S: Starved animals. F: Prefed animals

	H.g.	D.l.	C.q.	B.l.	D.b.	E.g.
dw (μg)	20.2–28.0	18.5–25.0	7.5–15.0	2.8–3.9	12.0–16.2	8.5–12.0
Dist. setae (μm)	6.6–10.0	1.8–5.2	1.5–3.7	2.5–5.5	2.3–3.0	–
Dist. setulae (μm)	1.8–3.9	0.5–0.9	0.4–0.8	1.1–1.4	0.2–0.3	–
1.0 μm /0.5 μm						
S	4.06 (3.82)	1.35 (0.16)	1.14 (0.19)	1.88 (0.30) ^a	1.18 (0.25)	–
F	1.08 (0.64)	1.51	1.47 (0.79)	–	–	–
5 μm /1.0 μm						
S	6.16 (2.91)	1.27 (0.58)	1.57 (0.55)	1.05 (0.67)	1.03 (0.10)	–
F	7.89 (2.35)	1.12	1.78 (0.74)	–	–	–
Yeast/ <i>E. coli</i>						
F	3.60 (0.73)	1.52 (0.38)	1.72 (0.28)	–	–	–

^a Calculated from autumn experiment

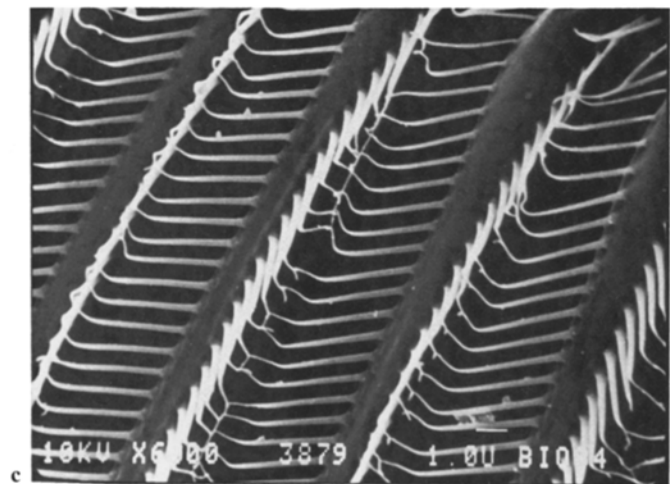
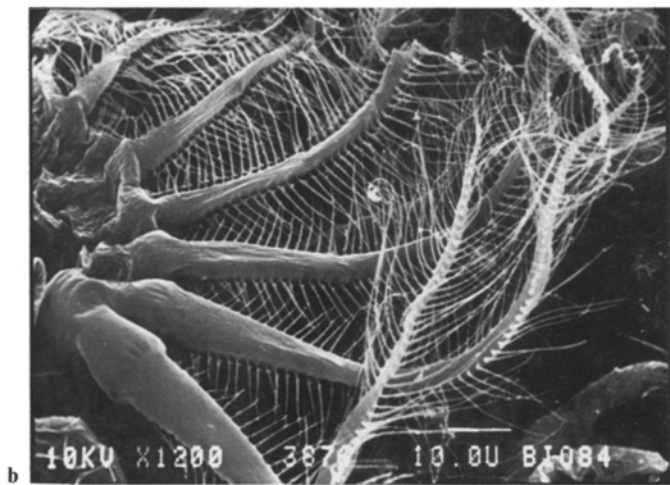
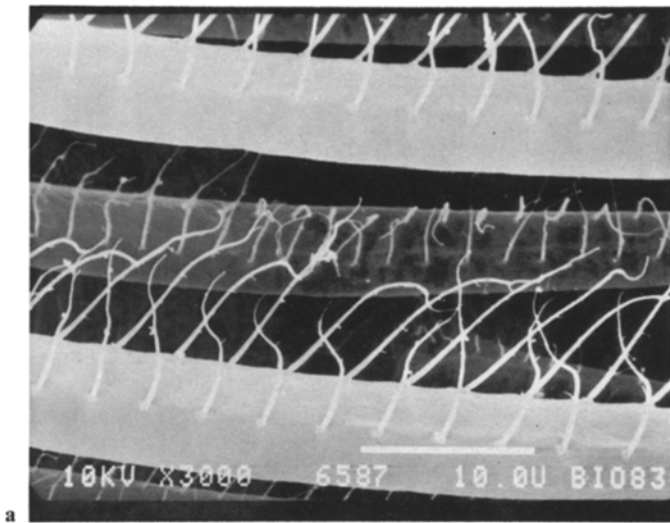


Fig. 1 a. Central part of the 3rd thoracic limb of *Holopedium gibberum*. (Length 1.5 mm). 2,160 ×. Scale bar = 10 μm. **b** Distal part of 3rd thoracic limb of *Bosmina longispina*, showing the “grasping” filter combs with cleaning bristle. (Length 0.6 mm). 758 ×. Scale bar = 10 μm. **c** Central part of 3rd thoracic limb of *Daphnia longispina*. (Length 1.4 mm). 3,900 ×. Scale bar = 1 μm

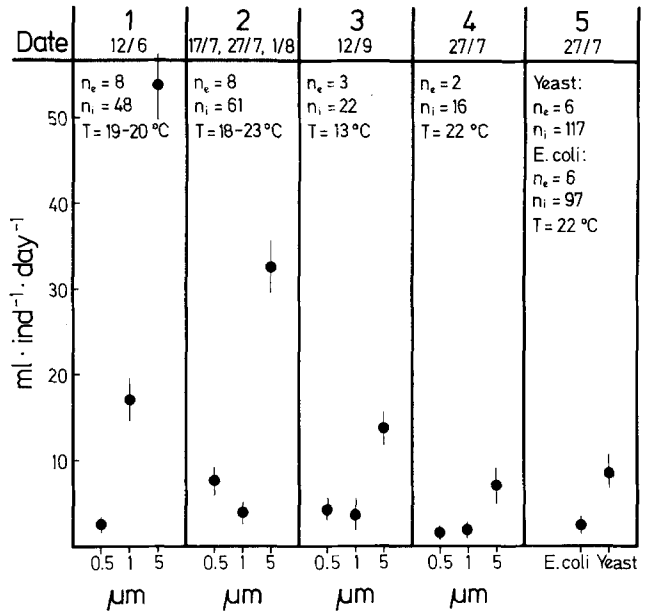


Fig. 2. Clearance rates ($\text{ml} \cdot \text{ind}^{-1} \cdot \text{day}^{-1}$) for *Holopedium gibberum* given different sizes of latex beads (0.5, 1 and 5 μm), *E. coli* and yeast. 1 Spring experiment with the earliest, egg-carrying individuals (ind from two different lakes). 2 Summer experiment (ind. from two different lakes). 3 Autumn experiments (ind. from one lake). 4 Prefed animals (see text). 5 Prefed animals fed *E. coli* and yeast. All animals in range 20.2–28.0 μg dw. n_e : number of experiments. n_i : total number of individuals. T : temperature in lake and in aquaria during experiments. Means and S.D. vertical bars refer to each series of experiments (N_e)

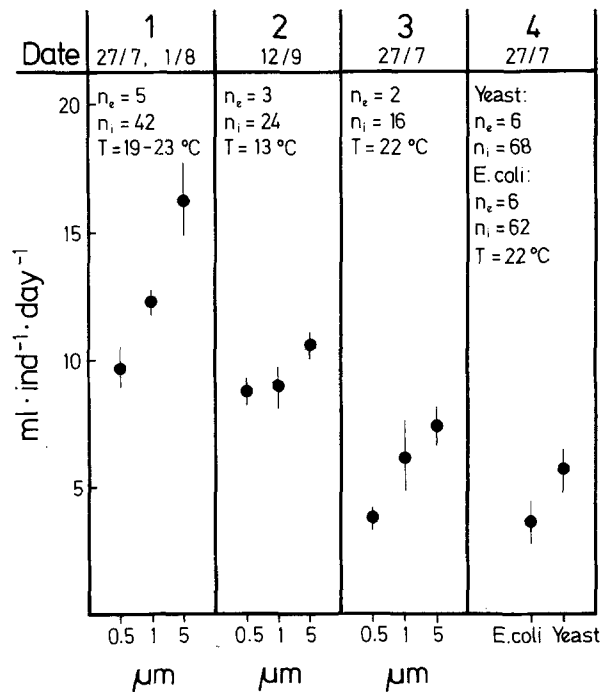


Fig. 3. Clearance rates for *Daphnia longispina*. 1 Summer experiments (ind. from two lakes). 2 Autumn experiments (ind. from two lakes). 3 Prefed animals. 4 Prefed animals fed on *E. coli* and yeast. All animals range 18.5–25.0 μg dw. Symbols as in Fig. 2

within at least some species, as these results are in close agreement with the findings of Geller and Müller (1981). Among some of the morphologically and genetically more unstable *Daphnia* species, one might expect greater local variations (Korinek and Machacek 1979).

The smallest intersetulae-distance can not be taken as an absolute limit for the smallest size of retainable particles, however. Even *Holopedium* was capable of retaining particles of 0.5 and 1.0 μm size, indicating that mechanisms besides the pure sieving process may be involved (cf. Rubenstein and Koehl 1977). It is noteworthy, however, that in

most of the experiments with *Holopedium*, both 0.5 and 1.0 μm beads were almost equally retained, which was not the case among the "microfiltrators". This might indicate that those "other mechanisms" involved (surface chemistry in food and filter combs, transferring processes from filter to gut etc.), does not discriminate well between particle sizes below 1.0 μm .

Gerritsen and Porter (1982) also observed a retention of particles smaller than the actual mesh size in *Daphnia*, and they argue against the sieving model due to this observation. In their careful study on four *Daphnia* spp., Gophen and Geller (1984) found a close relation between size of ingested particles and filter mesh sizes, concluding that simple mechanical sieving provides a sufficient explanation to particle retention. According to my results, where not only *Daphnia* was investigated, one can neither reject the sieving model, nor exclude the role of other mechanisms of particle capture within the filtering processes.

The fact that different species have evolved different mesh size, analogous to the gill-rakers in planktivorous fish, strongly indicates that these differences in morphology must be important. Otherwise one should expect a stabilization on an optimum mean mesh-size for all species.

The tested species could be arranged as following: *Eudiaptomus* > *Holopedium* > *Bosmina* > *Ceriodaphnia* > *Daphnia* > *Diaphanosoma*, according to intersetulae distance. With exception of *Bosmina*, this is in accordance with size-selection, thus supporting the hypothesis of Geller and Müller (1981).

The low ability of the calanoid *Eudiaptomus* to retain bacteria is known from several authors (Gliwicz 1969, 1977; Kibby 1971; Zankai and Ponyi 1974; Persson 1984). *Holopedium* is likewise found to be a poor filtrator on bacteria but having high clearance rates on larger particles (Johansson et al. 1976; Persson 1984), while most *Daphnia* species are regarded as potential bacteria feeders (Gliwicz 1969, 1977; Korinek and Machacek 1979; Peterson et al. 1978).

Ceriodaphnia is also supposed to be an efficient filtrator on bacteria (Manuilova 1958; Lynch 1978), while *Bosmina* species has a more uncertain status, and are by some au-

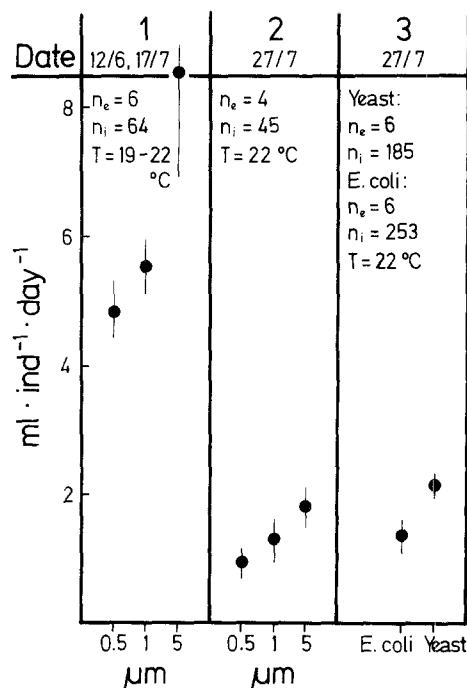


Fig. 4. Clearance rates for *Ceriodaphnia quadranqula*. 1 Summer experiments. 2 Prefed animals. 3 Prefed animals fed on *E. coli* and yeast. All animals in range 7.5–15.0 μg dw. Symbols as in Fig. 2

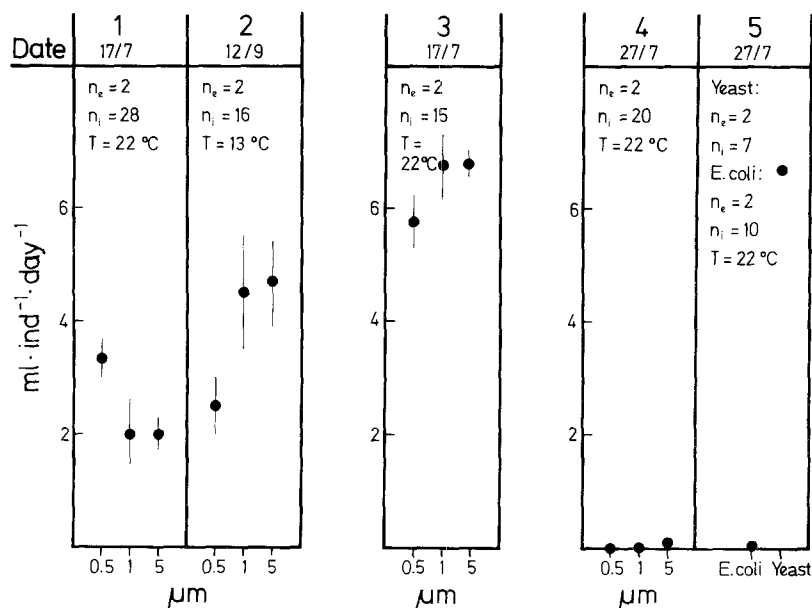


Fig. 5. Clearance rates for: 1 *Bosmina longispina*, summer experiment. 2 *B.l.*, autumn experiment. (Ind. weights: 2.8–3.9 μg dw.) 3 *Diaphanosoma brachyurum*, summer experiment. (Ind. weights: 12.0–16.2 μg dw.) 4 *Eudiaptomus gracilis*, summer experiment. 5 Prefed *E.g.*, fed on *E. coli* and yeast. (Ind. weights: 8.5–12.0 μg dw.) Symbols as in Fig. 2

thors ranged as a very efficient "microfiltrator" (Gliwicz 1969), by other as a more typical "macrofiltrator" (Geller and Müller 1981; Persson 1984). Probably, *Bosmina* may use different feeding strategies, due to the size of available food (DeMott 1982) which in part may explain the somewhat strange clearance rates and size-selection pattern found for *B. longispina* in this experiment. *Diaphanosoma*, which is known to have low clearance rates (Gulati 1978a; 1978b; Haney 1973), probably may compensate for this by its ability to retain very small-sized particles (Gliwicz 1977; Bern pers. comm.).

This might be regarded as three different strategies. A specialization on larger particles (*Holopedium*, which in lakes, most often are restricted to periods with suitable alga), the ability to select a broad range of particle sizes, including bacteria (*Daphnia* and *Ceriodaphnia*) or "taking what is left" (*Diaphanosoma*).

The competitive advantages of retaining bacteria would be obvious in brown-water lakes, where bacteria often make up more than 50% of the biomass potentially available to the herbivore zooplankton (Johansson 1983; Hessen in prep.). Although actual competition in natural habitats can not easily be derived from theory and culture experiments (Lynch 1978; Kerfoot and DeMott 1980), field data give evidence for competition, as *Holopedium* and *Daphnia* or *Holopedium* and *Ceriodaphnia* may overlap completely both in space and time, whereas e.g. *Daphnia* and *Ceriodaphnia* are separated either in space or time during periods of resource limitation (Hessen et al. in prep.).

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