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# Effects of stressors on growth and competition between different cryptic taxa affiliated with Ochromonadales (Chrysophyceae)

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**Abstract:** Morphologically similar flagellate taxa comprise a high cryptic diversity. This diversity evolved partly through parallel evolution in several phylogenetically distinct taxa. Here we investigate the effects of heat waves and salinization on growth and competition between cryptic taxa. For this purpose, we developed specific FISH probes targeting the phylogenetic clades comprising *Pedospumella encystans*, *Spumella rivalis*, and *Poteriospumella lacustris*, respectively. Exposure to salt resulted in a decreasing growth rate for all three taxa. In contrast, a sudden increase in temperature to 27 °C stimulated particularly the growth of *P. lacustris*. This species showed a high competitive strength and the taxon–specific responses to stressors lead to a shift of community composition. This turn–over of differently adapted cryptic species with presumably similar feeding preferences and predator–prey interactions may stabilize microbial food webs facing environmental change.

**Key words:** anthropogenic stressors, Chrysophyceae, cryptic species, fluorescence in situ hybridization, heat wave, heterotrophic flagellate, microbial food web, multiple stressors, salinization

## Introduction

In the face of rapid environmental change the stability of food webs and ecosystem functions becomes an increasingly pressing issue (Saint–Beat et al. 2015; van Meerbeek et al. 2021). In particular, microbial components are of special interest due to their high abundances and their link with geochemical cycles. On the one hand, microbial components may respond quickly to environmental changes due to short generation times, on the other hand, a high functional redundancy between taxa may dampen effects of environmental change. As cryptic diversity is high in many microbial groups the effects of stressors on taxon–turnover and the significance of stressor–induced shifts in the competitive strength of distinct (cryptic) taxa is largely unresolved. Here we address the competitive strength of such cryptic taxa using temperature and salinity as environmental stressors.

### Significance of heat waves and salinization

Freshwater ecosystems are under severe pressure by anthropogenic impacts and climate change, factors of particular concern in inland waters are heatwaves and salinization (Sun & Arnott 2022).

Salt is introduced to freshwater ecosystems by various routes including the mobilization of natural salts (Herczeg et al. 2001; Cañedo–Argüelles et al. 2013) and anthropogenic salinization including e.g., human–accelerated weathering, mining, vegetation removal and application of fertilizers, irrigation practices, and road de–icing salts (Cañedo–Argüelles et al. 2013; Dugan et al. 2017). Eukaryotic microbial community shifts caused by salinity are well documented (von Alvensleben et al. 2016; Castillo et al. 2018; Li et al. 2018; Nuy et al. 2018). Similarly, effects on the growth rates of individual taxa are well documented. The range of tolerated salt concentrations is highly variable (e.g., Boenigk 2008) but generally increasing salt concentrations are negatively correlated with growth rates (Jones et al. 2017; Lam et al. 2019; Park et al. 2020; Arnott et al. 2022). Salt stress can reduce the cell size, cease motility, and trigger morphological changes (Shetty et al. 2019; Kamakura et al. 2022).

Long–term temperature increases are expected due to the warming climate (Ipcc 2013; Rasconi et al. 2015). But heat waves, i.e., rapid short–term increases of temperature, may be more problematic and can impact all trophic levels including microorganisms (Szymczak et al. 2020; Polazzo et al. 2021). Again, severe eukaryotic

microbial community shifts following heat stress have been demonstrated (Hao et al. 2018; Nuy et al. 2018; Thomson et al. 2019). With respect to individual taxa increasing temperatures are expected to increase growth rate but above a critical threshold further temperature increases damage proteins and eventually cause cell death. Both, the extent of growth rate acceleration and the critical maximum temperatures are species– and strain–specific and may therefore affect the competitive strength of individual taxa even within the range of well–tolerated temperatures (Boenigk et al. 2007).

Even though salinization and heat waves are important stressors in freshwater ecosystems, effects of these stressors on the competitive strength and taxon–turn–over in cryptic species and with that the potential preservation of functions despite a considerable taxon turn–over stay secret.

**Significance of cryptic diversity**

Eukaryotic microbial communities are composed of numerous taxa which are in ecological studies often roughly categorized into functional groups such as heterotrophic nanoflagellates, microalgae, etc. (e.g., Fernandez–Leborans & Fernandez–Fernandez 2002; Latorre et al. 2021). Small protists, largely heterotrophic nanoflagellates (HNF) are the major link connecting dissolved organic material, bacteria, and higher trophic levels (Jürgens & Matz 2002; Sherr & Sherr 2002). They are consumers of suspended as well as of attached bacteria (for reviews see Fenchel 1986; Sandersa et al. 1992; Laybourn–Parry & Parry 2000). Despite their importance in food web architecture the functional role of heterotrophic nanoflagellates is often limited to black box approaches while the ecological importance of taxon diversity in this functional group still stays largely secret. During the last decades, molecular surveys proved the diversity behind these black boxes. On the one hand, many taxa comprise a high molecular diversity (e.g., Lowe et al. 2005), on the other hand, taxa from different lineages have similar morphological forms which evolved independently through parallel evolution (e.g., Graupner et al. 2018). The functional role of the high molecular (and taxon) diversity behind this similar morphology is not well understood. It is likely that different lineages are adapted to different environmental conditions. As current environmental change is speeding up deciphering the ecological significance of cryptic diversity may be a key for stabilizing ecosystem functions and services under changing conditions.

**Target group**

The chrysophytes (cf. Kristiansen & Škaloud 2017) are a particularly well–suited model flagellate group for addressing these issues: Chrysophytes are widespread and abundant, particularly in oligotrophic freshwaters and can reach abundances of several thousand cells.ml$^{-1}$ (Sandgren 1988; Carrick & Fahnenstiel 1989; Kristiansen & Preisig 2001; Boenigk & Arndt 2002). Further, they are among the most important grazers of bacteria–sized microorganisms (Finlay & Esteban 1998). Within the chrysophytes a high cryptic diversity of colourless non–scaled taxa evolved through parallel evolution in several chrysophyte lineages (Graupner et al. 2018). Colourless non–scaled forms comprise the genera *Spumella* Cienkowsky 1870, *Poteriospumella* Boenigk et Findenig 2010, *Pedospumella* Boenigk et Findenig 2010, *Atacamaspumella* Pietsch, Nitsche et Arndt 2022, *Vivaspumella* Pietsch, Nitsche et Arndt 2022, and many others (Grossmann et al. 2016; Pietsch et al. 2022). Specifically, the main target organisms of this study – members of the clades C1, C2, and C3 within Ochromonadales (in particular *Spumella* spp., *Pedospumella* spp., and *Poteriospumella* spp.), are abundant in many habitats (Boenigk et al. 2005; Pfandl et al. 2009; Nolte et al. 2010; Grossmann et al. 2016) and tolerate a wide range of physico–chemical conditions (Boenigk 2008).

Identifying and separating distinct cryptic nanoflagellate lineages is of particular significance in colourless non–scaled chrysophytes as a similar and in many cases microscopically indistinguishable morphological form evolved many times independently in several lineages (Grossmann et al. 2016; Graupner et al. 2018; Pietsch et al. 2022). Examples comprise the genera within Ochromonadales (e.g., *Poteriospumella*, *Atacamaspumella*, *Chlorospumella* Pietsch, Nitsche et Arndt 2022, *Spumella*, *Pedospumella*, and many so far not taxonomically assigned lineages), within the Apoikiida (e.g., *Apoikiospumella* Boenigk et Grossmann 2016, *Pseudapoikia* Pietsch, Nitsche et Arndt 2022), within the Chromulinales (e.g., *Chromulinospumella* Boenigk et Grossmann 2016), and in several other lineages (e.g., *Vivaspumella* Pietsch, Nitsche et Arndt 2022) (Grossmann et al. 2016; Pietsch et al. 2022). Here we address the competitive strength and ecophysiological differentiation between morphologically indistinguishable cryptic nanoflagellate taxa facing temperature and salt stress. We investigated the growth response at food concentrations in the range of field conditions, i.e., $2\times10^6$ bacteria.ml$^{-1}$, in single culture and mixed culture of cryptic taxa affilitated with *Spumella*, *Pedospumella*, and *Poteriospumella* using lineage–specific fluorescently labelled probes (FISH–probes) for differentiation.

Fluorescence in situ hybridization (FISH) is a robust and widely applied method for detection, identification, and quantification of microbes. The identification of single–celled organisms by hybridizing the ribosomal RNA with fluorescently labelled probes was first shown by Delong et al. (1989). Standard FISH probes are 15–25 nt long oligonucleotides that are labelled with a fluorescent dye at the 5'end and can be visualized by epifluorescence or confocal laser scanning microscopy (Amann et al. 1990; Wagner et al. 1994). Because the probes are designed to target specific phylogenetic groups,

FISH allows a more detailed and reliable identification than morphological characteristics.

We hypothesize that sudden shifts in environmental conditions as applied in our experiments decrease the growth rate. However, as the temperature stressor is within the optimal temperature range for the flagellates (Boenigk et al. 2007; Boenigk 2008) we expect that a potential negative effect is overcompensated by a positive relation of temperature and thus growth rates will increase. Based on experimental (Boenigk et al. 2007) and environmental seasonal abundance data (Nolte et al. 2010) we expect that the stimulating effect of temperature increase is strongest in *Poteriospumella*. With respect to salt we expect strong differences between the investigated strains as high variability in salt tolerance has been demonstrated for different strains of colourless chrysophytes (Pfandl et al. 2009). The study by Pfandl et al. (2009) showed that variability is similar in all three clades. We therefore cannot make predictions on the tolerance of the individual taxa but suspect that *Pedospumella* might be the least sensitive as this strain originated from soil while the other taxa originated from freshwater (Grossmann et al. 2016).

## Materials and Methods

**Strains and cultivation.** The strains *Pedospumella encystans* JBM/S11 Boenigk et Findenig 2010, *Spumella rivalis* AR4A6 Boenigk et Findenig 2010, and *Poteriospumella lacustris* JBM10 Boenigk et Findenig 2010 are affiliated with the 18S rRNA subclades C1, C2, and C3, respectively, and were obtained from the culture collection of the working group and the Central Collection of Algal Cultures (CCAC) at the University of Duisburg–Essen. The strains have previously been isolated from soil or freshwater samples from different geographical origins (Table 1). The xenic strains JBM/S11and AR4A6 were routinely grown in inorganic basal medium (IB Hahn et al. 2003) in cell culture flasks (25 $cm^3$ with filter screw cap, TTP Techno Plastic Products AG) at 15 °C with 14h:10h light–dark cycle in a climate chamber (SANYO Electric Co. Ltd., Osaka, Japan). The axenic strain JBM10 was routinely grown in NSY medium (Nutrient broth, Peptone from soybean (Bacto Soytone), Yeast extract; Hahn et al. 2003) in 100 ml Erlenmeyer flasks at 15 °C with 14h:10h light–dark cycle. The bacterial strain *Linmohabitans* spp. IID5 was grown in 100 ml Erlenmeyer flasks at room temperature (RT) with constant shaking (96 rpm, orbital shaker, LAUDA–Brinkmann, LP, New Jersey, USA).

**FISH–probe design and optimization.** Specific probes for fluorescence in situ hybridization (FISH) targeting the 18S rRNA were designed for the C1 clade, the C2 clade, the C3 clade, and the entire group of *Ochromonadales* using the DECIPHER's design web tool (Wright et al. 2014). To optimize the hybridization conditions a formamid series from 0% to 70% was carried out for all four probes in 10% steps. Pictures with a fixed exposure time of 1 s for the TRITC channel were taken for comparison of signal intensities in samples hybridized with different formamid concentrations. Signal intensities were measured with the program "Daime" (Daims et al. 2006) by measuring the mean signal intensity of all FISH–stained cells on each picture. Cells were identified by dark–light contrast with an object size threshold of 28 and including internal dark regions into the object area. In the range of optimal formamide concentrations, a second test in steps of 5% was applied in order to determine the optimal formamide concentration for each probe. In addition, formamide curves were generated using mathFISH (Yilmaz & Noguera 2007) for comparing calculated and experimentally determined concentrations for each newly designed probe. Sequences, specificity and hybridization conditions determined for each probe are

Table 1. Origin and affiliation of strains used in this study.

| Strain | Species designation | 18S rRNA clade | Geographical origin | Habitat origin | Media | Nutrition mode |
|---|---|---|---|---|---|---|
| JBMS11 | *Pedospumella encystans* | C1 | Austria, Mondsee, near Rauchhaus | soil | IB + WK | heterotrophic |
| AR4A6 | *Spumella rivalis* | C2 | Austria, River Fuschler Ache | freshwater | IB + WK | heterotrophic |
| JBM10 | *Poteriospumella lacustris* | C3 | Austria, Mondsee | freshwater | NSY (3g/L) | heterotrophic |

Table 2. Target region and hybridization conditions for FISH–probes.

| Probe | Sequence (5' to 3') | Specificity | Formamide (%) for hybridization at 46 °C | 18S rRNA position |
|---|---|---|---|---|
| O1C531 | CCGAGGATGGATTCAGACAACTGGT | C1 clade | 15 | 18S, 531–555 |
| O2C613 | GCCTGCTTTGAACACCCTATTT | C2 clade | 50 | 18S, 613–634 |
| O3C723 | ACCCCCAACTGTCCC | C3 clade | 50 | 18S, 723–737 |
| Och1268 | CTGTTATTGCCCCCAACTTC | Ochromonadales | 45 | 18S, 1268–1287 |

shown in Table 2. Coverage of the probes was evaluated with the Silva rRNA database (August 2022) using TestProbe 3.0 (http://www.arb–silva.de/search/testprobe; Quast et al. 2013).

**Stressor and competition experiments.** Food bacteria (*Linmohabitans* spp. strain IID5) were grown in NSY medium, collected by centrifugation for 10 min at 15000 g at RT and the pellet was resuspended in IB medium. Prior to the experiments, flagellate cultures were also collected by centrifugation for 10 min at 2820 g at RT to remove the initial medium. The resulting pellet was then resuspended in 50 ml IB medium and flagellate cultures were fed with one ml of washed bacteria every three to four days and incubated for 17 days at 23 °C with 14h:10h light–dark cycle.

All experiments were run in five replicates in 75 ml IB medium in 100 ml Erlenmeyer flasks following a three–day acclimatization period. At the beginning of the acclimatization phase, flagellates abundance was adjusted to 2400 cells.ml$^{-1}$. For the competition experiments density of each strain was adjusted to 800 cells.ml$^{-1}$, i.e., total flagellate abundance was the same for the single strain experiments and the competition experiments. Bacterial food concentration was adjusted to $2\times10^6$ bacteria.ml$^{-1}$. Then, all cultures were incubated at 23 °C (14h:10h hours light–dark cycle) for three days to acclimatize to lower food concentrations. On day 3, cultures were exposed to the respective stressors, i.e., the temperature treatments, as well as the combined treatments, were incubated at 27 °C, and sodium chloride was added to the salt and the combined treatment (final concentration corresponding to 2 g chlorid.l$^{-1}$). Subsamples were taken each day (on day 3 samples were taken before and after onset of stressors). Single–strain samples were fixed with Lugol's solution and stored at 4 °C until further use. Mixed–culture samples were fixed with 2% paraformaldehyde (PFA) for 1h at RT or overnight at 4 °C and 4–5 ml of each fixed sample were filtered onto white polycarbonate filters (diameter 25 mm, pore size 0.2 µm, Millipore GTTP 02500, Eschborn, Germany). The filters were air–dried and stored at –20 °C until further use.

**FISH protocol and cell enumeration.** Total flagellate abundance was counted in Sedgewick–Rafter chambers using 1 ml of the Lugol–fixed subsamples. In the competition experiment the fractions of C1, C2, and C3 clade were counted on the filters after FISH–staining as outlined in the following: sections of the filters were cut out with a razor blade and samples were dehydrated through an ethanol series of 50%, 80%, and 100%. For each step, the filters were incubated for 3 min at RT. The cells were then hybridized with fluorescently mono–labelled oligonucleotide probes (O1C531, O2C613, O3C723, or Och1268, 50 ng DNA. µl$^{-1}$, Eurofins Genomics Germany GmbH, Ebersberg, Germany) as described previously (Glöckner et al. 1996). To optimize signal detection rates the concentration of SDS was increased to 0.02% in hybridization buffer and hybridization time was extended to 3 h at 46 °C. To determine the total cell count in the mixed cultures, the filter sections were counterstained by incubating them in a 4,6–diamidino–2–phenylindole (DAPI) solution (0,1 mg.ml$^{-1}$) for 10 min at RT. To remove unspecific staining the filters were washed in sterile distilled water for 1 min at RT, then dehydrated with 100% ethanol for several seconds at RT and air–dried. The filters were mounted in the non–hardening and anti–bleaching mounting medium CitiFluor™ AF2 (Citifluor, Ltd., London, United Kingdom), and the cells were counted using a Nikon Eclipse 80i microscope (Nikon Corp., Tokyo, Japan) with a 20× objective and appropriate sets of fluorescence filters for detecting DAPI– and Cy3–signals. Images were acquired using the NIS–Elements BR ("Basic Research") software (Nikon Corp., Tokyo, Japan). Growth rates between day 3 and day 6 were calculated for single strains and mixed cultures. Statistical data processing was carried out with R version 4.2.1 (R core team 2022). Growth rates were statistically compared using ANOVA, post–hoc Tukey's test, and Welch's two–sample T–tests using R–packages „broom", „tidyverse", „dplyr" and base R (R core team 2022; Robinson et al. 2022; Wickham et al. 2022).

## Results

### Specificity of probes

We developed probes specific for the C1, the C2, and the C3 clade within Ochromonadales (Fig. 1). Tests of the probes using strains affiliated with other clades within Ochromonadales (Fig. 1) and outside Ochromonadales (data not shown) proved the specificity of the probes. Formamide concentration in the hybridization buffer affected the signal intensity and specificity (Fig. S1). Optimal hybridization conditions were similar for the probes O2C613 and O3C723, while for probe O1C531 the hybridization conditions differed. Additionally, the experimentally determined hybridization conditions notably differed from the calculated optimal formamide concentration (Fig. S1).

### Effect of temperature and salinization on growth rates

Growth rates were significantly different both for the application of stressors and between the taxa. This was consistent in the single strain experiments (ANOVA, stressors: $p < 0.001$; taxa: $p < 0.001$) as well as in the competition experiment (ANOVA, stressors: $p < 0.001$; taxa: $p = 0.009$). Further, the interaction between stressor application and taxa was also significant (single strains: $p = 0.002$; competition: $p < 0.001$).

In the single strain experiments, increased temperature alone did not have a significant effect on the growth rate for any of the strains (*Pedospumella*: $p = 1.0$; *Spumella*: $p = 0.92$; *Poteriospumella*: $p = 1.0$). The application of salt significantly reduced growth rate for *Spumella* ($p < 0.001$) but not for the two other taxa (*Pedospumella*: $p = 0.99$; *Poteriospumella*: $p = 0.99$). Similarly, the effects of combined stressors significantly decreased growth rate for *Spumella* ($p < 0.001$) but not for *Pedospumella* ($p = 0.99$) or *Poteriospumella* ($p = 1.0$).

In mixed cultures, higher temperature resulted in a significantly increased growth rate for *Poteriospumella* ($p < 0.001$) but not for the two other species (*Pedospumella*: $p = 0.99$; *Spumella*: $p = 0.97$). Increased salinity resulted in significantly lower growth rate for all taxa (*Pedospumella*: $p < 0.001$; *Spumella*: $p < 0.001$; *Poteriospumella*: $p < 0.001$). Application of both stressors reduced growth of *Pedospumella* ($p = 0.001$) and *Spumella* ($p = 0.03$). Growth rates for *Poteriospumella* were also lower but not significant ($p = 0.15$).

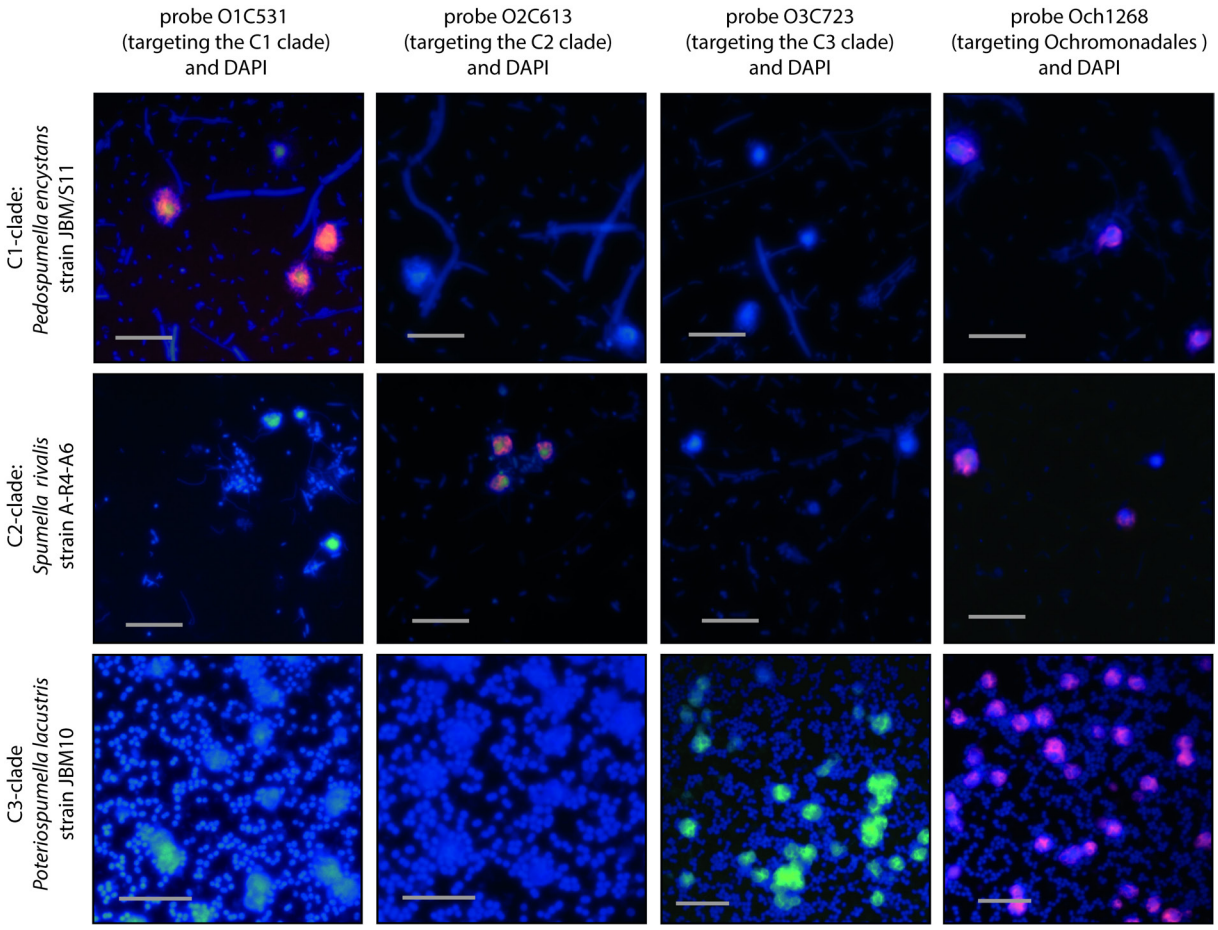


Fig. 1. Fluorescence overlay images (DAPI and Cy3 for probes O1C531, O2C613, and Och1268; DAPI and FITC for probe O3C723) using probes specific to the C1, C2, and C3 clade applied to *Pedospumella encystans* JBM/S11, *Spumella rivalis* A–R4–A6, *Poteriospumella lacustris* JBM10. Contrast was enhanced using the autocontrast function combined with a reduction of the blue channel range to 150 using Adobe Photoshop. All scale bars are 10 µm.

**Effect of temperature and salinization on competition in the mixed treatment**

Regarding competition between the three strains, the relative share of *Poteriospumella* increased in all treatments (Fig. 2) but the extent differed as well as the effect on the other species. The share of *Poteriospumella* slightly but significantly increased in the control ($p = 0.005$) but the associated decrease of both other taxa was not significant and could therefore not be attributed to any of the two species (*Pedospumella*: $p = 0.955$; *Spumella*: $p = 0.917$). In the temperature treatment, the share of *Poteriospumella* increased stronger ($p = 0.002$). This was largely compensated by a decrease of the share of *Spumella* ($p < 0.001$), while the decrease of the share of *Pedospumella* was not significant ($p = 0.103$). The salt treatment resulted in a significant increase of the share of *Poteriospumella* ($p = 0.001$), while it had no significant effect on both *Pedospumella* ($p = 0.969$) and *Spumella* ($p = 0.220$). The combination of both stressors (salt and temperature) also resulted in a significant increase of the relative share of *Poteriospumella* ($p = 0.002$). In contrast to the temperature treatment, the increased share of *Poteriospumella* was largely compensated by a decrease of the share of *Pedospumella* ($p = 0.004$) while the decrease of the share of *Spumella* was not significant ($p = 0.205$).

For all three species competition had a significant effect on growth (ANOVA; *Pedospumella*: $p = 0.001$; *Spumella*: $p = 0.008$; *Poteriospumella*: $p < 0.001$). Despite the significant overall effect of competition, results differed when comparing the effect of the treatments in single–strain experiments and in mixed cultures. For *Pedospumella* differences between single–strain and competition experiment were not significant (control: $p = 0.191$; temperature: $p = 0.564$; salt: $p = 0.080$; treatment combination: $p = 0.341$). For *Spumella* effects of competition were significant in the control ($p = 0.014$) and the combined treatment ($p = 0.005$) but not for temperature ($p = 0.093$) and salt ($p = 0.070$). For *Poteriospumella* the effect of competition was significant for the temperature treatment ($p < 0.001$) but not for the other treatments (control: $p = 0.06$; salt: $p = 0.47$; combination treatment: $p = 0.67$).

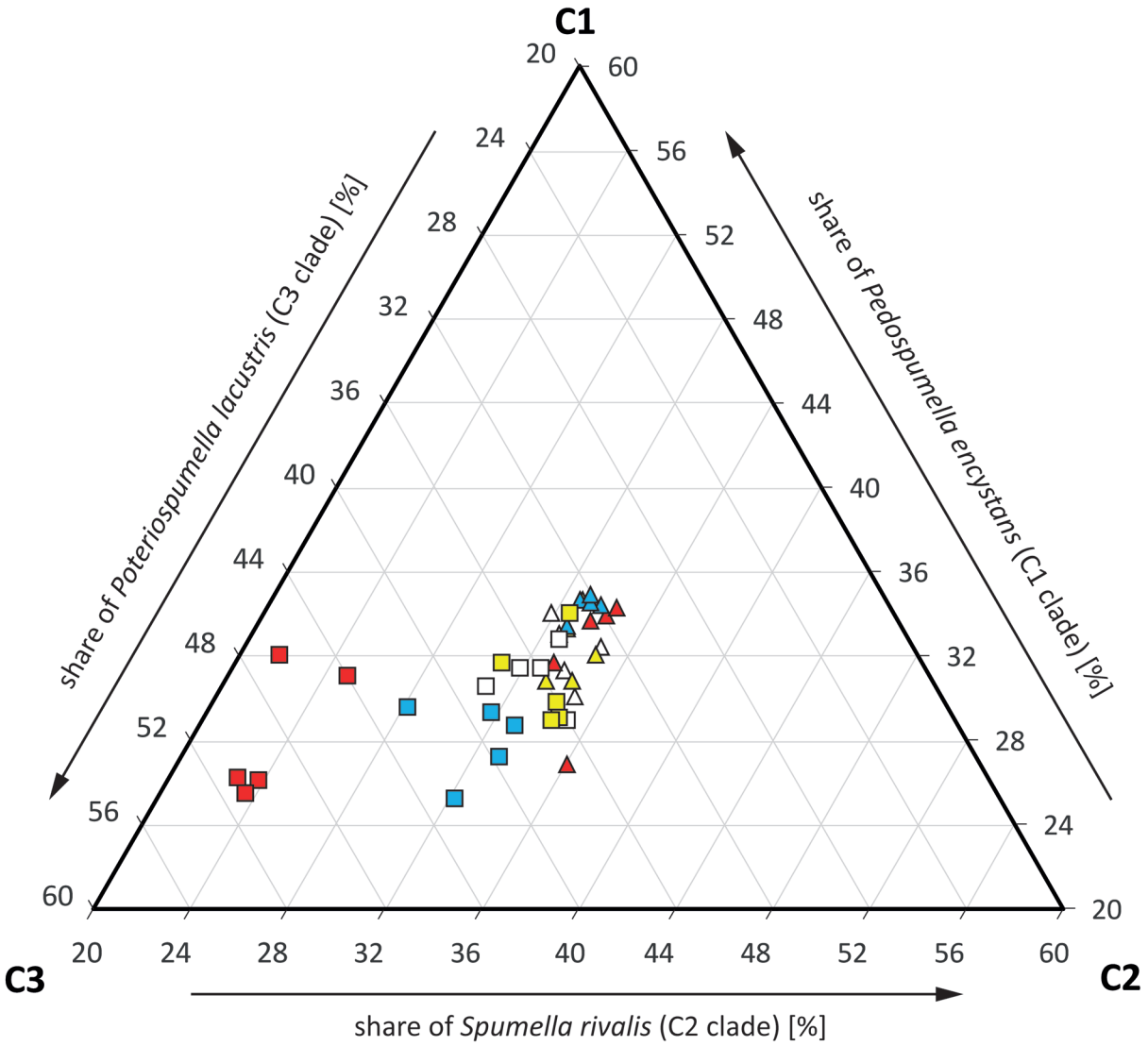


Fig. 2. Ternary plot showing the relative share of *Pedospumella encystans* (C1), *Spumella rivalis* (C2), and *Poteriospumella lacustris* (C3) in the competition experiments. Triangles refer to the community immediately before the application of stressors (day 3) and squares to day 6. White: control; red: temperature; yellow: salt; blue: combination of salt and temperature. Symbols of the same shape and colour represent replicates of the same treatment.

## Discussion

Colourless non–scaled chrysophytes are abundant in nearly any habitat, i.e., in soils, freshwater, and the marine habitats, and often make up a share of several percent of the total eukaryotic abundance (Foissner 1987; Sandgren 1988; Boenigk & Arndt 2002). As outlined above, this morphological form combines different distinct molecular lineages which are separated by taxa deviating in form and function such as e.g. the mixotrophic genus *Dinobryon*. Inferring the ecological role of this high molecular diversity and of the parallel evolution of this form requires a separation of these lineages based on molecular and morphological data. Quantifying different morphologically similar protist taxa both in the field and in competition experiments remains a tricky task. While morphologically such taxa can usually not be separated, molecular studies based on amplicon diversity do not provide absolute cell counts and the relative differences in read abundance are subjects to numerous biases including primer bias, different selectivity for sequences during processing and sequencing etc. (Medinger et al. 2010). The differentiation of cells by FISH offers a way for combining the advantage of molecular differentiating different cryptic taxa with absolute cell counts. We here focused on the order Ochromonadales as representatives of this order are often dominating in natural environments (Bock et al. 2022).

### Specificity of FISH–probes and suitability for competition experiments

We developed FISH–probes specific to clade C1 (comprising the genus *Pedospumella*), clade C2 (comprising

Table 3. Growth rates [day$^{-1}$] after stressor application (mean ± standard deviation). Negative growth rates correspond to mortality rates.

| | | ***Pedospumella* (C1)** | ***Spumella* (C2)** | ***Poteriospumella* (C3)** |
|---|---|---|---|---|
| Single–strain experiment | Control | 0.019 ± 0.052 | 0.164 ± 0.063 | –0.261 ± 0.099 |
| | Temperature | 0.061 ± 0.180 | 0.005 ± 0.068 | –0.254 ± 0.063 |
| | Salt | –0.041 ± 0.182 | –0.301 ± 0.034 | –0.344 ± 0.063 |
| | Combination | –0.075 ± 0.203 | –0.350 ± 0.033 | –0.256 ± 0.295 |
| Competition experiment | Control | –0.038 ± 0.039 | –0.053 ± 0.037 | –0.056 ± 0.034 |
| | Temperature | –0.014 ± 0.110 | –0.104 ± 0.076 | 0.139 ± 0.093 |
| | Salt | –0.366 ± 0.065 | –0.361 ± 0.034 | –0.379 ± 0.046 |
| | Combination | –0.220 ± 0.038 | –0.194 ± 0.064 | –0172 ± 0.048 |

the genus *Spumella* sensu stricto), and clade C3 (comprising the genus *Poteriospumella*). Tests of the probes with taxa affiliated to other clades prove the specificity of the probes (Fig. 1) and thus enable the separation of these clades in morphological community analyses. We successfully used the probes, following the standard FISH protocol (Glöckner et al. 1996), to specifically detect and quantify the differed clades in mixed cultures. The application of the probes on environmental samples, however, remains to be tested. Surprisingly, the predicted optimal formamid concentration differed notably from the optimal hybridization conditions that were determined experimentally (Fig. S1). The hybridization conditions resulting in highest fluorescence intensity, and consequently most efficient probe binding and clade–specific binding did not coincide with the predicted formamid concentration for all three clade–specific probes (Fig. S1). The general Ochromonadales probe did show high signal intensities when hybridized with the predicted formanid concentration in some but not all tested strains (data not shown). Signal intensities for Och1268, however, were stable when hybridized with a formamid concentration higher than predicted. Consequently, experimental testing enabled us to find the best hybridization conditions for the newly designed probes.

**Effects of stressors and food concentration on the growth of individual strains**

Data so far indicates that different members of the C1, C2, and C3 clades are ecophysiologically differently adapted (Boenigk et al. 2007; Boenigk 2008; Nolte et al. 2010). However, in our single–strain experiments, we found only low differences and significant effects only for *Spumella* exposed to salt. Before considering the effects of stressors and competition, a word of caution is needed with respect to the food concentrations applied in our experiments: all experiments were run at low food and potentially food–limiting conditions in order to mimic natural conditions and to focus on food–limiting conditions where competition should be expected to be more pronounced. Therefore, even slight changes in bacteria abundances affect growth rates and these differences need to be considered when comparing results from strains in the single strain experiments. We did not find any significant effects of the treatments on bacterial growth. Bacterial abundance was significantly higher ($p < 0.001$) in *Spumella* cultures that were exposed to increased salinity compared to the control, however, it is likely that this resulted from the reduced growth rate of *Spumella* during salt treatment. *Poteriospumella* generally proved to be the fastest–growing and potentially best–adapted strain to the experimental conditions. However, due to the stronger growth of this strain the food concentration was more strongly reduced, i.e., was lowest in the single–strain experiments with *Poteriospumella* ($2.8±3.5×10^6$ bacteria.ml$^{-1}$ on day 3), followed by the mixed community experiment ($5.79±0.6×10^6$ bacteria.ml$^{-1}$ on day 3) and the single–strain experiments using *Spumella* ($6.47±0.4×10^6$ bacteria.ml$^{-1}$ on day 3) and *Pedospumella* ($6.0±0.3×10^6$ bacteria.ml$^{-1}$ on day 3). In the direct comparison, this is reflected by lower growth rates of *Poteriospumella* in the single–strain experiments while this species proved to be best–adapted to the experimental conditions as reflected by the stronger growth during acclimatization (data not shown) and the higher growth rates in the competition experiment.

Corresponding to the expectations, *Poteriospumella* performed best under the experimental conditions and particularly profited from the temperature increase in the competition experiment, while the effect of temperature treatment on growth rates for the other taxa was less pronounced. This is in accordance with previous laboratory growth experiments that showed that *Poteriospumella* tolerated higher temperatures than members of the two other clades (Boenigk et al. 2007) and thus suggested a competitive advantage of the C3 clade at high temperatures. Except for strains originating from Antarctica (Boenigk et al. 2007) differences between members of the two other clades were not very pronounced but may

hint to a higher competitive strength of the C1 clade at low temperatures as compared to the C2 clade. In a seasonal study on Lake Fuschlsee the relative abundance of members of the C3 clade (*Poteriospumella* and allies) was highest in summer while the relative abundance of members of the C2 clade (*Spumella* and allies) was highest in the colder seasons (Nolte et al. 2010). The C1 clade (*Pedospumella* and allies) showed an intermediate pattern in this study. Regarding the C1 and C2 clades the results from environmental data and lab experiments thus slightly deviate. Although, a study focusing on the maximal temperature tolerated (Boenigk 2008) indicated a similar pattern as the environmental data, i.e., lowest temperature tolerance for the C2 clade (with some strains showing exceptions), followed by the C1 clade, and highest temperature tolerance for the C3 clade. However, this latter study contributes only data on the maximum temperatures survived but no data on growth rates of the distinct strains. In our experiments, the temperature treatment had no significant effect on growth rates for *Spumella* (C2) and *Pedospumella* (C1) in single or mixed cultures. As outlined above, taxa affiliated with the C2 and C1 clades seem to be adapted to lower or moderate temperatures, respectively.

Consequently, *Spumella* and *Pedospumella* did not profit from the temperature treatment but were able to tolerate it. Additionally, food concentrations in our experiments may have been too low to permit significantly increased growth rates for either of the two taxa (Boenigk et al. 2007). Competition experiments have so far not been conducted, the potential temperature dependence of the competitive strength can only be inferred from seasonal distribution data, which may, however, be affected by other factors. In our competition experiment increased share of *Poteriospumella* was largely compensated by a decreased share of *Spumella* which further implies a competitive advantage of the C3 clade and a competitive disadvantage of the C2 clade at high temperature.

In our study, salinity had a similar effect for all three species. In contrast, literature suggests strongly deviating salinity tolerances between protist taxa: while some taxa have been reported to tolerate freshwater as well as marine conditions, other taxa seem to have quite restricted tolerances (Finley 1930; Ekelund 2005). Brackish and at least some marine species tolerate a wide range of salinities while freshwater species seem more restricted to low salinities: some freshwater protist taxa show decreased growth rates or become non–viable already at concentrations around salinities of 1 $g.l^{-1}$ (e.g., Finley 1930; Lam et al. 2019), other taxa are only negatively affected at salinities above 5 $g.l^{-1}$ (Finley 1930; Zuo et al. 2014; Park et al. 2020). However, for some freshwater species exceptional tolerance ranges have been reported: for some freshwater bodonid taxa and some colourless non–scaled freshwater chrysophytes tolerance of marine conditions has been reported (Finley 1930). Our experiments showed less variability in salt tolerance between the investigated taxa as expected: in single strain cultures salinity of 2 $g.l^{-1}$ negatively affected the growth of *Spumella* (C2) but had no significant effect on growth rates for *Poteriospumella* (C3) or *Pedospumella* (C1) and in mixed cultures the effect on growth rates was similar for all investigated taxa. For distinct strains of colourless non–scaled chrysophytes salt tolerance has been tested but data so far are largely restricted to the maximum (short–term) tolerance of strains (Boenigk 2008). Salt tolerance of members of the C3 clade (including *Poteriospumella*) seem to be rather similar with a maximum around 5 $g.l^{-1}$ while the tolerance varied considerably (i.e., between 1 and 7 $g.l^{-1}$) for different members of the C2 and C1 clade. Bock et al. (2022) inferred from the distribution pattern in a pan–European data set that both *Pedospumella* and *Poteriospumella* were among the taxa with a wide tolerance to conductivity but the sampling site in this study covered only the lower of salinities. Ekelund (2005) suggested that soil protists may be more salt–tolerant than freshwater forms. But in contrast to our expectation pattern of soil–borne *Pedospumella* did not deviate from the water–borne taxa (*Poteriospumella lacustris* and *Spumella rivalis*). Comparative data on growth rate are not available for these strains and a potential niche separation with respect to salt concentration is therefore not clear. However, growth rates seem to be severely affected already at salt concentrations far below the maximum concentration survived (based on cultivation experience; unpublished data). Irrespective of a potential survival at high salinities we therefore expected a negative effect on growth rate already at moderate salt concentration. While older literature suggests a high salinity tolerance for many species (e.g., Finley 1930) more recent studies reported an increasing number of species with limited salinity tolerances (cf. Mylnikov 1983; Ekelund 2005). Several non–exclusive aspects may contribute to these findings: firstly, during progress in protistology the number of cultivated taxa and strains strongly increased, early isolates were often affiliated with 'ubiquitous' species and early cultivation protocols may have selected for taxa with broader tolerances. Secondly, evidence from environmental samples as well as evidence–based on mixed cultures (such as the study by Finley 1930) may contain several cryptic strains with different ecophysiological requirements as species identities in such mixed samples were hard to prove in early studies which rely on morphological inspection only. As most of these common species are now known to be highly diverse it is indeed likely that a high salinity tolerance inferred from (morphological) field studies erroneously concluded on a high salinity tolerance of the respective species while in fact, the distinct strains (and possibly species) had a much narrower (but different) salinity tolerance. Thirdly, strains from culture collections may show low salinity tolerances but may be able to

gradually acclimatize to higher salinities (Mylnikov 1983). In sum, literature findings over time may reflect to a certain extent the improving methodologies in isolation and cultivation as well as in the differentiation of taxa and strains. But even considering such potential uncertainties regarding some of the early studies the variation in salinity tolerance between taxa seems to be remarkably high. Many protist taxa originating from soil and freshwater survive salinities of 5 $g.l^{-1}$ or even of 10 $g.l^{-1}$ (Mylnikov 1983; Ekelund 2005). However, salinity tolerances of strains affiliated with the same species may considerably deviate and thus salinity may be an efficient reproductive barrier possibly questioning current species delimitations (Ekelund 2005).

Taken together the increasing growth rate in response to a sudden increase in temperature corresponded largely to our expectations while the similar effect of salinization for all three taxa was unexpected. Presumably, the applied salt concentration was in a range which effectively prevented growth but was not lethal. When both stressors were applied together, growth rates were higher as in the control and in the salt treatment but lower as in the temperature treatment. Temperature therefore is likely to stimulate growth but this effect is impaired by salinization.

**Effects of stressors on competitive strength**

Competition experiments using different lineages of colourless non–scaled chrysophytes have so far not been performed or were restricted to analyzing the relative shifts in amplicon abundances (e.g., Nolte et al. 2010). In lab experiments, the difficulties in separating cryptic taxa can leave unclear whether the presence of other taxa affects the observed pattern or not. Besides, the challenges of performing experiments at near–natural food concentrations further hamper such analyses. Both seems plausible: on the one hand, the investigated taxa are morphologically similar and most likely their ecological niches overlap to a considerable extent. Therefore, adverse effects due to competition might be expected. On the other hand, since the formulation of the 'paradox of the plankton' (Hutchinson 1961) numerous mechanisms for maintaining plankton diversity have been proposed (Roy & Chattopadhy 2007).

Our experiments provided evidence that the different cryptic taxa responded differently to the stressors and responded differently in competition as compared to single–strain experiments. It is likely that *Poteriospumella* can deal best with low food concentrations and thus outcompetes the two other taxa in particular at higher temperatures. At lower temperatures, i.e., in the control and the salt treatment, this relative advantage was less pronounced but still significant. Our results therefore imply that competition is a relevant issue but that its strength depends on the abiotic conditions. The different taxa responded differently to stressors thus shifting the relative abundance of these taxa in response to environmental change. Our results therefore suggest that the seeming persistence of common morphological flagellate types in natural communities masks a turn–over of cryptic taxa differently adapted to abiotic factors. Such a cryptic species turn–over affects food web stability: many aspects of predator–prey interactions such as food size preferences and feeding mechanisms largely correlate with morphology (Boenigk & Arndt 2000a, b) while adaptations to many abiotic environmental factors do not. Cryptic species diversity and turn–over thus stabilize trophic interactions and food web architecture despite a potentially high taxon turn–over due to environmental change or the (sudden) occurrence of stressors.

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## References

Amann, R.I.; Krumholz, L. & Stahl, D.A. (1990): Fluorescent–oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. – J. Bacteriol. 172: 762–770.

Arnott, S.E.; Fugère, V.; Symons, C.C.; Melles, S.J.; Beisner, B.E.; Cañedo–Argüelles, M.; Hébert, M.–P.; Brentrup, J.A.; Downing, A.L.; Gray, D.K.; Greco, D.; Hintz, W.D.; McClymont, A.; Relyea, R.A.; Rusak, J.A.; Searle, C.L.; Astorg, L.; Baker, H.K.; Ersoy, Z.; Espinosa, C.; Franceschini, J.M.; Giorgio, A.T.; Gobeler, N; Hassal, E.; Huynh, M.; Hylander, S.; Jonasen, K.L.; Kirkwood, A.; Langenheder, S.; Langval, O.; Laudon, H.; Lind, L.; Lundgren, M.; Moffett, E.R.; Proia, L.; Schuler, M.S.; Shurin, J.B.; Steiner, C.F.; Striebel, M.; Thibodeau, S.; Cordero, P.U.; Vendrell-Puigmitja, L.; Weyhenmeyer, G.A & Derry, A.M. (2022): Widespread variation in salt tolerance within freshwater zooplankton species reduces the predictability of community–level salt tolerance. – Limnol. Oceanogr. Letters: 10277. DOI: 10.1002/lol2.10277

Bock, C.; Olefeld, J.L.; Vogt, J.C.; Albach, D.C. & Boenigk, J. (2022): Phylogenetic and functional diversity of Chrysophyceae in inland waters. – Org. Div. Evol. 22: 327–341.

Boenigk, J. (2008): Nanoflagellates: Functional groups and intraspecific variation. – Denisia 23: 331–335.

Boenigk, J. & Arndt, H. (2000): Particle handling during interception feeding by four species of heterotrophic nanoflagellates. – J. Euk. Mic. 47: 350–358.

Boenigk, J. & Arndt, H. (2000): Comparative studies on the feeding behavior of two heterotrophic nanoflagellates: the filter–feeding choanoflagellate *Monosiga ovata* and the raptorial–feeding kinetoplastid *Rhynchomonas nasuta*. – Aquat. Microb. Ecol. 22: 243–249.

Boenigk, J. & Arndt, H. (2002): Bacterivory by heterotrophic flagellates: community structure and feeding strategies. – Antonie Van Leeuwenhoek 81: 465–480.

Boenigk, J.; Jost, S.; Stoeck, T. & Garstecki, T. (2007): Differential thermal adaptation of clonal strains of a protist morphospecies originating from different climatic zones. – Environ. Microbiol. 9: 593–602.

Boenigk, J.; Pfandl, K.; Stadler, P. & Chatzinotas, A. (2005): High diversity of the "*Spumella*–like" flagellates: An investigation based on the SSU rRNA gene sequences of isolates from habitats located in six different geographic regions. – Environ. Microbiol. 7: 685–697.

Boenigk, J.; Pfandl, K. & Hansen, P.J. (2006): Exploring strategies for nanoflagellates living in a 'wet desert'. – Aquat. Microb. Ecol. 44: 71–83.

Cañedo–Argüelles, M.; Kefford, B.J.; Piscart, C.; Prat, N.; Schäfer, R.B. & Schulz, C.–J. (2013): Salinisation of rivers: an urgent ecological issue. – Environ. Poll. 173: 157–167.

Carrick, H.J. & Fahnenstiel, G.L. (1989): Biomass, size structure, and composition of phototrophic and heterotrophic nanoflagellate communities in Lake Huron and Michigan. – Can. J. Fish. Aquat. Sci. 46: 1922–1928.

Castillo, A.M.; Sharpe, D.M.T.; Ghalambor, C.K. & De León, L.F. (2018): Exploring the effects of salinization on trophic diversity in freshwater ecosystems: a quantitative review. – Hydrobiologia 807: 1–17.

Daims, H.; Lücker, S. & Wagner, M. (2006): Daime, a novel image analysis program for microbial ecology and biofilm research. – Environ. Microbiol. 8: 200–213.

DeLong, E.F.; Wickham, G.S. & Pace, N.R. (1989): Phylogenetic stains: ribosomal RNA based probes for the identification of single cells. – Science 243: 1360–1363.

Ekelund, F. (2005): Tolerance of soil flagellates to increased NaCl levels. J. Eukaryot. Microbiol. 49: 324–328.

Fenchel, T. (1986): Protozoan filter feeding. – Prog. Protistol. 1: 65–113.

Fernandez–Leborans, G. & Fernandez–Fernandez, D. (2002): Protist functional groups in a sublittoral estuarine epibenthic area. – Estuaries 25: 382–392.

Finlay, B.J. & Esteban, G.F. (1998): Freshwater protozoa: biodiversity and ecological function. – Biodivers. Conservation 7: 1163–1186.

Finley, H. E. (1930): Toleration of freshwater protozoa to increased salinity. – Ecology 11: 337–347.

Foissner, W. (1987): Soil protozoa: fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators, and guide to the literature. – Prog. Protistol. 2: 69–212.

Glöckner, F.O.; Amann, R.; Alfreider, A.; Pernthaler, J.; Psenner, R.; Trebesius, K. & Schleifer, K.–H. (1996): An in situ hybridization protocol for detection and identification of planktonic bacteria. – Syst. Appl. Microbiol. 19: 403–406.

Graupner, N.; Jensen, M.; Bock, C.; Marks, S.; Rahmann, S.; Beisser, D. & Boenigk, J. (2018): Evolution of heterotrophy in chrysophytes as reflected by comparative transcriptomics. – FEMS Microbiol. Ecol 94: fiy039. DOI: 10.1093/femsec/fiy039

Grossmann, L.; Bock, C.; Schweikert, M. & Boenigk, J. (2016): Small but manifold – hidden diversity in '*Spumella*–like flagellates'. – J. Euk. Mic. 63: 419–439.

Hahn, M.W.; Lundsdorf, H.; Wu, Q.L.; Schauer, M.; Hofle, M.G.; Boenigk, J. & Stadler, P. (2003): Isolation of novel ultramicrobacteria classified as actinobacteria from five freshwater habitats in Europe and Asia. – Appl. Environ. Microb. 69: 1442–1451.

Hao, B.; Roejkjaer, A.F.; Wu, H.; Jeppesen, E.; Li, W. (2018): Responses of primary producers in shallow lakes to elevated temperature: a mesocosm experiment during the growing season of *Potamogeton crispus*. – Aquat. Sci. 80: 34.

Herczeg, A.L.; Dogramaci, S.S.; Leaney, F.W. (2001): Origin of dissolved salts in a large, semi–arid groundwater system: Murray Basin, Australia. – Mar. Freshwater Res. 52: 41–52.

Hutchinson, G.E. (1961): The paradox of the plankton. – Am Naturalist 95: 137–145.

Ipcc (2013): Climate change 2013: the physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. – 1535 pp., Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jones, D.K.; Mattes, B.M.; Hintz, W.D.; Schuler, M.S.; Stoler, A.B.; Lind, L.A.; Cooper, R.O. & Relyea, R.A. (2017): Investigation of road salts and biotic stressors on freshwater wetland communities. – Environ. Poll. 221: 159–167.

Jürgens, K. & Matz, C. (2002): Predation as a shaping force for the phenotypic and genotypic composition of planktonic bacteria. – Anton. Leeuw. Int. J. G. 81: 413–434.

Kamakura, S.; Ashworth, M.P.; Yamada, K.; Mikami, D.; Kobayashi, A.; Idei, M. & Sato, S. (2022): Morphological plasticity in response to salinity change in the euryhaline diatom *Pleurosira laevis* (Bacillariophyta). – J. Phycol. 58: 631–642.

Kristiansen, J. & Preisig, H.R. (2001): Encyclopedia of chrysophyte genera. – 260 pp., J. Cramer, Berlin.

Kristiansen, J. & Škaloud, P. (2017): Chrysophyta. In: Archibald, J.M.; Simpson, A.G.B. & Slamovits, C.H. (eds.): Handbook of the Protists. – pp. 331–366, Springer.

Lam, C.; He, L. & Marciano–Cabral, F. (2019): The effect of different environmental conditions on the viability of *Naegleria fowleri* amoebae. – J. Eukaryot. Microbiol. 66: 752–756.

Latorre, F.; Deutschmann, I.M.; Labarre, A. & Logares, R. (2021): Niche adaptation promoted the evolutionary diversification of tiny ocean predators. – PNAS 118: e2020955118.

Laybourn–Parry, J. & Parry, J. (2000): Flagellates and the microbial loop. – In: Leadbeater, B.S.C. & Green, J.C. (eds): The Flagellates. – pp. 216–239, Taylor & Francis, London.

Li, J.; Ye, W.; Wei, D.; Ngo, H.H.; Guo, W.; Qiao, Y.; Xu, W.; Du, B. & Wie, Q. (2018): System performance and microbial community succession in a partial nitrification biofilm reactor in response to salinity stress. – Bioresource Tech. 270: 512–518.

Lowe, C.D.; Day, A.; Kemp, S.J. & Montagnes, D.J.S. (2005): There are high levels of functional and genetic diversity in *Oxyrrhis marina*. – J. Eukaryot. Microbiol. 52: 250–257.

Mylnikov, A. P. (1983): Adaptation of zooflagellates to higher salinity. – Biol. Vnutr. Vod. Inform. Bull. 61: 21–24.

Nolte, V.; Pandey, R.V.; Jost, S.; Medinger, R.; Otten-

waelder, B.; Boenigk, J. & Schlötterer, C. (2010): Contrasting seasonal niche separation between rare and abundant taxa conceals the extend of protist diversity. – Mol. Ecol. 19: 2908–2915.

Nuy, J.K.; Lange, A.; Beermann, A.J.; Jensen, M.; Elbrecht, V.; Röhl, O.; Persoh, D.; Begerow, D.; Leese, F. & Boenigk, J. (2018): Responses of stream microbes to multiple anthropogenic stressors in a mesocosm study. – Sci. Tot. Environ. 633: 1287–1301.

Park, M.–H.; Park, C.–H.; Sim, Y.B. & Hwang, S.–J. (2020): Response of *Scenedesmus quadricauda* (Chlorophyceae) to salt stress considering nutrient enrichment and intracellular proline accumulation. – Int. J. Environ. Res. Pub. Health 17: 3624.

Pfandl, K.; Chatzinotas, A.; Dyal, P. & Boenigk, J. (2009): SSU rRNA gene variation resolves population heterogeneity and ecophysiological differentiation within a morphospecies (Stramenopiles,Chrysophyceae). – Limnol. Oceanogr. 54: 171–181.

Pietsch, T.; Nitsche, F. & Arndt, H. (2022): High molecular diversity in the functional group of small bacterivorous non–scaled chrysomonad flagellates. Eur. J. Protist. 86: 125915.

Polazzo, F.; Roth, S.K.; Hermann, M.; Mangold–Döring, A.; Rico, A.; Sobek, A.; Van den Brink, P.J. & Jackson, M.C. (2021): Combined effects of heatwaves and micropollutants on freshwater ecosystems: Towards an integrated assessment of extreme events in multiple stressors research. – Global Change Biol. 28: 1248–1267.

Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T. & Glöckner, F.O. (2013): The SILVA ribosomal RNA gene database project: improved data processing and web–based tools. – Nuc. Acids Res. 41: 590–596.

Rasconi, S.; Gall, A.; Winter, K. & Kainz, M.J. (2015) Increasing water temperature triggers dominance of small freshwater plankton. – PLoS One 10: e0140449.

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org.

Robinson, D.; Hayes, A. & Couch, S. (2022). broom: convert statistical objects into tidy tibbles. R package version 1.0.0. URL: https://CRAN.R–project.org/package=broom.

Roy, S. & Chattopadhyay, J. (2007): Towards a resolution of 'the paradox of the plankton': A brief overview of the proposed mechanisms. – Ecol. Complexity 4: 26–33.

Saint–Beat, B.; Baird, D.; Asmus, H.; Asmus, R.; Bacher, C.; Pacella, S.R.; Johnson, G.A.; David, V.; Vezina, A.F. & Niquil, N. (2015): Trophic networks: how do theories link ecosystem structure and functioning to stability properties? A review. – Ecol. Ind. 52: 458–471.

Sanders, R.W.; Caron, D.A. & Berninger, U.–G. (1992): Relationships between bacteria and heterotrophic nanoplankton in marine and fresh water: an inter–ecosystem comparison. – Mar. Ecol. Prog. Ser. 86: 1–14.

Sandgren, C.D. (1988): The ecology of chrysophyte flagellates: their growth and perenntion strategies as freshwater phytoplankton. In: Sandgren, C.D. (ed): Growth and reproductive strategies of freshwater phytoplankton. – pp. 9–104, Cambridge University Press, Cambridge.

Sherr, E.B. & Sherr, B.F. (2002): Significant of predation by protists in aquatic microbial food webs. – Antonie Van Leewenhoek 81: 293–308.

Shetty, P.; Gitau, M.M. & Maróti, G. (2019): Salinity stress responses and adaptation mechanisms in eukaryotic green microalgae. – Cells 8: 1657.

Sun, X. & Arnott, S.E. (2022):. Interactive effects of increased salinity and heatwaves on freshwater zooplankton communities in simultaneous and sequential treatments. – Freshw. Biol. 67: 1604–1617.

Szymczak, A.; Ryo, M.; Roy, J. & Rillig, M.C. (2020): Diversity of growth responses of soil saprobic fungi to recurring heat events. – Front. Microbiol. 11: 1–8.

Thomson, A.H. & Manoylov, K.M. (2019): Algal community dynamics within the Savannah River estuary, Georgia under anthropogenic stress. – Estuar. Coasts 42: 1459–1474.

von Alvensleben, N.; Magnusson, M. & Heimann, K. (2016): Salinity tolerance of four freshwater microalgal species and the effects of salinity and nutrient limitation on biochemical profiles. – J. Appl. Phycol. 28: 861–876.

van Meerbeek, K.; Jucker, T. & Svenning, J.C. (2021): Unifying the concepts of stability and resilience in ecology. – J. Ecol. 109: 3114–3132.

Wagner, M.; Assmus, B.; Hartmann, A.; Hutzler, P.; & Amann, R. (1994): In situ analysis of microbial consortia in activated sludge using fluorescently labelled, rRNA–targeted oligonucleotide probes and confocal scanning laser microscopy. – J. Microsc. 176: 181–187.

Wickham, H.; François, R.; Henry, L. & Müller, K. (2022): dplyr: A grammar of data manipulation. R package version 1.0.9, URL: https://CRAN.R–project.org/package=dplyr.

Wright, E.S.; Yilmaz, L.S.; Corcoran, A.M.; Ökten, H.E. & Noguera, D.R. (2014): Automated design of probes for rRNA–targeted fluorescence in situ hybridization reveals the advantages of using dual probes for accurate identification. – Appl. Environ. Microbiol. 80: 5124–5133.

Yilmaz, L.S. & Noguera, D.R. (2007): Development of thermodynamic models for simulating probe dissociation profiles in fluorescence in situ hybridization. – Biotech. Bioengineering 96: 349–363.

Zuo, Z.; Chen, Z.; Zhu, Y.; Bai, Y. & Wang, Y. (2014): Effects of NaCl and $Na_2CO_3$ stresses on photosynthetic ability of *Chlamydomonas reinhardtii*. – Biologia 69: 1314–1322.

Supplementary material

The following supplementary material is available for this article:

Fig. S1. Signal intensity as a function of formamide concentrations. The grey dashed line indicates the calculated optimal formamide concentration (mathFISH) and the black dashed line the selected formamide concentration in our protocols based on signal intensity and probe specificity.

This material is available as part of the online article (http://fottea.czechphycology.cz/contents)


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