Impact of salinity and growth phase on alkenone distributions in coastal haptophytes

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ABSTRACT

Batch cultures of *Isochrysis galbana* (strain CCMP 1323) and *Chrysotila lamellosa* (strain CCMP 1307) were grown at salinity values of ca. 10 to ca. 35 and the alkenone distributions determined for different growth phases. $U_{37}^{K'}$ values decreased slightly with salinity for *C. lamellosa* but were largely unaffected for *I. galbana* except during the decline phase. The values decreased with incubation time in both species. The proportion of $C_{37:4}$, used as a proxy for salinity, increased in both species at 0.16–0.20 % per salinity unit, except during the stationary phase for *I. galbana*. $C_{37:4}$ was much more abundant in *C. lamellosa* (30–44 %) than in *I. galbana* (4–12 %). Although our results suggest that salinity has a direct effect on alkenone distributions, growth phase and species composition should also have a marked impact, complicating the use of alkenone distributions as a proxy for salinity in the marine environment.

1. Introduction

Long chain alkenones are produced exclusively by a few species of marine (Volkman et al., 1980; Marlowe et al., 1984; Volkman et al., 1995) and lacustrine (e.g. Coolen et al., 2004; D'Andrea et al., 2006; Theroux et al., 2010) haptophyte algae and have been extensively studied because of the widespread application of the unsaturation indices U^{K}_{37} (Brassell et al., 1986) and $U^{K'}_{37}$ (Prahl and Wakenham, 1987) as palaeotemperature proxies. Besides temperature, alkenone distributions also vary with other factors including species (e.g. Marlowe et al., 1984), growth phase (Conte et al., 1998) and salinity (Ono et al., 2012).

There is interest in using the distributions as a proxy for salinity. An increased relative abundance of the $C_{37:4}$ alkenone as a proportion of the total abundance of C_{37} alkenones (%$C_{37:4}$) has been observed with decreased salinity in marine (Rosell-Melé, 1998; Schulz et al., 2000; Blanz et al., 2005) and lacustrine (Liu et al., 2008; 2011) environments and has been suggested as a salinity proxy. However, sometimes none or an opposite relationship with salinity has been observed (Sikes and Sicre, 2002; Theroux et al., 2010; Toney et al., 2010; Schwab and Sachs, 2011), possibly because %$C_{37:4}$ varies in different alkenone-producing species and strains (Marlowe et al., 1984; Conte et al., 1995). Interestingly, however, only one study has examined the direct effect of salinity on alkenone distributions in culture (Ono et al., 2012).

We have therefore grown batch cultures of the major coastal alkenone-producing haptophytes, *Isochrysis galbana* and *Chrysotila lamellosa*, which are phylogenically related to lacustrine alkenone producers (Coolen et al., 2004; Theroux et al., 2010), and studied their alkenone distributions at different salinity values and at different growth phases.

2. Material and methods

Batch cultures of *I. galbana* (strain CCMP 1323) and *C. lamellosa* (strain CCMP 1307) were grown in triplicate in f/2 media (Guillard, 1975) at six salinity values between 10.2 and 35.5. Cultures (600 ml) were grown at 15 °C receiving 60 μmol photons $m^{-2} s^{-1}$, with a light:dark cycle of 16:8 h. Cell density was monitored using flow cytometry (Accuri C6). *I. galbana* cell material was collected using filtration onto precombusted 0.7 μm GF/F filters, after 10 (exponential growth phase), 27 or 28 (stationary phase) and 69, 75, 83, or 89 days (decline phase). *C. lamellosa* was collected after 14 or 15 (early stationary phase) and 35 days (late stationary phase). After addition of an *n*-nonadecan-10-one internal standard, freeze-dried filters were extracted with 2:1 v/v dichloromethane (DCM)/MeOH (4 × 10 ml; 4 × 5 min sonication). The extract was separated using an Al_2O_3 column into three fractions: apolar (eluted with 9:1 v/v hexane/DCM), alkenone (1:1 v/v hexane/DCM) and polar (1:1 v/v DCM/MeOH). The alkenone fraction was analysed using gas chromatography (GC) with an Agilent 6890 instrument equipped with an Agilent CP-Sil 5 CB column (50 m × 0.32 mm i.d.; 0.12 μm film thickness). The temperature program was: 70 to 200 °C at 20 °C min^{-1}, then at 3 °C min^{-1} to 320 °C (held 25 min). Components were identified using GC–mass spectrometry (GC–MS) with an Agilent 7890A GC instrument and Agilent 5975C VL mass selective detector (MSD).

3. Results and discussion

The mean $U^{K'}_{37}$ and %$C_{37:4}$ of alkenones for *I. galbana* and *C. lamellosa* grown in triplicate over a range of salinity and sampled during different growth phases are displayed in Table 1 and Fig. 1.

Mean $U^{K'}_{37}$ for *I. galbana* was 0.11 ± 0.01 in the exponential phase, decreased to 0.07 ± 0.01

in the stationary phase and again to 0.03 ± 0.01 during the decline phase (Fig. 1a). There was no significant relationship between $U_{37}^{K'}$ and salinity in the exponential ($p = 0.08$) and stationary ($p = 0.19$) growth phases, in accord with a recent study (Ono et al., 2012). However, there was a significant decrease of 5×10^{-4} per unit salinity (S^{-1}) in the decline phase ($R^2 = 0.75$). Like *I. galbana*, $U_{37}^{K'}$ for *C. lamellosa* decreased with incubation time from 0.08 ± 0.01 in the early stationary phase to 0.07 ± < 0.01 in the late stationary phase (paired one-tailed t-test, $p < 0.001$; Fig. 1b; cf. Rontani et al., 2004). In contrast to results from *I. galbana*, $U_{37}^{K'}$ values for *C. lamellosa* decreased with increasing salinity during both the early and late stationary phase by $8 \times 10^{-4}\ S^{-1}$ ($R^2 = 0.66$) and $2 \times 10^{-4}\ S^{-1}$ ($R^2 = 0.26$; $p = 0.03$), respectively.

In *I. galbana*, $\%C_{37:4}$ increased with salinity by $0.19\%\ S^{-1}$ ($R^2 = 0.62$; Fig. 1c) and $0.20\%\ S^{-1}$ ($R^2 = 0.86$) in the exponential phase and decline phase, respectively, but there was no significant relationship during the stationary phase ($p = 0.49$). There was little change in $\%C_{37:4}$ between the exponential and decline phases (8 ± 2% and 7 ± 2%, respectively; paired two-tailed t-test, $p = 0.19$), although $\%C_{37:4}$ was lower (5 ± 1%) for the stationary phase. In *C. lamellosa*, $\%C_{37:4}$ increased from 35 ± 2% to 40 ± 2% from the early to late stationary phase and also increased with salinity in both the early ($0.16\%\ S^{-1}$; $R^2 = 0.44$; Fig. 1d) and late ($0.18\%\ S^{-1}$; $R^2 = 0.62$) stationary phase.

Our results show that both growth phase and salinity substantially affect alkenone distributions in coastal haptophytes. The $U_{37}^{K'}$ values remained constant or decreased by $< 1 \times 10^{-3}\ S^{-1}$ (equivalent to $< 0.03\ ^\circ C\ S^{-1}$ using the Müller et al., 1998 calibration), so changes in salinity are unlikely to affect temperature reconstruction. In contrast, growth phase had a large impact on $U_{37}^{K'}$ values, with a reduction of up to 0.08 observed after the exponential phase (cf. Prahl et al., 2003). Importantly, we have shown direct evidence for a physiological response of $C_{37:4}$ to changing salinity in individual algal strains: there was a consistent increase in $\%C_{37:4}$ with salinity for both species in most growth phases. The biochemical mechanisms behind this response are uncertain but may be related to an adaptation in buoyancy regulated by lipid bodies (cf. Pond, 2012). The increase in $\%C_{37:4}$ with salinity in our cultures contrasts with the general observation that $\%C_{37:4}$ decreases at higher salinity (e.g. Rosell-Melé, 1998), although increasing $\%C_{37:4}$ with salinity has been observed for Chesapeake Bay (Schwab and Sachs, 2011). These apparently contradictory observations might be reconciled by considering the large difference between $\%C_{37:4}$ for the two coastal species reported here (*C. lamellosa*: 30–44%; *I. galbana*: 4–12%) as well as $\%C_{37:4}$ for other marine species (*Emiliania huxleyi*, < 6%; *Gephyrocapsa oceanica*, < 1%; Conte et al., 1995). Changes in species composition may account for a decreasing trend in $\%C_{37:4}$ with salinity, whereas a relatively constant species composition may account for an increasing trend (Fig. 1c and d). Meanwhile, our results also show that the effect of growth phase on $\%C_{37:4}$ can be greater than that of salinity. Therefore, unless the species composition and growth phase of alkenone producers are known, the use of alkenone distributions as a salinity proxy is uncertain.

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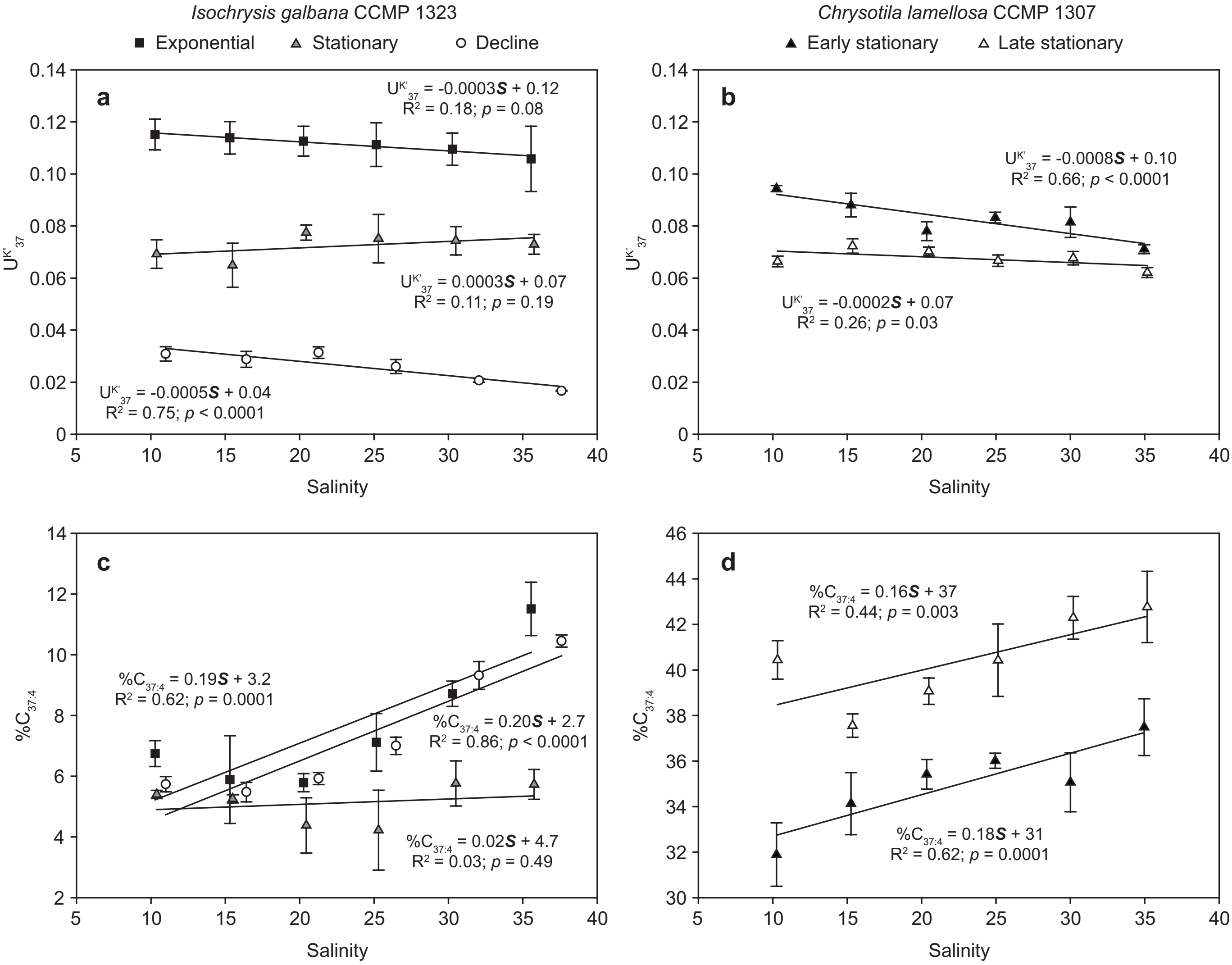

Fig. 1. $U^{K'}_{37}$ (a) and %$C_{37:4}$ (c; exponential phase data are from M'Boule et al., unpublished results) vs. salinity for *I. galbana*, and $U^{K'}_{37}$ (b) and %$C_{37:4}$ (d) vs. salinity for *C. lamellosa*. Values are the mean of triplicate cultures; error bars are ± 1σ.

Table 1

Results from *I. galbana* and *C. lamellosa* grown at different salinity at 15°C ($U_{37}^{K'}$ values are the mean ± 1σ of triplicate cultures; ± 1σ for growth rate and $U_{37}^{K'}$ are $0.01 \le \sigma \le 0.02$ and $\sigma \le 0.01$, respectively; %$C_{37:4}$ data for *I. galbana* at 10 days are from M'Boule et al., unpublished results).

Salinity	Incubation time (d)	Growth rate (d^{-1})	$U_{37}^{K'}$	%$C_{37:4}$	C_{37} alkenone concentration (pg $cell^{-1}$)
I. galbana					
10.3	10	0.63	0.12	6.7 ± 0.4	0.27 ± 0.05
15.3	10	0.60	0.11	5.9 ± 1.4	0.39 ± 0.14
20.3	10	0.64	0.11	5.8 ± 0.3	0.36 ± 0.12
25.2	10	0.62	0.11	7.1 ± 1.0	0.37 ± 0.05[a]
30.3	10	0.63	0.11	8.7 ± 0.4	0.42 ± 0.13
35.6	10	0.62	0.11	12 ± 0.9	0.33 ± 0.03
10.3	28		0.07	5.4 ± 0.1	1.08 ± 0.11
15.4	28		0.06	5.3 ± 0.1	1.32 ± 0.24[a]
20.4	27		0.08	4.4 ± 0.9	0.95 ± 0.30
25.3	28		0.08	4.2 ± 1.3	0.69 ± 0.25[a]
30.5	28		0.07	5.8 ± 0.7	0.90[b]
35.8	27		0.07	5.7 ± 0.5	0.74 ± 0.03
10.7	69		0.03	5.7 ± 0.3	0.91 ± 0.21[a]
16.0	69		0.03	5.5 ± 0.3	1.23 ± 0.13
21.1	75		0.03	5.9 ± 0.2	1.94 ± 0.38
26.3	83		0.03	7.0 ± 0.3	1.89 ± 0.30
31.8	89		0.02	9.3 ± 0.5	1.84 ± 0.34
37.4	89		0.02	10 ± 0.2	1.63 ± 0.05
C. lamellosa					
10.2	14		0.09	32 ± 2	
15.3	14		0.09	34 ± 2	
20.3	14		0.08	35 ± 1	
25.0	15		0.08	36 ± < 1	
30.0	15		0.08	35 ± 1	
35.0	15		0.07	37 ± 1	
10.2	35		0.07	40 ± 1	
15.3	35		0.07	38 ± 1	
20.5	35		0.07	39 ± 1	
25.1	35		0.07	40 ± 2	
30.2	35		0.07	42 ± 1	
35.2	35		0.06	43 ± 2	

[a] Mean from two cultures; reported error is the range; [b] from one culture.