HELGOLÄNDER MEERESUNTERSUCHUNGEN
Helgoländer Meeresunters. 41, 323–327 (1987)

Lethal exposure times and preconditioning to upper temperature limits of some temperate North Atlantic red algae

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ABSTRACT: Using the red alga *Polyneura hilliae* as an example, the minimum time taken for lethal temperature exposure, with no regeneration capacity left, was 2 weeks. Employing this exposure time, the upper temperature limits of the following 13 red algal species belonging to four biogeographical distribution groups were determined: *Callophyllis laciniata, Polyneura hilliae, Hypoglossum hypoglossoides, Halurus equisetifolius, Lomentaria articulata, Cryptopleura ramosa, Calliblepharis ciliata* (warm-temperate Mediterranean-Atlantic group); *Callithamnion tetragonum, Lomentaria orcadensis* (amphiatlantic-temperate group); *Grinnellia americana, Lomentaria baileyana, Agardhiella subulata* (northeast American tropical-temperate group), *Solieria tenera* (amphiatlantic tropical-temperate group). Pre-incubation temperatures of 10 and 20 °C for one month (or 15 and 25 °C for the two last-mentioned distribution groups) did not measurably affect the critical survival temperature.

INTRODUCTION

Investigators who have attempted to correlate the temperature requirements for survival of benthic marine algae with their phytogeographic distribution have employed differing exposure times to limiting temperatures. To give a few examples, Montfort et al. (1957) used 3 h, Biebl (1958, 1962) 12 h, Lüning (1984) 1 week, Bolton (1983) 3 weeks, McLachlan & Bird (1984), Yarish et al. (1984, 1986) 5 weeks, and Cambridge et al. (1984) 2 months. The first objective of the present paper was to provide a complete time series at least for one alga in order to obtain some idea about the minimum exposure time after which the upper lethal temperature remains constant for the next few months.

The second objective concerned another uncertainty of the experimentator, namely the selection of the temperature at which algae should be kept and propagated prior to treatment at the experimental temperatures. Does pre-incubation at temperatures differing by as much as e.g. 10 °C change the upper lethal temperature? Such cases of "non-genetic resistance adaptation" (Precht et al., 1955, 1973; Kinne, 1970) or "plastic resistance", in the terminology of Levitt (1980), have been investigated more in marine animals (e.g. Newell, 1976). The few experiments reported on marine algae (for review of the older literature see Gessner, 1970) stressed the stability of the upper lethal survival limit and the inability of the experimentator to shift these limits by choosing differing pre-incubation temperatures.

The last objective was to determine precisely, in temperature steps by 1 °C, the upper survival limits of 13 species of red algae. For these the phytogeographic boundaries, temperature demands for growth and reproduction, and the survival limits in temperature steps by 5 °C have been presented earlier (Yarish et al., 1984, 1986).

MATERIAL AND METHODS

The inocula for experimentation consisted of cloned cultures (cf. Yarish et al., 1984, 1986 for collection data) and were propagated at 15 °C in von Stosch's enriched seawater medium (Ott, 1966) having a salinity of 33 ‰. Standard light conditions: cool fluorescent light, photon fluence rate of 30 µmol m^{-2} s^{-1}, 16 h light per day. Tests on temperature survival were performed, as described in detail by Lüning et al. (1987). In short, isolates (0.2–0.3 g fresh weight) were incubated in Plexiglas cylindrical jars (140 ml of culture medium; Von Stosch's Enriched Seawater at half strength; cf. Guiry & Cunningham, 1984) contained in illuminated bath-cryostat units. The culture medium was replaced automatically once a day. After a standard treatment of 2 weeks the plants were post-incubated under good growing conditions (15 °C) for 4 weeks to check for survival and for regenerated thalli from a few living cells in the otherwise bleached tissue. In cases where there had been any doubt about the viability of the treated material, the post-incubation was prolonged for another 4 weeks.

RESULTS AND DISCUSSION

The survival responses of the gametophytes of *Polyneura hilliae* are given for a period of up to 56 days (Table 1) at temperatures of 20, 24–30 °C. After a six-hour

Table 1. Survival responses of *Polyneura hilliae* gametophytes over eight weeks. xxx plants alive (with sight observation and confirmation after 4 weeks post-incubation at 15 °C; reg. regeneration of purportedly "dead" tissue after four weeks at 15 °C; - - - plants dead

	Temperature over eight weeks (°C)							
Time (d)	20	24	25	26	27	28	29	30
1/4	xxx	xxx	xxx	xxx	reg.	reg.	- - -	- - -
1	xxx	xxx	xxx	xxx	reg.	- - -	- - -	- - -
2	xxx	xxx	xxx	reg.	- - -	- - -	- - -	- - -
3	xxx	xxx	xxx	reg.	- - -	- - -	- - -	- - -
5	xxx	xxx	xxx	reg.	- - -	- - -	- - -	- - -
7	xxx	xxx	reg.	- - -	- - -	- - -	- - -	- - -
10	xxx	xxx	reg.	- - -	- - -	- - -	- - -	- - -
14	xxx	xxx	- - -	- - -	- - -	- - -	- - -	- - -
21	xxx	xxx	- - -	- - -	- - -	- - -	- - -	- - -
28	xxx	xxx	- - -	- - -	- - -	- - -	- - -	- - -
37	xxx	xxx	- - -	- - -	- - -	- - -	- - -	- - -
42	xxx	xxx	- - -	- - -	- - -	- - -	- - -	- - -
56	xxx	xxx	- - -	- - -	- - -	- - -	- - -	- - -

Table 2. Upper survival temperature and geographical boundaries of selected temperate North Atlantic red algae. Exposure time to experimental temperatures was 2 weeks. Temperature values in brackets following the geographical boundaries indicate the mean August isotherms (according to Sverdrup et al., 1942) of the surface seawater at the corresponding locations. Nomenclature according to South & Tittley (1986). [1]restricted to shallow embayments where summer temperatures exceed 17 °C thereby permitting growth and reproduction Pre-incubation temperatures: Distribution groups 1 and 2: continuously at 15 °C (*); transferred for one month to 10 or 15 °C (**; identical upper survival temperature). Distribution groups 3 and 4: continuously at 20 °C (***), or transferred for one month to 20 or 25 °C (****; identical upper survival temperature)

Species	Northern boundary	Southern boundary	Upper survival temperature (°C)
(1) Warm-temperate Mediterranean-Atlantic group			
	Europe	Europe/Africa	
Callophyllis laciniata (Huds.) Kütz.	Norway (11)	Morocco (21)	24*
Polyneura hilliae (Grev.) Kylin	Ireland (14)	Portugal (19)	24**
Hypoglossum hypoglossoides (Stackh.) F. Coll. et Hervey	Shetland Is. (12)	Cameroun (27)	25**
Halurus equisetifolius (Lightf.) Kütz.	Ireland (13)	Canaries (22)	26*
Lomentaria articulata (Huds.) Lyngbye	Norway (11)	Madeira Is. (21)	27**
Cryptopleura ramosa (Huds.) Kylin ex Newton	Faeröes (11)	Madeira Is. (21)	27**
Calliblepharis ciliata (Huds.) Kütz.	Ireland (14)	Portugal (19)	27**
	Europe or N. America	Europe/Africa or N. America	
(2) Amphiatlantic-temperate group			
Callithamnion tetragonum (With.) S. F. Gray	Iceland (10) Norway (10) Newfdl. (12)	Madeira Is. (21) Virginia (25)	25**
Lomentaria orcadensis (Harvey) F. Collins ex W. Tayl.	Iceland (10) Norway (10) Nova Scotia (15)	Portugal (20) N. Carolina (25)	27**
(3) Northeast American tropical-temperate group			
Grinnellia americana (C. Ag.) Harvey	Massachusetts (17)	Tropical margins (Florida)	31***
Lomentaria baileyana (Harvey) Farlow	s. Gulf of (15) St. Lawrence[1]	Tropical margins (s. Florida)	33***
Agardhiella subulata (C. Ag.) Kraft et Wynne	s. Gulf of (15) St. Lawrence[1]	Tropics	33****
(4) Amphiatlantic tropical-temperate group			
Solieria filiformis (= *tenera*) (Kütz.) Gabrielson	N. Carolina (25)	Tropics	33****

treatment in the water baths, temperatures exceeding 28 °C were lethal. Plants incubated at 27–28 °C appeared dead; however, they began to regenerate after being reincubated at 15 °C. After 1-day treatment, the regeneration capacity was further diminished to 27 °C, after treatments of 2–5 days duration to 26 °C, and after treatment duration of 7–10 days to 25 °C. The upper survival limit at 24 °C was constant from an exposure time of 14 days onward.

It is interesting to note that a two-week incubation period was sufficient for determining the isolates' upper survival limits and that the difference between survival and death of the complete thallus may be a matter of 1 °C (Table 1). The precision of the technique employed must be emphasized as one-degree intervals were used for each taxon in contrast to other experiments, which are often set in 5 °C intervals. The importance of post-incubation instead of visual inspection right after the treatment cannot be over-emphasized. Post-incubation was particularly needed for *Hypoglossum hypoglossoides, Lomentaria articulata, Callithamnion tetragonum,* and *Polyneura hilliae,* as each was able to regenerate entire thalli from only a few cells. It is suggested for future research in the determination of the upper lethal limits of algal taxa that a 5 °C interval should be established encompassing the lethal temperature. After the interval has been found out, then the precise lethal limit should be determined by incubation at 1 °C intervals for at least two weeks with frequent change of culture medium.

Table 2 exhibits the survival responses of all investigated algal species. The upper survival limits of the warm-temperate species (groups 1 and 2 in Table 2) range at 24–27 °C, whereas species with tropical affinities (groups 3 and 4) are clearly differentiated by values ranging at 31–33 °C.

Nine out of the 13 species investigated were tested after pre-incubation for one month at temperatures differing by 10 °C (Table 2). Identical values for the upper survival temperature were obtained in all cases. Apparently, the isolates had no ability to shift their upper lethal temperature. This is in accordance with results reported for most of the smaller marine algal species from Helgoland, whereas seasonal shifts of upper temperature tolerance were readily detected in larger algae, e.g. *Laminaria* spp. and *Desmarestia aculeata* (Lüning, 1984 and older literature cited herein). During the cold season, these algae form new, heat-sensitive tissue, which subsequently "hardens" and exhibits a 2–5 °C higher temperature tolerance in summer. It may well be possible that in smaller algae the youngest cells are more heat-sensitive and that this effect is easily overlooked for technical reasons, because in larger algae one can easily treat old and young tissue separately. The upper survival temperatures listed in Table 2 cannot be directly related to the positions of the species' southern limits, as other factors (e.g. temperatures limiting growth and/or reproduction) may be important. Moreover, extreme temperature values are more important than average values (see Yarish et al., 1984, 1986).

Acknowledgements. The authors thank P. Kadel for her invaluable technical assistance. C. Yarish and H. Kirkman are indebted to the Director of the Biologische Anstalt Helgoland for his hospitability and working facilities. Thanks are also due to Prof. C. van den Hoek for helpful suggestions in regard to the manuscript. C. Yarish is also indebted to support from the Connecticut Sea Grant Program.

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