Temperature and light responses of alga *Caulerpa taxifolia* introduced into the Mediterranean Sea

Teruhisa Komatsu\(^1,2,\star\), Alexandre Meinesz\(^1\), Daphne Buckles\(^1,3\)

\(^1\)Laboratoire Environnement Marin Littoral, CNRS UMR 6540, Université de Nice-Sophia Antipolis, F-06108 Nice Cedex 2, France

\(^2\)Ocean Research Institute, University of Tokyo, 1-15-1, Minamidai, Nakano-ku, Tokyo 164, Japan

\(^3\)Division of Natural Science, Southampton College, Long Island University, Southampton, New York 11968, USA

ABSTRACT: Cuttings of Mediterranean *Caulerpa taxifolia* were cultured under controlled temperature and light conditions in culture chambers. The upper lethal temperature was between 31.5 and 32.5°C and the lower lethal temperature between 9 and 10°C. Between 10 and 12.5°C, the alga survived without any growth; new stolons and new fronds developed at 15 and 17.5°C, respectively. Stolon growth was strongly correlated with the temperature increase. No morphological changes were observed when the cuttings were cultured within the vital temperature range (10 to 31.5°C). The new fronds and stolons developed on the cuttings under a very weak light intensity of photosynthetically active radiation (27 \(\mu\text{mol m}^{-2} \text{s}^{-1}\) at a photoperiod cycle of 14 h light:10 h dark). The most favorable range of light intensity was between 88 and 338 \(\mu\text{mol m}^{-2} \text{s}^{-1}\) (14 h light:10 h dark). These light and temperature responses confer to the Mediterranean strain *C. taxifolia*, introduced in 1984, a large potential for expansion throughout the Mediterranean Sea and also in adjacent tropical and temperate seas.

KEY WORDS: *Caulerpa taxifolia* · Mediterranean Sea · Temperature · Light

INTRODUCTION

Introduced into the Mediterranean Sea at Monaco in 1984 (Meinesz & Boudouresque 1996), the tropical *Caulerpa taxifolia* (Vahl) C. Agardh is continuously spreading each year (Meinesz & Hesse 1991, Meinesz et al. 1993, 1994a). The affected areas were estimated at 1 \(\text{m}^2\) in 1984, 3 \(\text{ha}\) in 1990 (year of discovery in France), 30 \(\text{ha}\) in 1991, 427 \(\text{ha}\) in 1992 (year of discovery in Italy and Spain), 1300 \(\text{ha}\) in 1993 and 1500 \(\text{ha}\) at the end of 1994. In February 1995 it was first discovered in Croatia (Adriatic Sea). Actual findings showed that the principal mode of reproduction of *C. taxifolia* in the Mediterranean Sea is vegetative (Meinesz 1992). Furthermore, the dispersion of cuttings may occur either naturally in the immediate area of a colonized site, or artificially at greater distances by means of pleasure boat anchorings or fishing nets (Meinesz 1992, Sant et al. 1994).

To define potential habitat range limits, we analyzed the effects of temperature and light on the growth and development of cuttings of *Caulerpa taxifolia* cultured in culture chambers. Furthermore, culturing of *C. taxifolia* under these various conditions will allow us to assess possible morphological ecotypical variations.

MATERIAL AND METHODS

The material was collected using SCUBA on the day before the start of each experiment. The sampling site was in the recreational harbour of La Darse (Villefranche-sur-Mer, France: 43° 41.98' N, 7° 18.60' E), where a colony, which in May 1996 covered more than 1 \(\text{ha}\), has developed since 1992 between 3 and 6 \(\text{m}\) depth.

A complete thallus of *Caulerpa taxifolia* is composed of a ramified stolon which can reach more than 2 \(\text{m}\) in length and can carry more than 100 fronds and as many as 100 rhizoid pillars (Meinesz et al. 1993). These individuals are strongly fixed to the substrate by a dense network of fine rhizoids. It is therefore difficult to remove entire individuals of this alga without breakage. The effects of light and temperature on the develop-
opment and growth were evaluated from generated parts on stolons and fronds of 2 types of cutting units: 'isolated frond segments' (5 cm apical part of a frond) and 'thallus fragments' (8 or 10 cm of stolon carrying 2 to 4 fronds) (Fig. 1).

The experimental specimens were set in aquaria containing 4 l of seawater, previously filtered and sterilized in an autoclave. The seawater, analyzed at the start of the experiments, contained less than 0.16 mg l⁻¹ of nitrates and less than 0.1 mg l⁻¹ phosphates.

The aquaria were kept in culture chambers equipped with 3 fluorescent lamps (Thorn, Model 16H06) attached to the upper panel of the culture chamber. The underwater light intensity was measured with a LI-COR waterproof spherical sensor at the level at which the alga lay in each of the aquaria which were placed on 3 different shelves in the culture chamber. Photosynthetically active radiation (PAR) varied from 27 to 356 μmol m⁻² s⁻¹ depending on the different configurations (number of lamps, and position and number of aquaria) in the culture chamber. Different photoperiod cycles of 8:16, 10:14, 12:12, 14:10, and 16:10 h (light:dark) were tested.

Experimental temperatures ranged from 6 to 34°C. The temperature of the water in the aquaria, measured every day, varied ±0.2°C in relation to the desired temperature. To avoid thermal shock, we always acclimated the cuttings; 2 to 5 d were necessary to progressively decrease or increase the temperature until the desired temperature was reached. Such a precaution is recommended for this type of experiment (e.g. Novaczek et al. 1989). When the acclimation period was completed, an initial state of cutting morphology was established; photographs of the samples were used as references.

The cultures, under precise light and temperature conditions, were maintained for periods between 10 and 90 d in duration; the samples were again photographed or fixed in a herbarium.

A total of 24 aquaria in 3 culture chambers were used, and 20 isolated frond segments were used per aquarium. We used 2 or 4 thallus fragments per aquarium, maintaining them in their natural position (stolon along the bottom and fronds vertical) using transparent, plastic separators.

The following measurements were taken: (1) the quantity, length, and diameter of the new stolons; (2) the quantity of the new rhizoid pillars and their spacing on the stolon; and (3) the quantity and size (length) of the new fronds.

RESULTS

Lethal temperature experiments

To determine the maximum and minimum temperatures at which the alga can survive, we used a long day photoperiod cycle (16:8 h) with high temperatures (30, 31, 31.5, 31.7, 32, 32.5°C) and a short day photoperiod cycle (8:16 h) with low temperatures (6, 7, 9, 10, 12°C). In both cases, a weak light intensity was used (between 27 and 95 μmol m⁻² s⁻¹). This was recommended by Cambridge et al. (1984), Yarish et al. (1984, 1986) and Novaczek et al. (1989). Twenty cuttings of isolated frond segments were used for each temperature tested.

Between 30 and 31°C, the cuttings grew and developed. At 31.5°C, 8 cuttings of isolated frond segments were dead after 1 wk of culture; the other frond segments developed new stolons and survived a 20 d culture period. At 31.7°C, only 4 cuttings of isolated frond segments survived after 20 d, and at 32°C, only 1 frond survived. At 32.5°C, all of the fronds died within the first week of culture. At this temperature, the same result was obtained with photoperiod cycles of 12:12 and 16:8 h.
For the lower temperatures, we first noticed the absence of development and growth of the cuttings at 10 and 12°C. Resistance to the lowest temperatures was observed for short periods of time because the cuttings could not remain alive for longer. After 10 d at 7°C or 1 wk at 6°C, algal growth resumed when the temperature was slowly brought back to 18°C. The isolated frond segments resisted for 3 mo at 10°C and then developed after raising of the temperature to 18°C. However, all of the isolated frond segments maintained at 9°C were dead in less than 2 mo. Thus, the coldest experimental lethal temperature for a period of 3 mo is between 9 and 10°C.

The temperature responses of the *Caulerpa taxifolia* introduced into the Mediterranean Sea are summarized in Fig. 2. It gives the thermal tolerances of the alga that grew between 15 and 31.5°C and of those that were able to survive between 10 and 15°C for 3 mo, which demonstrated a very marked polarity. We also noticed that the diameter of the new stolons decreased as the temperature increased (Fig. 4). The 1-way ANOVA test rejected the 2 null hypotheses, homo-

Effects of temperature on growth and development

We tested the effect of temperature under defined light conditions (193 ± 26 μmol m⁻² s⁻¹). The period was 12:12 h for the following temperatures: 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, 30 and 32.5°C. Twenty isolated frond segments and 6 thallus fragments were used for each temperature. Results were recorded after a 10 d culture period.

Development and growth of the stolons

Growth rates of new stolons were similar on both types of cuttings (isolated frond segments and thallus fragments) (Fig. 3). Nevertheless, the average total length of new stolon for 1 cutting was close to 4 times greater on the thallus fragments (larger cuttings) than on the isolated frond segments.

At 10 and 12.5°C, no development of new stolons or fronds was observed on the cuttings which remained in a good condition (no necrosis). At 32.5°C, all of the cuttings had died after 5 d. Within the temperature range of 15 to 30°C, new stolons appeared on all the cuttings.

The length of the new stolons increased in direct proportion to the temperature (Fig. 3). The largest mean length of new stolons was 61.7 mm (SD = 16.8 mm) at 30°C on isolated frond segments over 10 d and was 240.7 mm (SD = 42.13 mm) at 27.5°C on thallus fragments for the same culture period.

On the isolated frond segments, most of the stolons (95%) appeared on the distal cut section (Fig. 1A),
Fig. 4. Mean diameter ± SD of the new stolons on the 2 types of cuttings at different temperatures after a 10 d culture period. Light: 12:12 h (light:dark), 193 ± 26 μmol m⁻² s⁻¹.

genesis of means of diameter of new stolons on the isolated frond segments and on the thallus fragments among 5 temperature groups (df = 4, F = 36.68, p < 0.0001 and df = 4, F = 20.36, p < 0.0001, respectively), with the SAS statistical package program (SAS Inc.). Then, we made multiple comparison tests for means to examine which temperature groups equally affected growth of stolons on the 2 types of cuttings using the Student-Newman-Keuls test in the SAS statistical package program. It was suggested that the abrupt decrease in the mean diameter of new stolons above 27.5°C on the isolated frond segments and above 30°C on the thallus fragments was statistically significant (Table 1). Thus it was concluded that the most favorable temperature for new stolon growth was 27.5°C on a basis of length and diameter of new stolons.

Development of the fronds

The development of new fronds on the cuttings was less regular and more sensitive to temperature variations (Fig. 5). At 10, 12.5, and 15°C, there was no development of new fronds on the 2 types of cuttings tested. For the cuttings composed of isolated frond segments, we obtained a small number of new fronds at 17.5°C (4 of 20 isolated frond segments) and none at 20°C (Fig. 5a). For the thallus fragments, new fronds appeared only at 20°C (Fig. 5b). These fronds appeared as much on the initial fragment of the stolon as on the new stolons (Fig. 1B). Of all water temperatures tested, numbers of new fronds on the 2 types of cuttings cultured (isolated frond segments and thallus fragments) were the greatest, 1.3 and 4 respectively, at 27.5°C after a 10 d culture period.

Table 1. Results of the Student-Newman-Keuls multiple comparison test to examine the differences between all of the possible pairs of the means of diameter of new stolons on isolated frond segments and isolated thallus fragments cultured at different water temperatures. Light: 12:12 h (light:dark), 193 ± 26 μmol m⁻² s⁻¹. Isolated frond segments: df = 103, MSE = 0.012377, α = 0.05. Thallus fragments: df = 54, MSE = 0.023109, α = 0.05. A, B and C represent statistically different groups.

![Fig. 5. Mean number ± SD of new fronds on (a) each isolated frond segment (n = 20) and (b) each thallus fragment (n = 6) after a 10 d culture period at different temperatures. Light: 12:12 h (light:dark), 193 ± 26 μmol m⁻² s⁻¹. Dashed lines show that all the cuttings died after the culture period.](image-url)
Growth of the fronds

The average length of the new fronds was similar between 20 and 30°C (Fig. 6), but most of the new fronds did not terminate their growth after the 10 d culture period. The greatest lengths were obtained at 30°C, where some new fronds reached lengths of 10 cm (Fig. 7).

Effects of light on growth and development

To test the effect of light, cuttings were cultured at a temperature favorable to growth and development: 25°C.

Fig. 8 shows that the growth of new stolons on thallus fragments showed higher development of new stolons in the range of light intensities above 88 μmol m⁻² s⁻¹ (photoperiod cycle of 14:10 h). Development of new stolons on isolated frond segments decreases at light intensities greater than 17.94 mol m⁻² d⁻¹, equivalent to 356 μmol m⁻² s⁻¹ (14:10 h) (Fig. 9). The 1-way ANOVA test rejected the null hypothesis, homogeneity of means of length of new stolons on the thallus fragments and of means of length of new stolons on the isolated frond segments among 9 light intensity groups (df = 8, F = 5.12, p < 0.0001 and df = 8, F = 10.87, p < 0.0001, respectively), with the SAS statistical program package. Then, multiple comparison tests for means were conducted to examine which light intensity groups equally affect growth of stolons on the 2 types of cutting units (data sets of Figs. 8 & 9) using the Student-Newman-Keuls test in the SAS statistical package program. The mean lengths of new stolons on the thallus fragments cultured above 88 μmol m⁻² s⁻¹ were statistically the largest of all the new stolons (Table 2). Table 3 suggests that the mean length of new stolons on the isolated frond segments cultured at 17.94 mol m⁻² d⁻¹ was statistically smaller than those between 12.8 and 15.4 mol m⁻² d⁻¹. The growth of new stolons on the thallus fragments was observed at the weak light intensities of 27 and 55 μmol m⁻² s⁻¹ (photoperiod cycle of 14:10 h) equivalent to 1.36 and 1.98 mol m⁻² d⁻¹.

On the thallus fragments (n = 2), cultured for 40 d (photoperiod cycle of 14:10 h), some new fronds developed on the new stolons. Fig. 10 shows that the number of new fronds increased with stronger irradiation.
On the other hand, the lengths of the new fronds under weak light were greater (Fig. 10).

The variation of irradiation equally affected the morphogenesis of the fronds (Fig. 11). We also observed an elevated development of dichotomously ramified rachis of fronds under weak light on the thallus fragments (after a 10 d culture period), although the results are not shown here. Likewise, the isolated frond segments developed far more dichotomous ramifications at their apex under weak light (after a 10 d culture period).

Weak irradiation, for example 1.36 mol m$^{-2}$ d$^{-1}$, had a positive effect on the number and length of the new pillars of rhizoids on the thallus fragments (n = 2) (after a 40 d culture period) (Fig. 12).

**DISCUSSION**

In spite of the large spectrum of temperatures and light intensities used in our experiments, their effects have not indicated great morphological modifications such as those Peterson (1972) and Ohba & Enomoto (1987) observed for another species of *Caulerpa* (*C. racemosa* (Forsskal) J. Agardh). In all the different experimental conditions, the newly developed fronds all present the defined characteristics of the species *C. taxifolia* (Meinesz et al. 1994b). The morphological plasticity of this species remains weak, this explains the small variations of irradiation equally affected the morphogenesis of the fronds (Fig. 11). We also observed an elevated development of dichotomously ramified rachis of fronds under weak light on the thallus fragments (after a 10 d culture period), although the results are not shown here. Likewise, the isolated frond segments developed far more dichotomous ramifications at their apex under weak light (after a 10 d culture period).

Weak irradiation, for example 1.36 mol m$^{-2}$ d$^{-1}$, had a positive effect on the number and length of the new pillars of rhizoids on the thallus fragments (n = 2) (after a 40 d culture period) (Fig. 12).

**DISCUSSION**

In spite of the large spectrum of temperatures and light intensities used in our experiments, their effects have not indicated great morphological modifications such as those Peterson (1972) and Ohba & Enomoto (1987) observed for another species of *Caulerpa* (*C. racemosa* (Forsskal) J. Agardh). In all the different experimental conditions, the newly developed fronds all present the defined characteristics of the species *C. taxifolia* (Meinesz et al. 1994b). The morphological plasticity of this species remains weak, this explains the small variations of irradiation equally affected the morphogenesis of the fronds (Fig. 11). We also observed an elevated development of dichotomously ramified rachis of fronds under weak light on the thallus fragments (after a 10 d culture period), although the results are not shown here. Likewise, the isolated frond segments developed far more dichotomous ramifications at their apex under weak light (after a 10 d culture period).

Weak irradiation, for example 1.36 mol m$^{-2}$ d$^{-1}$, had a positive effect on the number and length of the new pillars of rhizoids on the thallus fragments (n = 2) (after a 40 d culture period) (Fig. 12).
number of ecological phenotypes found in open seas which, in the past, were wrongly described as varieties or forms (Meinesz et al. 1994b). Carruthers et al. (1993) showed also the lack of morphological variation under different culture conditions of 2 taxa of the Sedoideae section (C. racemosa var laetevirens (Montagne) Weber-van-Bosse form C. cylindracea (Sonder) Weber van Bosse and C. lagara Carruthers, Walker & Huisman).

Between 15 and 31.5°C, the alga developed rapidly; the growth and development increased as a function of the temperature. The best conditions were observed between 20 and 30°C. At these temperatures, and with highly varied lighting (27 to 338 μmol m⁻² s⁻¹ at a photoperiod cycle of 14:10 h and 88 to 356 μmol m⁻² s⁻¹ at 3 photoperiod cycles of 10:14, 12:12 and 14:10 h), cuttings composed of isolated frond segments and thallus fragments produced new stolons and new fronds within 10 d.

The alga grew with very weak irradiation (between 1.36 and 1.98 mol m⁻² d⁻¹). This could explain the distribution of the alga in the Mediterranean Sea at depths ranging from 50 to 99 m (Belsher & Meinesz 1995).

It has clearly been established that weak light favors the lengthening of the fronds and dichotomous ramifications of the fronds, which was already obvious in situ (Meinesz & Hesse 1991, Meinesz et al. 1995). The experiments with the 2 types of cuttings, thallus fragments and isolated frond segments, give the same results; but the use of isolated frond segments is more convenient.

Fig. 10. Frequency distribution of the new frond length on 2 thallus fragments after a 40 d culture period at different light intensities. Photoperiod: 14:10 h (light:dark) at 25°C.

Fig. 11. Total number of dichotomous ramifications on new fronds at the apex of the original 20 isolated frond segments after a 10 d culture period with 3 light intensities (75, 147 and 281 μmol m⁻² s⁻¹) and 3 different photoperiods (14:10, 12:12 and 10:14 h light:dark) at 25°C.

Fig. 12. Frequency distribution of the new pillar of rhizoids (developed on the new stolons) after a 40 d culture period of 2 thallus fragments at 4 light intensities. Photoperiod: 14:10 h (light:dark) at 25°C.
for testing the various conditions of growth and development (easier handling, more homogenous samples, and 20 cuttings cultured in the same aquarium).

The upper lethal temperature (between 31.5 and 32.5°C) is well marked. This temperature, never observed in the open Mediterranean Sea, corresponds to the warmest water temperatures of the tropical seas (in open sea) where the alga exists.

The lower lethal temperature has been more difficult to define because it varies greatly as a function of the number of days of exposure to cold water temperatures. To experimentally define the lower lethal temperature of the different groups of algae, the following culture periods were taken into consideration: 1 mo for van den Hoek (1982 a, b); 1½ mo for Cambridge et al. (1990). Novaczek et al. (1989) and Cambridge et al. (1991) define the minimum vital temperature experimentally: it is determined when the algae resists a 3 mo culture period.

Van den Hoek (1982b) defined the relationship between the minimum vital temperature obtained experimentally and the isotherms of sea surface temperature. According to him, the experimental vital minimum temperature corresponds to the average temperature of the surface water during the coldest month (February for the Northern Hemisphere).

We could thus define the area of theoretical extension of the introduced strain of Caulerpa taxifolia in the Mediterranean Sea based on this study. It can live between the isotherms of 10 and 31°C. Noticing that growth and development occur above 15°C and that sexual reproduction does not play a major role in the ability of the alga to grow perennially and extensively (Meinesz 1992), the development of populations of the alga can extend in all the regions of Mediterranean Sea. The experimental results concerning the lowest lethal temperature were confirmed by the extension of C. taxifolia colonies in open sea at the northern part of the Golfe du Lion (harbor of Saint-Cyprien, Pyrénées-Orientales, France) and at the northern part of the Adriatic Sea (harbor of Malinska, Isle of Krk, Croatia) where the average water temperature of the coldest winter months is between 10 and 11°C. The latter station is the highest northern latitude (45° N) at which a population of the genus Caulerpa has been found in the world.

Throughout the year, the greater the number of days above 15°C (especially between 20 and 30°C), the more substantial are the growth and development. This experimental report allows one to anticipate an extension of the species into all of the Mediterranean, with a more rapid invasion in the southern regions.

According to the experimental lethal thermal boundaries, the potential thermal distribution of the Mediterranean strain of Caulerpa taxifolia covers all the tropical regions and the warm temperate adjacent seas; it overlapped those of the cosmopolitan red alga species Centroceras clavatum (C. Agardh) Montagne (van den Hoek & Bremner 1990, van den Hoek et al. 1990).

The lower lethal temperature found experimentally and observed in situ in the Mediterranean strain of Caulerpa taxifolia is much lower (nearly 10°C) than that of the isothermal limit (about 20°C) corresponding to the distribution of tropical strains of C. taxifolia (Meinesz & Hesse 1991, Meinesz et al. 1994b). Such a difference, observed in this pantropical species which does not present anomalies of distribution, can only be explained by a genetic characteristic (Meinesz et al. 1994b, Caye et al. 1996). Thus, it is advisable to consider the risk of extension of this vigorous strain in the tropical regions and in the adjacent temperate seas.

Acknowledgements. This work was supported by an EEC grant (DGXI - Life), the French Ministry of Environment, the GSI Positionne and the Association 'Route des hautes technologies' - Region Provence-Alpes-Côte d'Azur with the cooperative research program of the 'Japanese Society for the Promotion of Science' (JSPS) and French 'Centre National de la Recherche Scientifique' (CNRS). T.K. acknowledges the French Ministry of Foreign Affairs for the scholarship of 'Haut niveau' for his research undertaken at the Université de Nice-Sophia Antipolis. We thank Christian Visscher of Université de Nice-Sophia Antipolis for critical reading of the English manuscript and Dr Tom Nishida of Japanese National Institute for Far Seas Fisheries for his help with the statistical analysis.

LITERATURE CITED


This article was submitted to the editor


Manuscript first received: July 2, 1996
Revised version accepted: November 15, 1996