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Growth and survival of the invasive alga, *Caulerpa taxifolia*, in different salinities and temperatures: implications for coastal lake management

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Abstract The alga *Caulerpa taxifolia* is an invasive pest species in many parts of the world and has recently become established in several estuaries in south eastern Australia. A major infestation has occurred in Lake Conjola, an intermittently open and closed coastal lagoon in southern NSW. Short term (1 week) laboratory experiments were carried out to investigate growth and survival of fragments of *C. taxifolia* collected from this outbreak, under a range of salinities (15–30 ppt) and water temperatures (15–30°C). Fronds, stolons and thalli of the alga all displayed similar responses. Many of the algal fragments doubled in size over the week and a maximum growth rate of 174 mm/week was recorded. Fragments showed good growth (>20 mm/week) at salinities >20 ppt and temperatures >20°C. Almost total mortality occurred at salinities lower than 20 ppt and temperatures less than 20°C. Historical records of water quality demonstrate that prior to entrance manipulation in 2001, salinities in Lake Conjola had often dropped to below 17 ppt for extended periods (up to 2 years). This suggests that management of the alga may be improved if the lake was allowed to undergo its normal cycles of opening and closing to the ocean, and that entrance manipulation may be one factor that has influenced the success of this invasive species.

Keywords *Caulerpa taxifolia* · Salinity · Temperature · Growth · Coastal lagoon · Management

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Lagoons and Coastal Wetlands in the Global Change Context: Impacts and Management Issues

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Introduction

Caulerpa taxifolia is a fast growing green alga, native to tropical waters of the Indian, Pacific and Atlantic Oceans (Phillips and Price, 2002). *C. taxifolia* has been popularly dubbed the 'killer algae' due to its success as an introduced noxious weed in several temperate locations, including Europe, USA and Australia (Jousson et al., 2000). Its high profile as an invasive species arises from its introduction to, and now widespread occurrence throughout, the Mediterranean Sea and its ability to out-compete important native species

(Boudouresque et al., 1995; Ribera et al., 1996; Ceccherelli and Cinelli, 1999b; Ceccherelli et al., 2002). The first reported introduction of *C. taxifolia* to non-tropical waters was a one square metre patch in the Mediterranean, off the Monaco coast, in 1984 (Meinesz et al., 1993). The species has now been reported widely throughout the Mediterranean at depths ranging from a few metres to around 100 m in clear water (Belsher and Meinesz, 1995). By 2000, *C. taxifolia* had infested approximately 131 km^2 composed of 103 independent colonies along 191 km of coastline in six countries: Spain, France, Monaco, Italy, Croatia, and Tunisia (Meinesz, 2002). In Australia, *C. taxifolia* is native to sheltered tropical waters throughout the north, ranging as far south as 28°15′ S on the eastern coastline (Phillips and Price, 2002). In April 2000, *C. taxifolia* was discovered in the temperate waters of New South Wales (NSW) and is now present in nine waterways.

C. taxifolia, along with most other species of siphonous green algae, can spread quickly and reach high densities because they grow rapidly relative to the seagrasses they replace (Ceccherelli and Cinelli, 1999a; Vroom and Smith, 2001; Ceccherelli et al., 2002). *C. taxifolia* is able to reproduce both sexually and asexually (Silva, 2002). However, sexual reproduction has not yet been observed in invasive populations (Zuljevic and Antolic, 2000). The success of *C. taxifolia* as an invasive species has therefore been largely attributed to its ability to reproduce asexually via vegetative fragments (Ashton and Mitchell, 1989; Ceccherelli and Cinelli, 1999b; Smith and Walters, 1999; Boudouresque and Verlaque, 2002). Very small fragments of *C. taxifolia*, some as small as 1 cm, can survive and have been successfully grown (Smith and Walters, 1999). Fragments can be produced by natural disturbances, such as storms, or created by human activities, such as boat propellers or anchors (Meinesz, 2002). The ability of *C. taxifolia* to fragment readily, successfully re-attach, to form new colonies, and to grow quickly, are key factors contributing to the rapid establishment and vegetative spread of the alga (Smith and Walters, 1999; Vroom and Smith, 2001).

In order to assist in the management of this pest alga, it is important to understand the environmental conditions that favour establishment and growth, particularly of fragments, which are the prime mode of spreading. Smith and Walters, (1999) examined growth of native *C. taxifolia* in laboratory experiments and found differences depending on the size of fragments and the position within the plant. Laboratory experiments carried out on the invasive *C. taxifolia* in the Mediterranean, have shown that *C. taxifolia* had little growth at temperatures between 10 and 15°C (Gayol et al., 1995; Komatsu et al., 1997). However, there is doubt about the similarity in the strains of *C. taxifolia* throughout the world (Boudouresque et al., 1995; Meinesz and Bouderesque, 1997; Schaffelke et al., 2002), and it is therefore important to understand the environmental conditions that favour growth of the invasive strain of *C. taxifolia* in the south east Australian region. Currently, there are no published experimental investigations on the growth and survival of *C. taxifolia* in this region. Here we examine the effects of salinity and temperature on the growth and survivorship of frond, stolon and thallus fragments of *C. taxifolia*. The findings are used to discuss the implications for the management of Lake Conjola, a medium sized coastal lagoon in this region, which is heavily infested with *C. taxifolia*.

Materials and methods

To examine the effects of temperature and salinity on the growth and survival of *C. taxifolia* fragments, experiments were done under controlled conditions in a growth cabinet (Thermoline Refrigerated Incubator), with controlled temperature (0–35°C) and lighting (neon tubes with PAR ~100 μmol m^{-2} s^{-1}). A 12-h day/night cycle was maintained during each experiment. *C. taxifolia* fragments and seawater were collected from Lake Conjola, NSW, Australia (34°05′ S, 151°08′ E), and transported to the laboratory at the beginning of each experiment so that fresh material was used each time. Lake

Conjola, which is a coastal barrier lagoon located approximately 210 km south of Sydney, is one of many lakes in this region that are characterised by entrance channels which open and close to the ocean depending on climatic conditions (West et al., 1985). *C. taxifolia* was first recorded at Lake Conjola in 2000, and the lake is now the most severely infested location in south east Australia.

A short pilot study was done to select the appropriate salinities and replication for the main laboratory experiments. In the pilot study, five replicate fragments were placed in the growth cabinet at 25°C at four salinities (10, 15, 25 and 30 ppt). These salinities were chosen after examination of published historical records of salinity recorded for Lake Conjola (SCC, 2003). It was found that the five replicates at 10 and 15 ppt were all dead after 1 week but that all replicates at 25 and 30 ppt grew both fronds and stolons during this short period. On the basis of these results, a range of salinities between 15 and 30 ppt was chosen for the main laboratory experiments.

For the main study, growth and survival of *C. taxifolia* fragments were assessed in a series of four experiments in the growth cabinet (described above). Each experiment was carried out over a week and at one of the following temperatures: 15, 20, 25 or 30°C. In each of the four experiments, 54 plastic containers were filled with 1 l of water, into which a fragment of *C. taxifolia* was placed. The water was a mixture of seawater from Lake Conjola and distilled water, adjusted to one of six salinities, namely: 15, 17.5, 20, 22.5, 25 and 30 ppt.

Growth and/or mortality were compared among three different fragment types, stolon (fragment of stolon only), frond (fragment of frond only) and thallus (fragment of stolon with one frond). There were three replicate fragments (<200 mm in length and <0.05 g dry weight) for each combination of fragment type and salinity. Single fragments were cut from relatively large and healthy plants kept at 30 ppt, immediately placed into open vessels containing water at the range of salinities listed above, and then randomly positioned in the growth cabinet. After one week, new fronds and stolons were counted and measured (length, mm) for each fragment. Total new growth (mm) was calculated by adding the new frond and stolon growth together for each fragment. This new growth was a combination of cell division and elongation. The catchment of Conjola Lake is largely cleared, and the estuarine waters of the lake have levels of dissolved nutrients (SCC, 2003) and inorganic carbon that are unlikely to be growth limiting for *C. taxifolia*. Since very small fragments of *C. taxifolia* were used in each container, and growth was measured after only 1 week, the effect of dilution on growth and mortality was considered to be primarily a response to salinity change. Mortality of fragments was measured as the proportion of the fragment that went a clear/white colour during the course of the experiments (% bleached).

Two-way ANOVAs were done to test for significant differences in total new growth [$\log_{10}(x + 1)$ transformed] and/or mortality [% bleached, $\arcsin(x/100)$ transformed] among salinities (fixed factor) and fragment type (fixed factor). Data were tested for homogeneity of variances using Cochran's test. Where all replicates of a treatment showed no growth or died, these data were excluded from the analyses to improve normality. Where some deviations from normality remained in the data, ANOVA was still adopted as this test is quite robust under such circumstances (Zar, 1984). Where significant differences were found, Tukey's HSD test was used for means comparison. Statistical comparisons were not made among temperatures because experiments were done at different times with different batches of *C. taxifolia* and therefore confounded by psuedoreplication (Hurlbert, 1984). Long term salinity and temperature records for Lake Conjola were obtained from the Shoalhaven City Council (SCC, 2003).

Results

Fragments of *C. taxifolia* were grown in the laboratory for periods of 1 week, during which time they had rapid growth rates at salinities >22.5 ppt and temperatures >15°C. The most growth displayed by a single fragment was 174 mm, and many fragments doubled in size during the 1-week study period. All fragments types displayed growth under suitable conditions.

In the majority of cases, fragments produced new stolons and fronds, rather than simply displaying repair and expansion of the damaged areas.

There was no growth of *C. taxifolia* at salinities of 20 ppt or less, regardless of temperature or fragment type (Fig. 1a). Fragments displayed some growth at salinities of 22.5 ppt and above, regardless of temperature, except for temperatures of 15°C where growth rate was negligible or small (Fig. 1a). At 20°C, highest growth rates were at 25 and 30 ppt; at 25°C, there was similar growth at the salinities greater than 20 ppt; while, at 30°C, the amount of growth was significantly higher at 30 ppt (Fig. 1a, Table 1). At salinities of

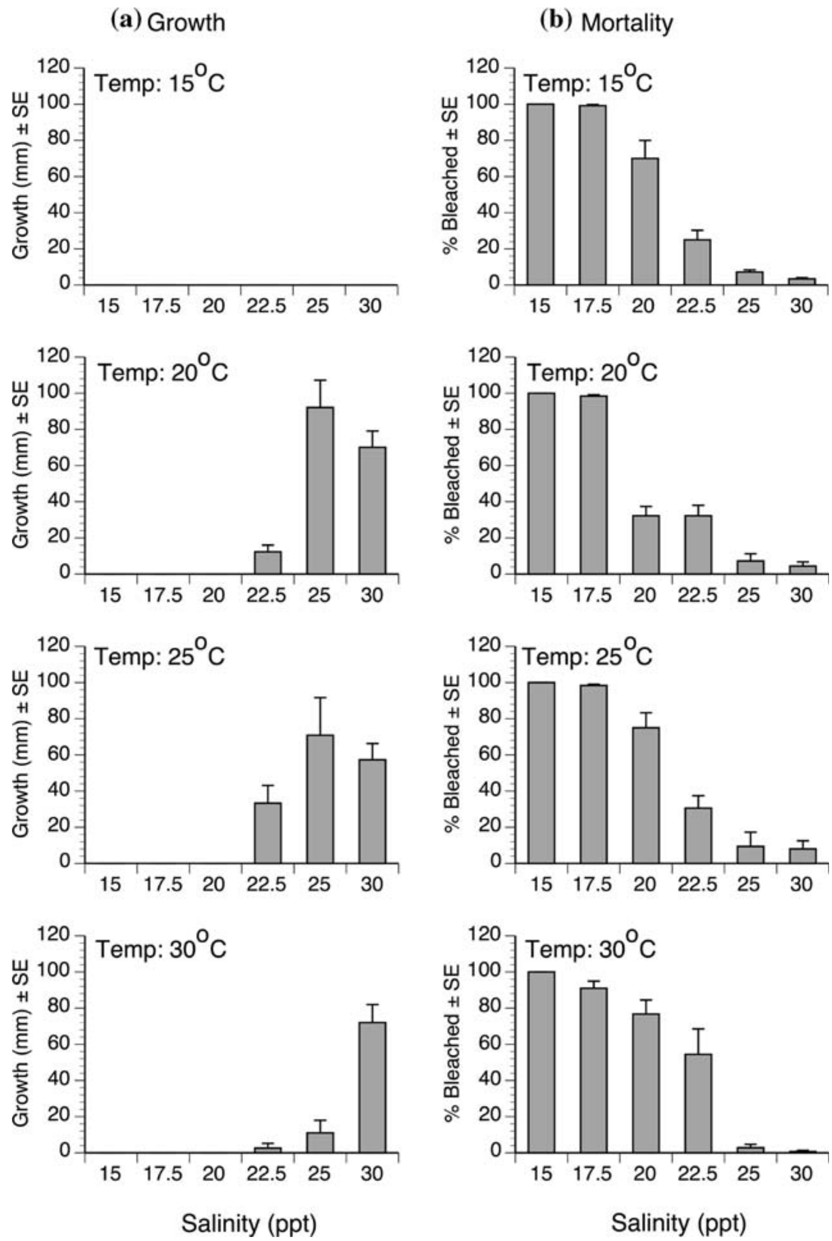

Fig. 1 (**a**) Growth (mm) and (**b**) Mortality (% bleached) of *C. taxifolia* fragments kept at different salinities and temperatures under laboratory conditions for 1 week. Data has been pooled over three fragment types (stolons, fronds and thallus). Mean and standard error shown (n = 9)

Table 1 Results of ANOVAs to test for differences in new growth (mm) of *C. taxifolia* fragments grown in laboratory conditions at four separate temperatures

Temperature		15°C		20°C		25°C		30°C	
Source	df	MS	F	MS	F	MS	F	MS	F
Salinity (S)	2	n.a.	n.a.	3.23	16.1***	0.37	0.7^{ns}	6.88	23.0***
Fragment (F)	2			0.04	0.2^{ns}	0.39	0.7^{ns}	0.24	0.8^{ns}
S × F	4			0.06	0.3^{ns}	1.24	2.3^{ns}	0.04	0.2^{ns}
Residual	18			0.20		0.55		0.30	
Cochran's		ns		ns		ns		ns	
Tukey's HSD				22.5 < 25 = 30 ppt		22.5 = 25 = 30 ppt		22.5 = 25 < 30 ppt	

^{ns} not significant; *<0.05; **<0.01; ***<0.001

Tukey's HSD test has been used to indicate significantly different means. Notes: (1) data for salinities of 15, 17.5 and 20 ppt were excluded from the analyses as there was no growth; (2) growth data has been log 10(x + 1) transformed

15 and 17.5 ppt, there was nearly 100% mortality of all *C. taxifolia* fragments, regardless of temperature (Fig. 1b). Mortality decreased at salinities 20 and 22.5 ppt, and the lowest mortality of fragments was at 25 and 30 ppt regardless of temperature (Fig. 1b; Table 2).

Long-term data for the salinity and temperature for Lake Conjola have been summarised for a representative site, located near the *C. taxifolia* outbreak, namely Roberts Point (Fig. 2).

Discussion

In general, *C. taxifolia* fragments grew rapidly in the laboratory at temperatures above 15°C and at salinities above 22.5 ppt. For example, several individual fragments doubled in size in 1 week. Successful growth of *C. taxifolia* fragments has also been documented in laboratory experiments elsewhere, where the rapid growth of fragments has been suggested to be a major contributing factor in the 'bloom' of *C. taxifolia* at new locations (Smith and Walters, 1999). Growth and survivorship of *C. taxifolia* fragments varied with salinity and temperature. In order to consider the relevance of these laboratory experiments in relation to existing conditions in Lake Conjola, long-term information of temperatures and salinities were examined using water quality data collected for Lake Conjola by Shoalhaven City Council (SCC, 2003).

Under laboratory conditions, the growth of fragments was negligible at 15°C but was high (>50 mm week^{-1}) at 20 and 25°C. Other laboratory experiments have similarly shown that other strains of *C. taxifolia* had little growth at temperatures between 10 and 15°C (Gayol et al., 1995;

Table 2 Results of ANOVAs to test for differences in mortality of *C. taxifolia* fragments (% bleached) grown in laboratory conditions at four separate temperatures

Temperature		15°C		20°C		25°C		30°C	
Source	df	MS	F	MS	F	MS	F	MS	F
Salinity (S)	4	3.78	41.1***	3.02	147.6***	3.26	59.4***	2.92	21.7***
Fragment (F)	2	0.01	0.10^{ns}	0.01	0.3^{ns}	0.08	1.5^{ns}	0.24	1.8^{ns}
S × F	8	0.04	0.42^{ns}	0.02	1.2^{ns}	0.04	0.8^{ns}	0.11	0.8^{ns}
Residual	30	0.09		0.02		0.05		0.13	
Cochran's		ns		ns		ns		*	
Tukey's HSD		17.5 > 20 > 22.5 = 25 = 30 ppt		17.5 > 20 = 22.5 > 25 = 30 ppt		17.5 > 20 > 22.5 = 25 = 30 ppt		17.5 = 20 = 22.5 > 25 = 30 ppt	

^{ns} not significant; *<0.05; **<0.01; ***<0.001

Tukey's HSD test has been used to indicate significantly different means. Notes: (1) data for salinity of 15 ppt was excluded from the analyses as there was 100% mortality; (2) growth data has been arcsin(x/100) transformed

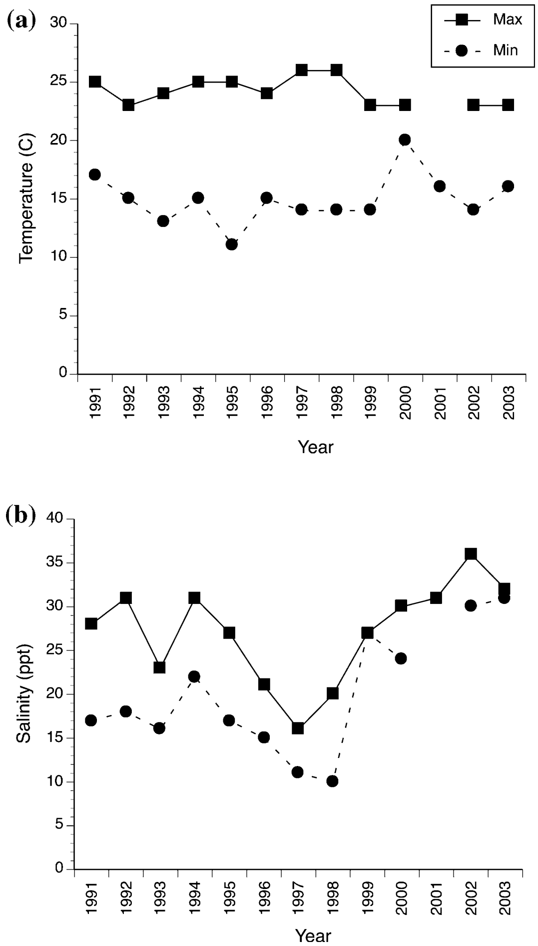

Fig. 2 Long-term maximum and minimum temperature (**a**) and salinity (**b**) records for Lake Conjola, NSW, Australia. Data have been made available from the Shoalhaven City Council (SCC, 2003). These data are for Roberts Point, located in the central basin of Lake Conjola (SCC, 2003, Site 43), for the period between May 1989 and June 2003

Komatsu et al., 1997; Chrisholm et al., 2000). The natural water temperatures of Lake Conjola are seasonal, with summer temperatures of approximately 23–25°C and winter temperature of between 12 and 16°C (Fig. 2a; SCC, 2003). Therefore, the results of the laboratory experiments in this study indicate that the *in situ* growth of *C. taxifolia* in Lake Conjola would be expected to be negligible at the recorded range of winter water temperatures, but high at the range of summer water temperatures. Field observations confirm this seasonality in the growth of *C. taxifolia* both locally (E. West, pers obs.) and in the Mediterranean situation (Ceccherelli and Cinelli, 1999a, b). These initial laboratory experiments proved very useful in determining the effect of salinity change on growth and mortality of *C. taxifolia*, and suggest that a range of experiments investigating other environmental factors, such as temperature, light climate, nutrient additions and so on, would be beneficial in understanding the limitations of this pest species in the region.

These experiments used *C. taxifolia* from Lake Conjola, which has an entrance that is intermittently open and closed to the Pacific Ocean (West et al., 1985; Pollard, 1994). Historically, Lake Conjola has undergone periods of entrance opening and closing, including closures that have lasted several years. Long term data for water quality reflects these periods of opening and closing in that salinity is close to marine (35 ppt) when open and falls during periods of closure (Fig. 2). In 2001, Lake Conjola was artificially opened to the sea and, despite drought conditions, has remained permanently opened since that time. For the 12 years of data prior to 2001, salinity at most sites was 30 ppt or below. During a long period of lake closure from 1995 to 1998, salinity fell to below 20 ppt and for about 2 years during this period was below 17 ppt (Fig. 2).

Since the lake has been permanently open, salinity has remained above 30 ppt at most sites and the lake is mostly marine. The laboratory experiments demonstrated that *C. taxifolia* fragments taken from the lake have a low growth rate and higher mortality at salinities below 22.5 ppt. Salinities in the range of 15–17.5 ppt resulted in almost total mortality of all fragments, at temperatures between 15 and 30°C, under growth cabinet conditions. This would suggest that *C. taxifolia*, which is primarily a marine alga, would be severely impacted by long periods of low salinity, similar to those that have occurred naturally in previous years prior to entrance manipulation (Fig. 2). Overall, the combination of laboratory experiments on the growth of *C. taxifolia* fragments and the long-term data for water quality, indicate that manipulation of the entrance may be an important factor in the long-term survival of this invasive alga at Lake Conjola.

Artificial opening of the entrance has maintained salinities that are close to seawater, which appear optimum for the growth of this predominantly marine alga. While it is uncertain that this has directly caused the establishment of *C. taxifolia*, there is little doubt that the continuation of these marine conditions assists in its survival and growth. One option for management that could be considered is the return of Lake Conjola to its natural opening regimes, which may in the future include long periods of closure and lowered salinity. On the evidence of these laboratory experiments and existing literature, entrance closure, if associated with lowered salinity, would have a negative impact on growth and may lead to mortality of large areas of the invasive alga. Prior to taking such a potentially controversial and unpopular decision, further laboratory and fieldwork may be required. These findings should also be considered when deciding on artificially opening any estuaries near infested sites in the region, because permanently opening an estuary could create nearly marine conditions, which appear to be ideal for establishment and further invasion of *C. taxifolia*. It is somewhat ironic that a managerial strategy used to enhance the health of the lake is potentially sustaining one of its greatest threats.

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