Culture Studies on Two Morphological Types of *Caulerpa* (Chlorophyta) from Perth, Western Australia, with a Description of a New Species

T. J. B. Carruthers, D. I. Walker and J. M. Huisman*

Department of Botany, The University of Western Australia, Nedlands, Western Australia 6009, Australia

* *School of Biological and Environmental Sciences, Murdoch University, Murdoch, Western Australia 6150, Australia*

Abstract

The phenotypic plasticity and performance of two growth-forms of *Caulerpa racemosa* (Forsskål) J. Agardh, one found in estuarine conditions, the other growing in a coastal reef environment was studied in laboratory culture. There was no change in the morphology of either growth-form when cultured through a range of salinity (10–40‰) and light (20–140 $\mu Em^{-2}s^{-1}$) conditions. The estuarine growth-form performed better than the coastal one in low salinities (20‰), which suggests physiological adaptation to the estuarine environment. It was concluded that differences in morphology of both types were fixed rather than plastic. The taxonomic status of the estuarine growth-form was raised from a phenotypic variant of *C. racemosa* (Forsskål) J. Agardh to a new species, *Caulerpa lagara* sp. nov.

Introduction

The genus *Caulerpa* Lamouroux presents taxonomic difficulties due to high phenotypic plasticity. In *C. racemosa* (Forsskål) J. Agardh extreme morphological variation has been observed under different environmental conditions. Some twenty-two species of the genus *Caulerpa* Lamouroux (Chlorophyta, Caulerpaceae) have been recorded from the temperate waters of south-west Western Australia (Womersley 1984, Huisman and Walker 1990). One of the more abundant taxa on the Perth coastline is *Caulerpa racemosa* var. *laetevirens* (Montagne) Weber-van Bosse f. *cylindracea* (Sonder) Weber-van Bosse (Fig. 1a), which occurs from the intertidal reef flats to depths of at least 6 m. In the intertidal region it is an opportunistic species and grows during winter on reef flats covered with sand during the summer months. This taxon has also been recorded (as *Caulerpa cylindricea* Sonder) from the Swan River Estuary (Perth) by Royce (1955). An examination of Royce's material and more recent collections of the same taxon has shown, however,

that the estuarine plants differ significantly from *C. racemosa*, and could represent an autonomous species (Fig. 1b). The two populations are geographically distinct, one in a tidal estuary and the other on coastal reefs (Fig. 2). An occasional specimen of *C. racemosa* has a distichous arrangement of ramuli, but in these individuals the ramuli are always clavate. These specimens have not been found to occur in the transition between coastal and estuarine environments and are not considered to be intermediates.

Species of *Caulerpa* have shown high morphological plasticity when subjected to variations in environmental factors (Tandy 1934, Peterson 1972, Enomoto and Ohba 1987, Ohba and Enomoto 1987, Coppejans and Beeckman 1989) culture studies were necessary to determine the degree of environmentally induced morphological change possible in the estuarine plants. The culture studies undertaken were duplicated with the coastal *C. racemosa* var. *laetevirens* f. *cylindracea* to provide a comparison with a supposedly legitimate form of *C. racemosa*.
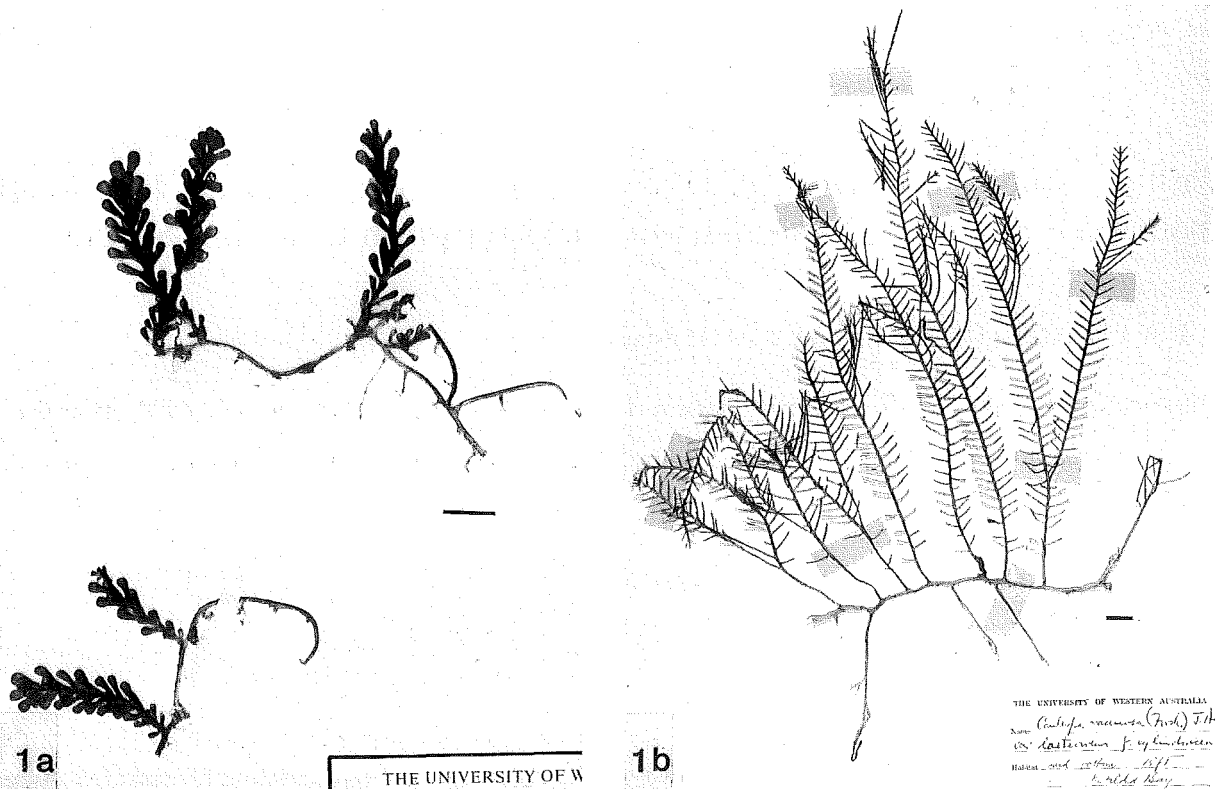
Fig. 1a. *Caulerpa racemosa* var. *laetevirens* form. *cylindracea* (scale bar = 1 cm). Fig. 1b. Holotype of *Caulerpa lagara* sp. nov. (UWA 3284) (scale bar = 1 cm).

Estuarine and coastal environments in south-west Western Australia display differences in several factors presently perceived as being important in the control of morphology in *Caulerpa*. Svedelius (1906) stated that substratum and light intensity (depth) can control morphology in *Caulerpa*. Morphological variation due to different light and temperature conditions was shown in laboratory culture by Ohba and Enomoto (1987). Coppejans and Beeckman (1989) suggested that the influences of salinity, water movement and emersion time may be valuable in further describing the relationship between environment and morphology in *Caulerpa*. The Swan River estuarine environment has mud substratum, seasonally brackish water (13−34.2‰), very low light (Sechii depths to 0.3 m) and cold temperatures (12.8−17.2 °C) (John 1983), whereas the coastal intertidal environment has solid limestone substratum, constant oceanic salinities, very high light intensities and potentially high temperatures.

This research was carried out to investigate whether estuarine and coastal growth-forms of *C. racemosa* are determined by environment factors or whether differences in morphology are fixed genotypically. Culture techniques were used to assess the short term morphological and performance (health) responses of both growth-forms to a range of environmental parameters, allowing assessment of their taxonomic sta-

tus. The null hypothesis was that both growth-forms were of the same genotype. It was thus expected that the morphological variation observed was only a short term response to the environmental stimuli varying between the two habitats and that both growth-forms would perform equally well under the range of experimental conditions.

Material and Methods

Caulerpa racemosa (estuarine growth-form) was collected at 1.5−2.5 m depth from Pelican Point in the Swan River during the winter of 1990 (Fig. 2). These estuarine specimens were cut into portions with two to five assimilators and at least one growing tip. To lessen numbers of bacteria and epiphyte spores, specimens were washed in seawater containing 'Miltons' anti-bacterial solution. The algae were left for 6 days before culture, to allow the wounds to heal (see Dreher *et al.* 1978). Specimens were slowly acclimatised to their experimental salinities. *Caulerpa racemosa* (coastal growth-form) was collected from the intertidal/subtidal reef to the south of the main Cottesloe groyne (Fig. 2). Material preparation was the same as that of the estuarine material.

Light and salinity were varied in a 4 × 4 culture experiment with five replicates of each treatment.

Light levels of 20, 60, 100 and 140 μEm^{-2}s^{-1} (12 : $\overline{12}$ h D/L cycle) and salinities of 10, 20, 30 and 40‰ were used. Temperature was maintained at 20 °C. One coastal and one estuarine specimen was placed into each 1 L conical flask. Due to the short term of the experiment it was not thought to be necessary to exchange the water in the flasks. Nutrients were not added to the flasks as there was found to be no enhancement of growth rate by addition of nutrients over the short term of the experiment (see Results) implying that cultures were not nutrient limited.

Seawater was filtered by a membrane filter (pore size: 1 μm). The required salinities were obtained by dilution of seawater (10, 20 and 30‰) and by evaporation (40‰). These salinities were measured using a Hand Refractometer (Nippon Optical Works, Japan). Light intensities remained constant over the experimental period ($\pm$ 5 μEm^{-2}s^{-1}). All light measurements were made with a Quantum sensor (Licor, Lamda Instrument Corp., U.S.A.).

Photographs of the algae were examined to determine whether any stolon, assimilator or rhizoid tissue had grown for each *Caulerpa* specimen. For each thallus it was noted whether the assimilators were branched or not. Shape and arrangement of ramuli were also recorded.

After 20 days under experimental conditions, the ecological performance (or survival potential) of all specimens was determined. Assimilator and stolon tissues were scored in terms of proportion of bleached thallus for this purpose. A score of four indicated that tissue was still all green ranging down to a score of zero when the tissue was entirely bleached. Each alga then had a total score out of eight indicating the performance, or health, of that alga under the particular experimental conditions. The performance score was changed to a proportion and these values were transformed using the $p' = \arcsin \sqrt{p}$ transformation (Zar 1984). These values were found to be normal or very close to normal using the 'Studentised Range Test' (Madansky 1988), and homogeneous using 'Cochran's Test' (Eisenhart *et al.* 1947). Two way ANOV's were then performed on each growth-form using 'Super ANOVA', and subsequently 'Tukey's multiple range test' was carried out on factors displaying significant effects. To assess the validity of the performance measure, chlorophyll content of specimens throughout the range of scores was measured. Thalli of ten specimens of each growth-form were divided into bleached and non-bleached tissue. Each (0.5 – 1.0 g) sample was placed immediately into 5 mL of cold 90% acetone and extracted at -15 °C for 24 h. Samples were then ground and the supernatant made up to a total volume of 5 mL. After centrifuging for 5 mins (relative centrifugal force 1500 $\times$ g) to remove all cell wall material from solution, absorbance was read at 665, 645 and 630 nm against a 90% acetone standard (on an LBK 'NOVASPEC'). Chlorophyll *a* concentration (mg/g) was calculated by the formula of Parsons and Strickland (1963).

Results

Initial experiments indicated that assimilator morphology of *Caulerpa racemosa* (coastal growth-form) was not influenced by substratum type: sand, limestone plate and limestone rubble were tested. Over all substratum treatments, 80% of individuals had strong rhizoid attachment after 4 days, acclimatisation to experimental conditions was thus assumed to be rapid. There was found to be no change in growth rates of *Caulerpa* when experimental flasks were nutrient enriched, aerated or enriched and aerated.

Morphology

The coastal growth-form of *Caulerpa racemosa* grew in 25 of the 64 scored cases, compared to 8 of those of the estuarine growth-form under culture conditions (Table I). When assimilator growth did occur, the morphology of the new thallus was always similar to that of the original thallus (Figs 3a and 3b). Both growth-forms of *C. racemosa* grew under a range of light and salinity treatments, showing most growth at 30 and 40‰ salinity (Table II). Neither growth-form
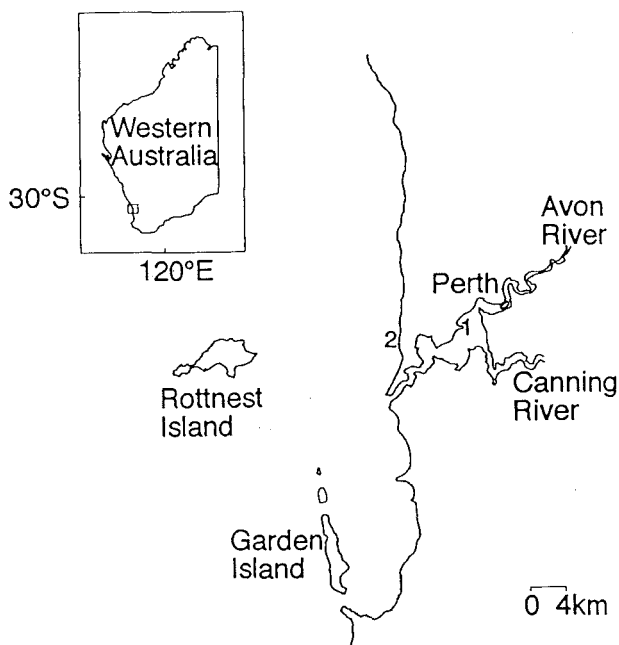

Fig. 2. Map of the Perth region showing estuarine (Pelican Point — site 1) and coastal (Cottesloe reef — site 2) collection sites.
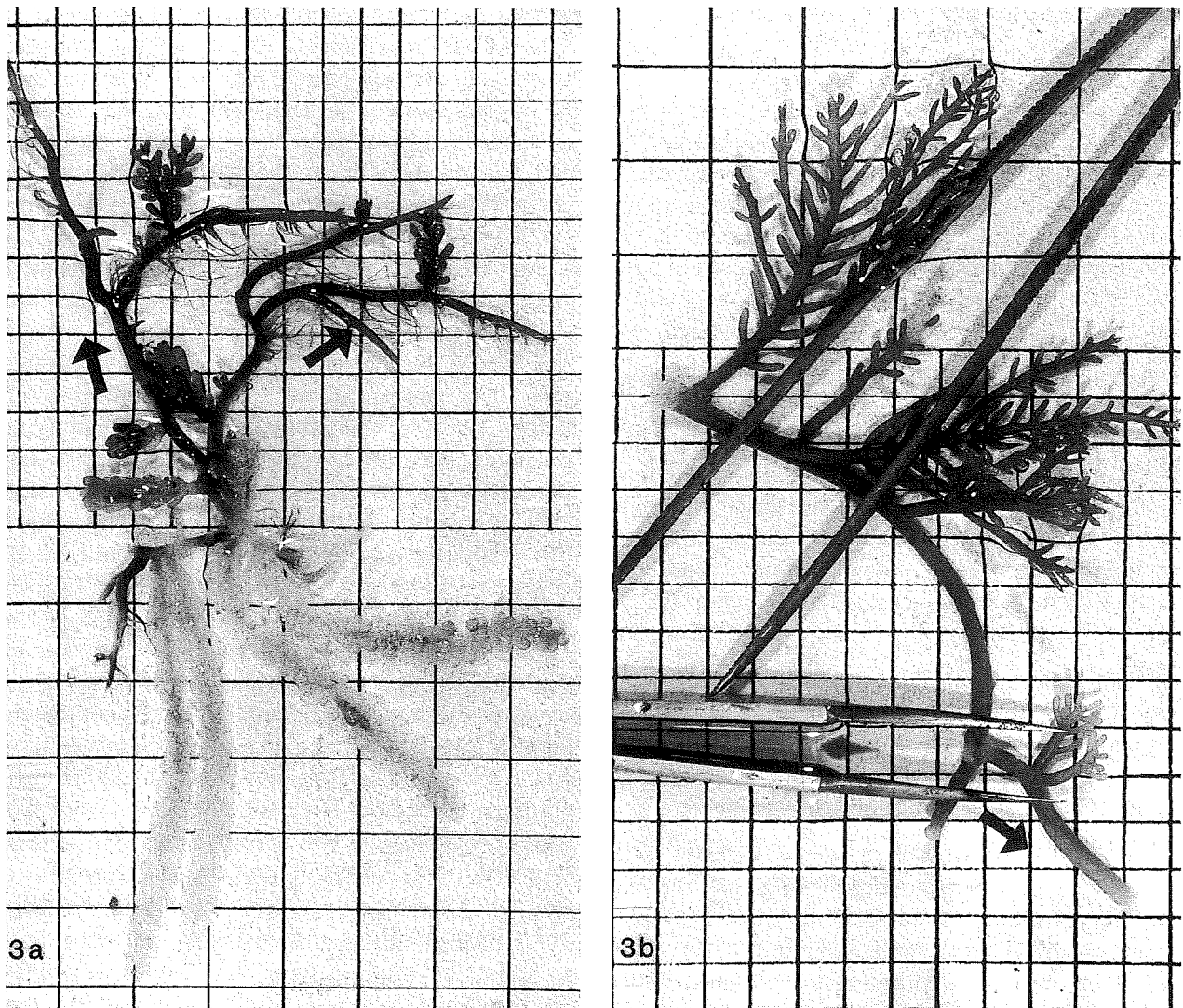
Fig. 3a. Coastal growth-form after culture at 60 $\mu Em^{-2}s^{-1}$ light and 30‰ salinity (large squares = 1 cm). Arrows indicate new growth, starting at the base of the arrow, in the direction of the arrow. Fig. 3b. Estuarine growth-form after culture at 20 $\mu Em^{-2}s^{-1}$ light and 30‰ salinity (large squares = 1 cm). Arrows indicate new growth, starting at the base of the arrow, in the direction of the arrow.

grew at 20‰ salinity. The coastal growth-form showed slightly higher growth at 20 and 60 $\mu Em^{-2}s^{-1}$ light, but there was no apparent influence on the estuarine growth-form (Table III).

Both growth-forms of *C. racemosa* showed very low levels of morphological variation for a range of light and salinity conditions in culture. The new ramuli of coastal growth-form algae were clavate and distinctly radially arranged (Fig. 3a). The new growth on the estuarine growth-form had the robust appearance of the parent tissue (see stolon and stem of assimilator). The ramuli were terete, notice particularly the lowest (oldest) ramuli on the new growth of the estuarine growth-form and compare to the ramuli on the parent assimilators (Fig. 3b). The density of ramuli reduced slightly in the coastal growth-form under experimental conditions, however in the estuarine growth-form, ramuli density appears to have remained constant (Figs 3a and 3b).

Table I. Number of algae showing growth of different organs under culture conditions.

Growth-form	No. of scored algae	No. showing growth	Organ showing growth		
			Stolon	Assimilator	Rhizoid
Coastal	64	25	25	11	22
Estuarine	64	8	6	7	1

Table II. Number of algae showing growth under various salinity conditions.

Growth-form	Salinity (‰)			
	10	20	30	40
Coastal	1	0	12	12
Estuarine	1	0	4	3

Table III. Number of algae showing growth under various light conditions.

Growth-form	Light ($\mu Em^{-2}s^{-1}$)			
	20	60	100	140
Coastal	7	10	3	5
Estuarine	3	1	2	2

Performance

A significant correlation was determined between chlorophyll concentration and performance ($R = 0.552$, $p = 0.0116$) (Fig. 4).

Neither growth-form of *C. racemosa* showed a significant performance response to the interaction of light and salinity (coastal: $F = 1.478$, $p = 0.175$, df = 9; estuarine: $F = 0.356$, $p = 0.951$, df = 9). The estuarine growth-form performed equally well at lower and higher light intensities ($F = 0.156$, $p = 0.9257$, df = 3) (Fig. 5). ANOVA detected a significant difference in performance of coastal growth-form with light treatment ($F = 5.072$, $p = 0.0033$, df = 3) (Fig. 5), however Tukey's test was unable to discriminate a significant difference.

Performance of the estuarine growth-form was significantly affected by salinity ($F = 97.284$, $p = 0.0001$, df = 3) and so was that of the coastal growth-form ($F = 55.451$, $p = 0.0001$, df = 3) (Fig. 6). Turkey's tests showed that salinities of 10 and 20‰ resulted in lower performance than 30 and 40‰ for the coastal growth-form. The performance of the estuarine growth-form was least at 10‰ salinity, and was less at 30‰ than at 20 or 40‰. Most importantly, at 20‰ salinity, performance of the estuarine growth-form was better than the coastal growth-form.

Discussion

The lack of morphological variation of both coastal and estuarine growth-forms under the conditions tested here, contrasts with many studies on *C. racemosa* (Tandy 1934, Peterson 1972, Calvert 1976, Enomoto and Ohba 1987, Ohba and Enomoto 1987). Peterson (1972) obtained growth of new morphology after six to ten days, so the present experiment was considered long enough for potential growth. Lack of variation in the coastal growth-form may be partly because the experiment was carried out at 20 °C, although variations in levels of plasticity have been recorded before (Calvert 1976). Calvert (1976) observed a retention of radial ramuli arrangement in *C. racemosa* var. *uvifera* (Turner) Weber-van Bosse in response to low light that contrasted the study by Nasr (1947 in Calvert 1976) and Peterson (1972) on the same variant. Calvert (1976) suggested that this difference was due to the evolutionary history of the geographically separated populations. The present
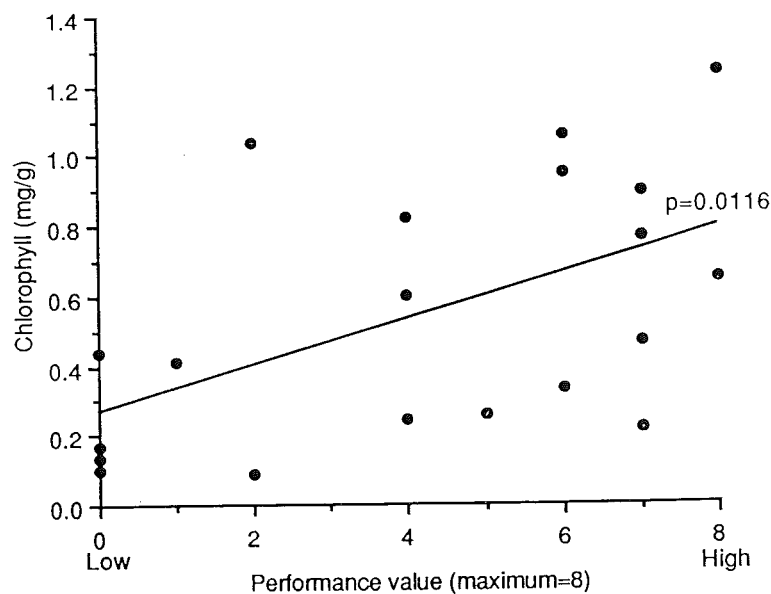

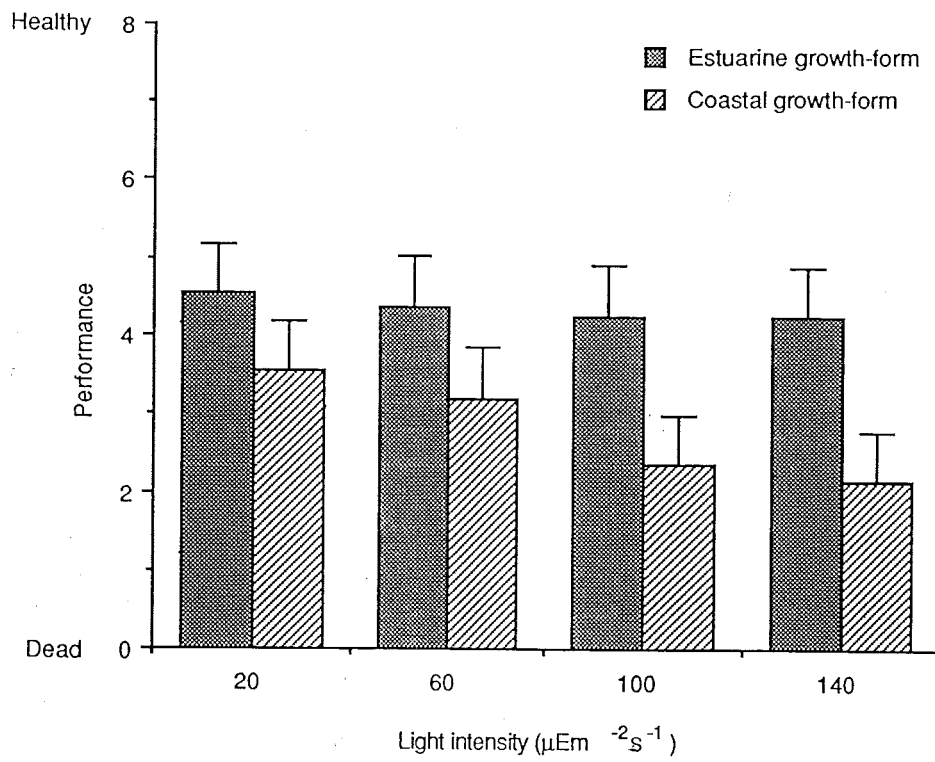Fig. 4. Correlation of scored performance value with average Chlorophyll concentration per plant (R = 0.552).

Fig. 5. The performance of both coastal and estuarine growth-forms of *Caulerpa* in response to light intensity (mean ± Standard Error).

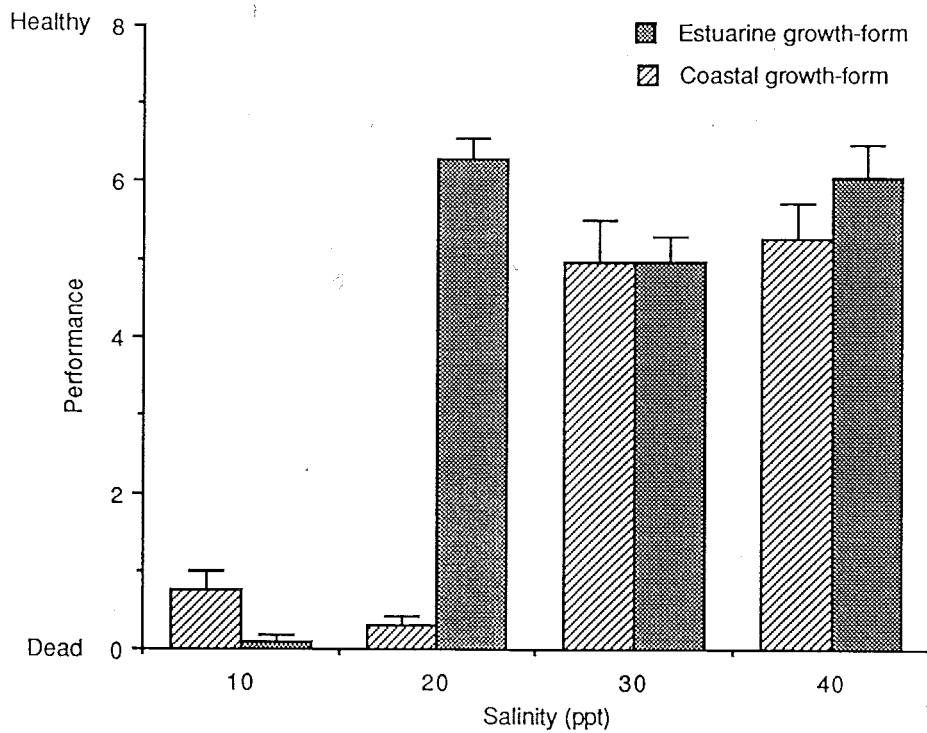Fig. 6. The performance of both coastal and estuarine growth-forms of *Caulerpa* in response to salinity (mean ± Standard Error).

study supports Calvert's idea that geographic and evolutionary differences may result in varying degrees of plasticity in *Caulerpa* spp.

Distinct differences were observed in the physiological responses of the two different forms of *C. racemosa*. The slight increase in performance (*i. e.* Chlorophyll *a* content) of the coastal growth-form at lowest light intensities may indicate a physiological response to low light. This is consistent with the low levels of morphological variation observed as it may be an alternate response to low light conditions. Overall, light was not a large influencing factor of morphology or performance for either growth-form of *Caulerpa* used in this study.

The estuarine growth-form of *Caulerpa* shows adaptation to periods of low salinity. While no growth was observed, the good performance at 20‰ salinity indicates that this growth-form can survive at least short periods of reduced salinity and presumably continue growth once salinity levels increase.

The present study has shown that the morphology of the estuarine plants is genetically fixed and unaffected by environmental conditions. The morphological distinction between these two algae has apparently occurred in the longer term rather than the short term and thus implies evolutionary adaptation rather than phenotypic plasticity. Thus we propose that the estuarine plants be recognised as a separate species from *C. racemosa*, *Caulerpa lagara* sp. nov.

Diagnosis

Caulerpa lagara Carruthers, Walker *et* Huisman, sp. nov.

Plantae medio- ad atrato-virides in arena leptogranulosa crescens; stolones grossi, nudi, cartilagini, 1−2(−3)mm diametro; axes erecti simplices vel irregulariter ramosi, (5−)9−13(−26) cm alti, 1−2 mm diametro, ramulis alternis vel oppositis distichis ferentibus; rami ex ramulis orientibus; ramuli vesciculati, teretes, generatim ubique diametro similari et apicibus obtusis vel aliquando clavatis vel acutis, 5−13.5(−23.5) mm longi et (0.4−)0.5−1(−1.1) mm diametro.

Plants medium to dark green, growing on fine sand or mud; stolon course, naked, cartilaginous, 1.0−2.0(−3.0) mm in diameter; erect axes simple or irregularly branched, (5−)9−15(−26) cm high, 1.0−2.0 mm in diameter, bearing alternate or opposite, distichous ramuli; branches developing from ramuli; ramuli vesiculate, terete, generally of similar diameter throughout with obtuse tips, occasionally clavate or tapering to a point, 5.0−13.5(−23.5) mm long, (0.4−)0.5−1.0(−1.1) mm in diameter.

Etymology: The specific epithet 'lagara' is Greek for 'lanky', and refers to the tall and sparsely branched habit of the species.

Type specimen: UWA 3284, 1957. (Carlyle and Smith) (Fig. 1b).

Type locality: Pelican Point, Swan River, Perth Western Australia.

Remarks: The culture studies undertaken have clearly demonstrated that *Caulerpa lagara* is distinct from (at least) one variety of *Caulerpa racemosa* (var. *laetivirens* form *cylindracea*) and on that basis warrants recognition as a distinct species. The morphology of the new species approaches that of *C. corynephora* Montagne (type locality, Torres Strait, Australia), which is presently regarded as a variety of *C. racemosa* [var. *corynephora* (Montagne) Weber-van Bosse]. The two taxa apparently also share similar habitat preferences, both are generally found on unconsolidated sand or mud. Published illustrations of *C. racemosa* var. *corynephora*, however, invariably show a plant bearing clavate ramuli with regularly rounded apices (*e. g.* Weber-van Bosse 1898, pl. XXXIII, Coppejans 1992, fig. 4A, Coppejans and Prud'Homme van Reine 1992, fig. 15A) as is typical of ramuli in most varieties of *C. racemosa*. Although occasional plants of *C. lagara* bear slightly clavate ramuli, the plants generally produce terete ramuli of similar diameter throughout, mostly tapering to a point. This morphology has not been reported for any varieties of *C. racemosa*, including var. *corynephora*.

Other species possibly related to *C. lagara* include *C. faridii* Nizamuddin (1964, p. 210), which differs in the smaller diameter of its rhizome and flattened erect axes (Nizamuddin 1964), and *C. veravalensis* Thivy *et* Chauhan, which (as described by Coppejans and Meinesz 1988, p. 188), also produces flattened erect axes. Erect axes in *C. lagara* are consistently terete.

References

Calvert, H. E. 1976. Culture studies on some Florida species of *Caulerpa*: morphological responses to reduced illumination. *Phycol. J. 11*: 203–214.

Coppejans, E. and A. Meinesz. 1988. Marine algae of Papua New Guinea (Madang, Prov.) 1. Caulerpaceae (Chlorophyta — Caulerpales). *Blumea 33*: 181–196.

Coppejans, E. and W. F. Prud'Homme van Reine. 1992. Seaweeds of the Snellius — II Expedition (E. Indonesia): the genus *Caulerpa* (Chlorophyta — Caulerpales). *Bull. Séanc. Acad. r. Sci. Outre-Mer Meded. Zitt. K. Acad. Overzeese Wet. 37*: 667–712.

Coppejans, E. and T. Beeckman. 1989. *Caulerpa* section Sedoideae (Chlorophyta, Caulerpales) from the Kenyan coast. *Nova Hedwigia 49*: 381–393.

Dreher, T. W., B. R. Grant and R. Wetherbee. 1978. The wound response in the siphonous alga *Caulerpa simpliciuscula* C. Ag.: Fine structure and cytology. *Protoplasma 96*: 189–203.

Eisenhart, C., M. W. Hastay and W. A. Wallis. 1947. *Techniques of Statistical Analysis*. Chapter 15. McGraw-Hill, New York.

Enomoto, S. and H. Ohba. 1987. Culture studies on *Caulerpa racemosa* (Caulerpales, Chlorophyceae) I. Reproduction and development of *C. racemosa* var. *laetevirens*. *Jpn. J. Phycol. 25*: 167–177.

Fritsch, F. E. 1935. *The Structure and Reproduction of the Algae. Volume I*. Cambridge University Press, London. 791 pp.

Huisman, J. M. and D. I. Walker. 1990. A catalogue of the marine plants of Rottnest Island, Western Australia, with notes on their distributions and biogeography. *Kingia 1*: 349–459.

John, J. 1983. *The Diatom Flora of the Swan River Estuary Western Australia*. Bibliotheca Phycologia, 64. J. Cramer, Hirschberg, Germany. pp. 8–12.

Macraild, G. N. 1981. Chlorophyta. *In*: (Clayton, M. N. and R. J. King, eds) *Marine Botany — an Australasian Perspective*. Longman, Cheshire. pp. 180–199.

Madansky, A. 1988. *Prescriptions for Working Statisticians*. Springer-Verlag, Berlin. pp. 34–36.

Nizamuddin, M. 1964. Studies on the genus *Caulerpa* from Karachi. *Bot. Mar. 6*: 204–222.

Norton, T. A., A. C. Mathieson and M. Neushul. 1981. Morphology and environment. *In*: (Lobban, C. S. and M. J. Wynne, eds) *The Biology of Seaweeds*. Blackwell Sci. Publ., Oxford. pp. 421–454.

Ohba, H. and S. Enomoto. 1987. Culture studies on *Caulerpa* (Caulerpales, Chlorophyceae) II. Morphological variation of *C. racemosa* var. *laetevirens* under various culture conditions. *Jpn. J. Phycol. 25*: 178–188.

Parsons, T. R. and Strickland, J. D. H. 1963. Discussion of spectrophotometric determination of marine-plant pigments, with revised equations for ascertaining Chlorophylls and Carotenoids. *J. Mar. Res. 21*: 155–163.

Peterson, R. D. 1972. Effects of light intensity in the morphology and productivity of *Caulerpa racemosa* (Forsskål) J. Agardh. *Micronesica 8*: 63–86.

Royce, R. D. 1955. Algae of the Swan River. *In*: *Swan River Reference Committee — Report by Sub-committee on Pollution of the Swan River*. Government Printer, Western Australia. pp. 41–50.

Svedelius, N. 1906. Reports on the marine algae of Ceylon. 1. — Ecological and systematic studies of the Ceylon species of *Caulerpa. Rep. Ceylon Mar. Biol. Lab.* Pt. 2, No. 4: 81–144.

Tandy, G. 1934. Experimental taxonomy in marine algae, with special reference to *Caulerpa. Proc. Linn. Soc. London 146*: 63–64.

Weber-van Bosse, A. 1898. Monographie des *Caulerpa. Ann. Jard. Bt. Buitenzorg. 4*: 243–401, 15 pls.

Womersley, H. B. S. 1984. *The Marine Benthic Flora of Southern Australia. Part I*. Government Printer, Adelaide, South Australia. pp. 253–274.

Zar, J. H. 1984. *Biostatistical Analysis* second edition. Prentice-Hall Inc., Inglewood Cliffs. 718 pp.