

Temperature tolerances of two southern African *Ecklonia* species (Alariaceae: Laminariales) and of hybrids between them

J. J. Bolton¹ and R. J. Anderson²

¹ Botany Department, University of Cape Town; 7700 Rondebosch, Republic of South Africa

² Seaweed Unit, Sea Fisheries Research Institute; Private Bag X2, Rogge Bay, Cape Town, Republic of South Africa

Abstract

The temperature tolerances in culture of *Ecklonia maxima* (Osbeck) Papenf., the dominant kelp in the Benguela upwelling region on the west coast of southern Africa, and the smaller *E. biruncinata* (Bory) Papenf., growing on the warmer south coast, have been investigated. Vegetative growth and reproduction of gametophytes show very similar patterns in the two species, although temperature optima in *E. biruncinata* are consistently 2° to 3°C higher than those of *E. maxima*. Reciprocal crosses between clonal female and male gametophytes of the two species produced healthy juvenile sporophytes, indistinguishable from the parent crosses. All parents and hybrids grew well over a wide range of temperatures (8° to 22°C), with *E. biruncinata* sporophytes again having slightly higher optima than *E. maxima*. Both reciprocal crosses exhibited optima intermediate between the parent crosses. The results are discussed with respect to the origins and evolution of *Ecklonia* species in the region.

Introduction

It has recently been put forward (Bolton, 1986) that southern Africa has two warm temperate seaweed floras, with the major disjunction between them in the region of Cape Agulhas, the most southerly point of the continent (Fig. 1). The distribution of warm temperate kelps of the genus *Ecklonia* reflects this pattern. The largest species of the genus *E. maxima* occurs on the west coast of the region, has smooth fronds and is characterised by hollow stipes which may be over 10 m in length, with a distal swollen gas-filled bulb (see Papenfuss, 1942). Extensive inshore beds of this kelp occur from Aasfontein, 15 km west of Cape Agulhas, westwards, at least as far as Swakopmund in Namibia (Fig. 1). On the south coast, corresponding to the other warm temperate marine phyto-

geographic region, the much smaller *E. biruncinata* is common in littoral rock pools and the sublittoral. This species is commonly less than 50 cm-tall, with a short, solid stipe (although in a few individuals, some breakdown of the medullary tissue has been observed). The plants are characterised by a dense cover of spines on the sporophylls, and usually also the primary blade, although an individual population may have plants with only a few or sometimes no spines. The taxonomy of *Ecklonia* spp. in the region is in need of review, and it is likely that the species here referred to as *E. biruncinata* (Papenfuss, 1944) should be included in *E. radiata* (C. Ag.) J. Ag., as plants of the latter species in Australia and New Zealand can also be smooth or spinous (Womersley, 1967; I. Novacek, *personal communication*). Although drift plants of *E. biruncinata* are common at Die Dam (30 km west of Cape Agulhas), attached intertidal plants of this species occur

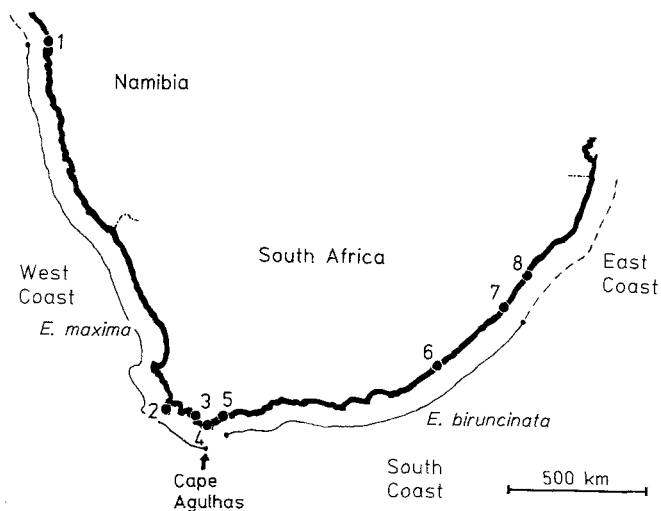


Fig. 1. Map of southern Africa showing distribution of *Ecklonia* species (dotted line = possible distribution). Numbered sites are: 1. Swakopmund; 2. Kommetjie; 3. Die Dam; 4. Cape Agulhas; 5. De Hoop; 6. Qolora (Kei Mouth); 7. Port Edward; 8. Durban

only from De Hoop Nature Reserve (60 km east of Cape Agulhas) eastwards. This species grows intertidally as far east as Port Edward (Fig. 1). The subtidal eastward extension of this species is not known, and populations of an as yet undetermined *Ecklonia* species, which appears to fit in the *E. radiata* complex, occur in deep water at sites off the coast of Natal (G. Lambert, *personal communication*). Gametophytes of *E. maxima* were first described by Papenfuss (1942), and their growth in various environmental conditions in the laboratory has been studied by Branch (1974) and Bolton and Levitt (1985). In this investigation we compare temperature tolerances of south coast *E. biruncinata* with those of *E. maxima* from the colder Benguela upwelling region on the west coast of southern Africa. The temperature tolerances of small sporophytes of these two species in culture, and of hybrids between them, are also described in order to gain information on the genetic basis of the link between distribution and temperature tolerance in southern African *Ecklonia*.

Materials and methods

Fertile sporophylls of the two species were collected at Kommetjie, Lat. 34° 8' S; Long. 18° 20' E [*Ecklonia maxima* (Osbeck) Papenf.] and Glengarriff, 32° 55' S, 28° 6' E [*E. biruncinata* (Bory) Papenf.] in early summer (November). Zoospore release was stimulated by first wiping the fertile area clean, and then placing the sporophylls together with a moistened paper towel in closed crystallizing dishes at 8°C in the dark overnight. Zoospores were released on placing the plants in enriched seawater medium (PES; Provasoli, 1968) the following morning. One ml of spore suspension was added to 200 ml of PES medium in crystallizing dishes containing glass slides where spores could settle. For the gametophyte experiments, these dishes were then placed in experimental conditions. To inhibit diatom contamination, germanium dioxide (0.1 ml of an aqueous saturated solution per litre of PES) was added to the initial medium. Experimental media were renewed weekly, and subsequent changes of medium had no added germanium.

For sporophyte experiments, unialgal cultures of gametophytes were first obtained by growing cultures of settled zoospores in red light of ca $5 \mu\text{E m}^{-2} \text{s}^{-1}$, produced by white light through red Plexiglas, followed by the isolation of clean, individual male and female gametophytes (Lüning and Dring, 1975). Single species and hybrid sporophytes were produced by gently grinding the gametophytes with a mortar and pestle to produce suspensions of male and female filaments 1 to 10 cells long. These were placed in crystallizing dishes containing a glass slide and PES medium, and placed in white light. All experiments were conducted at an irradiance of $55 (\pm 5) \mu\text{E m}^{-2} \text{s}^{-1}$ provided by cool white fluorescent lamps. Control cultures containing only female gametophytes of the two species were set up to investigate whether sporophytes in the hybrid cultures were being produced parthenogenically.

All cultures were grown, with weekly change of medium, until the hybrid sporophytes were approximately 1 mm in length, (measured from transition region to apex), after five weeks. The length of 20 plants of each cross was measured to serve as an 'initial length'. Ten plants of the crosses were then placed in each experimental dish. Three replicates were placed in each experimental temperature and grown for 14 d, after which time the 'final length' of the 30 sporophytes was measured. Relative growth rate was then calculated using the formula:

$$\text{Relative growth rate} = \log_e (\text{final length}/\text{initial length})/T$$

Experimental dishes were incubated in controlled temperature rooms at 4°, 8° and 15°C. Other temperatures were obtained in 8° and 15°C controlled temperature rooms using water baths in which the dishes were immersed to the level of the medium. Temperatures of 4°, 8°, 12°, 15°, 19°, 22°, 26° and 30°C (each $\pm 1^\circ\text{C}$) were thus achieved.

Results

Gametophyte tolerances

In Figs. 2 and 3, the temperature tolerances of *Ecklonia biruncinata* gametophytes are compared with previously published information on *E. maxima* (Bolton and Levitt, 1985). Vegetative growth of female gametophytes (as length at 7 d, Fig. 2) shows a similar pattern in the two species, except that the *E. biruncinata* plants are slightly larger and their optimal temperature is slightly warmer (19° to 22°C) than is the case for *E. maxima* (17.5° to 20°C). The individual temperatures used in the present investigation were slightly different than those of Bolton

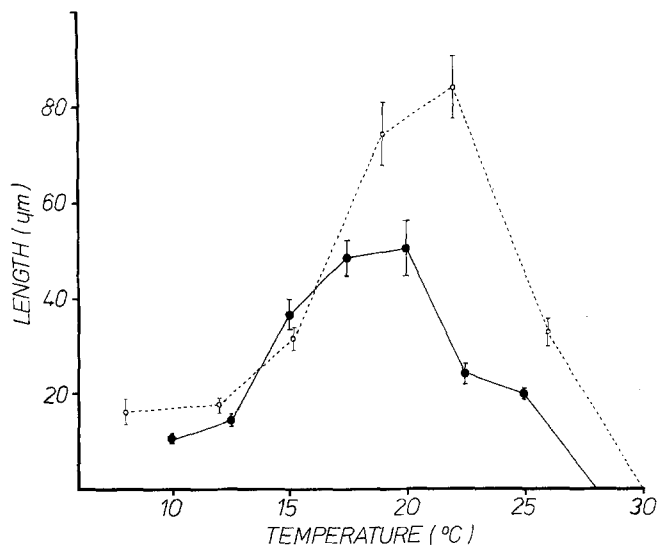


Fig. 2. Length of female gametophytes of *Ecklonia maxima* (solid line) and *E. biruncinata* (dotted line) after 7 d ($\pm 95\%$ confidence limits)

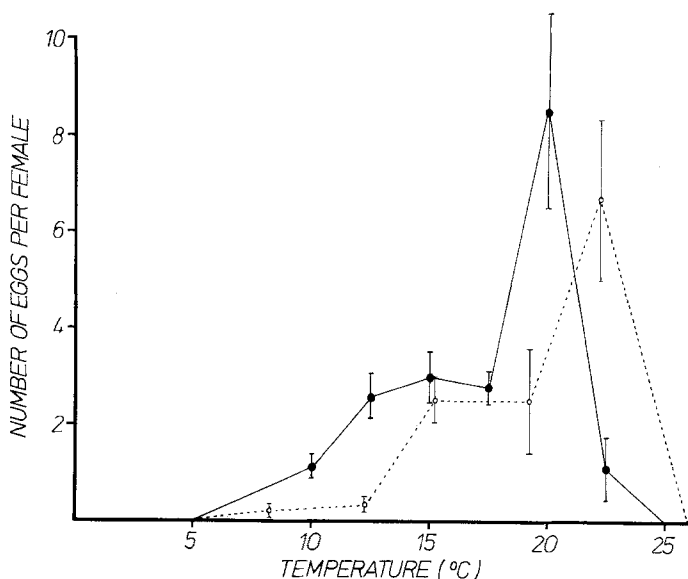


Fig. 3. Numbers of eggs produced per female gametophyte of *Ecklonia maxima* (solid line) and *E. biruncinata* (dotted line) after 20 d (\pm 95% confidence limits)

and Levitt (1985), although they covered a similar range. The number of eggs produced per female gametophyte (after 20 d, Fig. 3) again shows a very similar pattern in the two species over the range of temperatures, with a region of consistent egg production of around 2.5 to 3 eggs per female (12.5° to 17.5°C in *E. maxima*, and 15° to 19°C in *E. biruncinata*), and a large peak of egg production at slightly higher temperatures (20°C in *E. maxima* and 22°C in *E. biruncinata*).

Parent crosses and both reciprocal hybrid crosses were equally successful. The small sporophytes produced in the four crosses could not be distinguished from each other based on morphological grounds. Cultures containing only female gametophytes of each of the two species produced very small numbers of twisted sporophytes, showing poor development. Optimal growth rate of all parent and hybrid crosses were in the range 10.9 to 12.6% per day (Fig. 4). All parents and hybrids grew well over a wide range of temperatures (8° to 22°C), and poorly at 4°C. At 26°C, the pure strain *Ecklonia maxima* sporophytes began disintegrating distally after 7 d, and were dead after 14 d. The *E. biruncinata* pure strain plants and both reciprocal crosses survived, with slow growth, at this temperature. The temperature/growth curve for the pure *E. biruncinata* plants (Fig. 4a) is skewed to the right, with optimum growth in the range 15° to 19° (-22°C). The pure *E. maxima* plants grew marginally better at temperatures at the lower end of the optimal range, i.e. 8° to 15° (-19°C). The two reciprocal crosses (Fig. 4b) show very similar temperature growth curves, although the hybrid with the *E. maxima* female parent (M×B) grew at a slightly faster rate at all temperatures. Both show a broad temperature preference, although with a slight peak at 15°C.

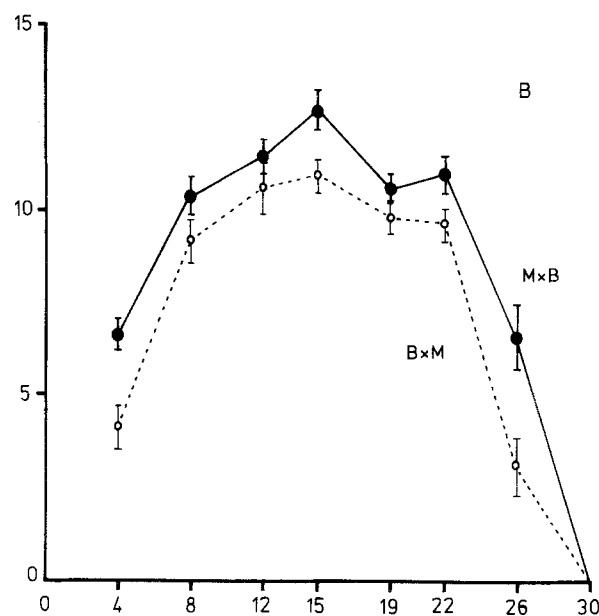
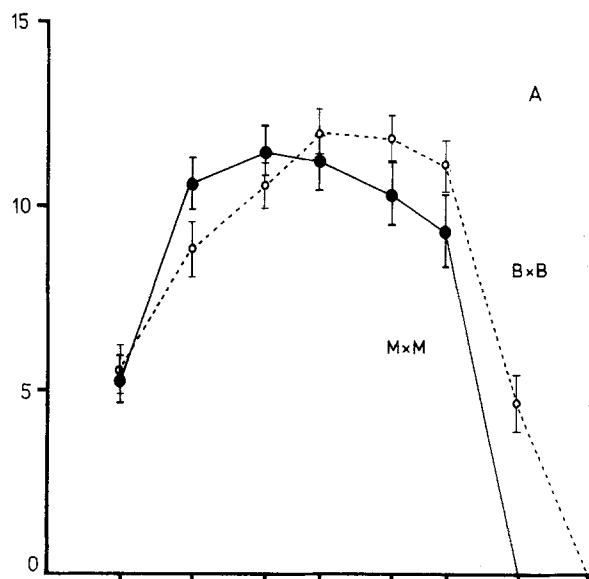


Fig. 4. Relative growth rate of small hybrid sporophytes (% per day) over two weeks at temperature. B=*Ecklonia biruncinata*, M=*E. maxima*; Females of cross mentioned first (\pm 95% confidence limits)

Discussion

The west and south coast marine phytogeographic regions in the southern African inshore can be defined by their temperature regimes (Bolton, 1986). The west coast (with large beds of *Ecklonia maxima*) is a region with annual mean inshore sea water temperatures in the range of 12° to 16°C, mean in the warmest month in the range of 13° to 19°C, and mean in the coldest month in the range 11.5° to 14.5°C; all temperatures are 10-year means supplied by the South African Maritime Weather Office, and extracted from Bolton, 1986. The south coast (coinciding with the distribution of *E. biruncinata*) has annual

mean sea temperatures in the range of 17° to 19°C, mean in the warmest month in the range of 19° to 21.5°C, and mean in the coldest month in the range of 13.5° to 17°C. These warmer ambient temperatures in the *E. biruncinata*, as compared to the *E. maxima* habitats, are reflected in the *in-vitro* temperature tolerances of both gametophytes (vegetative growth and reproduction) and small sporophytes (vegetative growth).

Female gametophytes of *Ecklonia maxima* show the fastest vegetative growth at 20°C, but very poor growth at 22.5°C (Fig. 2, data from Bolton and Levitt, 1985). This can be compared with a maximum monthly mean in the species' natural range of 19°C. Similarly, *E. biruncinata* females show most rapid growth at 22°C, and very poor growth at 25°C, compared with a maximum monthly mean of 21.5°C. A previous study with nine species of Laminariales (Lüning and Neushul, 1978) revealed a correlation between geographical distribution and vegetative growth of female gametophytes, in that species from southern California had higher temperature optima (17°C) than those from central California (12°C). The *Ecklonia* spp. data follow this pattern, with these two species of warm temperate kelps having optima much higher than any of the Californian Laminarians, and the upper limit for good vegetative growth of gametophytes being closely related to the maximum ambient temperatures over the range of the species.

The pattern of egg production with temperature in *Ecklonia maxima* was explained in detail by Bolton and Levitt (1985). In the range 12.5° to 17.5°C, this species produced eggs most rapidly, resulting in 2.5 to 3.0 eggs per female. At a slightly higher temperature (20°C), the onset of reproduction was slightly delayed, although eventual egg production per gametophyte was much higher. It was thought that this may be an ecological adaptation which improves survival prospects towards the limits of the geographical distribution of the species. The same pattern is evident for *E. biruncinata* gametophytes, with most rapid egg production in the range of 15° to 19°C, but eventual maximum egg production per female at 22°C (Fig. 3). A similar pattern has also been reported for New Zealand *E. radiata* (Novaczek, 1984). If we follow the explanation of Bolton and Levitt (1985), and also of some previous authors working with Laminarian gametophytes (for a discussion of this see Kain, 1979), we can suggest that egg production is optimal when it is most rapid, even though fewer eggs are eventually produced per female gametophyte, i.e. at 12.5° to 17.5°C for *E. maxima* and 15° to 19°C for *E. biruncinata*. These suggested optimal temperatures for gametophyte development are close to the range of annual mean temperatures over the distributions of the two species, i.e. 12° to 16°C (*E. maxima*) and 17° to 19°C (*E. biruncinata*).

Small sporophytes of both species (Fig. 4a) show reasonable growth over a wide range of temperatures (8° to 22°C), although the species can be distinguished at this stage only by a slight preference for lower temperatures in *Ecklonia maxima* and for higher temperatures in *E.*

biruncinata. These patterns are interesting for a comparison with the range of monthly means over the distributions of the two species, i.e. 11.5° to 19°C (*E. maxima*) and 13.5° to 21.5°C (*E. biruncinata*). Sporophytes of *E. maxima* are fertile all year round, although sorus production and spore release per plant have a distinct seasonal pattern (Joska and Bolton, 1987). It is thus not surprising that small sporophytes should be capable of good growth over the whole range of habitat temperatures. The optimum for growth of young sporophytes of kelp species corresponds to temperature regions, i.e. Arctic to cold temperate kelps-*Laminaria longicruris* de la Pyl., 10°C (Bolton and Lüning, 1982); *Saccorhiza dermatodea* (Pyl.) J.Ag., 10°C (Norton, 1977); *Laminaria saccharina* (L.) Lamour., *Laminaria digitata* (Huds.) Lamour., 10° to 15°C; Cold temperate to warm temperate kelp-*Laminaria hyperborea* (Gunn.) Fosl., 15°C (Bolton and Lüning, 1982); warm temperate kelps-*Undaria pinnatifida* (Harv.) Suringar, 15° to 20°C (Akiyama, 1965); *Saccorhiza polyschides* (Lightf.) Batters, 23°C (Norton, 1977). The very broad optimal temperature range for the growth of small sporophytes of *Ecklonia maxima*, 8 to 19 (–22°C) may reflect the adaptation of a warm temperate plant to a temperature regime which is only marginally warm temperate in comparison with other world regions (see Bolton, 1986).

Although these differences in pattern between the two species are slight, there is a strong suggestion that the temperature tolerances of the hybrid sporophytes in both reciprocal crosses (Fig. 4b) have a pattern intermediate between those of the two parent kelps. Intraspecific (ecotypic) variation in temperature tolerance has been observed on a number of occasions in the brown algae, notable in *Laminaria saccharina* (L.) Lamour. (Lüning *et al.*, 1978) and *Ecklonia radiata* (Novaczek, 1984). Also, crosses between strains of *Ectocarpus siliculosus* (Dillw.) Lyngb. with different temperature tolerances (Bolton, 1983) showed a narrower optimum than either parent, intermediate between the parent optima. In this latter study, as with the present *Ecklonia* spp. data, the optimum of the hybrids also corresponded with the original temperature of the stock cultures (20°C for *E. siliculosus* 15°C for *Ecklonia* spp.). The possibility of selection of the hybrids for temperature tolerance can thus not be ruled out.

The ability for hybridisation between species of closely related Laminarians has been recorded on a number of occasions, i.e. between *Laminaria ochotensis* Miyabe, *L. japonica* Aresch., and *L. religiosa* Miyabe (Tokida and Yabu, 1962) and between *Laminaria saccharina*, *L. longicruris* and *L. ochotensis* (Lüning *et al.*, 1978; Bolton *et al.*, 1983). An intergeneric cross between *Macrocystis pyrifera* (L.) C.Ag. and *Pelagophycus porra* (Leman) Setchell also produced healthy sporophytes in the laboratory, similar to apparent hybrid sporophytes which are occasionally found in nature (Sanbonsuga and Neushul, 1978; Coyer and Zaugg-Haglund, 1982), although these plants are infertile. Only in crosses between *Laminaria longicruris* and *L. saccharina* have fertile hybrids between species been re-

corded (Bolton *et al.*, 1983), although these species have been considered synonymous by some authors (as *L. saccharina* ssp. *longicuris*, see Lüning, 1985). Thus, the production of small rapidly-growing sporophytes between two species of *Ecklonia* does not demonstrate complete interfertility. The success of these hybridisation experiments nevertheless provides further evidence that *E. maxima* is a relatively recent evolutionary development from smaller forms of the *E. radiata* type which occur in other regions, and are still represented on the east coast of southern Africa by *E. biruncinata* (see Bolton and Anderson, 1985; Hommersand, 1986). The influence of cool upwelled water from the Benguela current is a fairly recent geological event (for a review see Shannon, 1985). Persistent upwelling (the proto-Benguela) was initiated in the middle to late Miocene (14 to 11 million years ago), although the formation of the Benguela system similar to the present is considered to have occurred less than three million years ago. The evolution of a large hollow-stiped *Ecklonia* sp., capable of forming large forests dominating the inshore regions, from a smaller, short-stiped, more warm tolerant plant must have occurred within this time scale.

Acknowledgements. The authors would like to thank the University of Cape Town, the South African CSIR (FRD), and the Director of the Sea Fisheries Research Institute, for financial assistance and use of facilities, and Mr. Sciocatti and the S. A. Maritime Weather Office for sea temperature data.

Literature cited

- Akiyama, K.: Studies of ecology and culture of *Undaria pinnatifida* (Harv.) Sur. II. Environmental factors affecting the growth and maturation of gametophyte. Bull. Tohoku reg. Fish. Res. Lab. 25, 143–170 (1965)
- Bolton, J. J.: Ecoclinical variation in *Ectocarpus siliculosus* (Phaeophyceae) with respect to temperature growth optima and survival limits. Mar. Biol. 73, 131–138 (1983)
- Bolton, J. J.: Marine phytogeography of the Benguela upwelling region on the west coast of southern Africa; a temperature dependent approach. Bot. mar. 29, 251–256 (1986)
- Bolton, J. J. and R. J. Anderson: The *Ecklonia* species of southern Africa: variation, distribution and temperature tolerances. Br. Phycol. J. 20, 182 (1985)
- Bolton, J. J., I. Germann and K. Lüning: Hybridization between Atlantic and Pacific representatives of the Simplicies section of *Laminaria* (Phaeophyta). Phycologia 22, 133–140 (1983)
- Bolton, J. J. and G. J. Levitt: Light and temperature requirements for growth and reproduction in gametophytes of *Ecklonia maxima* (Alariaceae: Laminariales). Mar. Biol. 87, 131–135 (1985)
- Bolton, J. J. and K. Lüning: Optimal growth and maximum survival temperatures of Atlantic *Laminaria* species (Phaeophyta) in culture. Mar. Biol. 66, 89–94 (1982)
- Branch, M. L.: Limiting factors for the gametophytes of three South African Laminariales. Invest. Report Sea Fish. Branch S. Afr. 104, 1–38 (1974)
- Coyer, J. A. and A. C. Zaugg-Haglund: A demographic study of the elk kelp *Pelagophycus porra* (Laminariales, Lessoniaceae), with notes on *Pelagophycus* + *Macrocystis* hybrids. Phycologia 21, 399–407 (1982)
- Hommersand, M. H.: Biogeographic affinities of the South African red algal flora. Bot. mar. 29, 257–270 (1986)
- Joska, M. A. P. and J. J. Bolton: *In situ* measurement of spore release, and seasonality of reproduction in *Ecklonia maxima*. Br. Phycol. J. 22, 209–214 (1987)
- Kain, J. M.: A view of the genus *Laminaria*. Oceanogr. mar. Biol. A. Rev. 17, 101–161 (1979)
- Lüning, K.: Meeresbotanik, 375 pp. Stuttgart: Georg Thieme Verlag 1985
- Lüning, K., A. R. O. Chapman, and K. H. Mann: Crossing experiments in the non-digitate complex of *Laminaria* from both sides of the Atlantic. Phycologia 17, 293–298 (1978)
- Lüning, K. and M. J. Dring: Reproduction, growth and photosynthesis of gametophytes of *Laminaria saccharina* grown in blue and red light. Mar. Biol. 29, 195–200 (1975)
- Lüning, K. and M. Neushul: Light and temperature demands for growth and reproduction of Laminarian gametophytes in southern and central California. Mar. Biol. 45, 297–309 (1978)
- Norton, T. A.: Experiments on the factors influencing the geographical distributions of *Sacchorhiza polyschides* and *Sacchorhiza dermatodea*. New Phytol. 78, 625–635 (1977)
- Novacek, I.: Response of gametophytes of *Ecklonia radiata* (Laminariales) to temperature in saturating light. Mar. Biol. 82, 241–246 (1984)
- Papenfuss, G. F.: Studies of South African Phaeophyceae: *Ecklonia maxima*, *Laminaria pallida* and *Macrocystis pyrifer*. Am. J. Bot. 29, 15–24 (1942)
- Papenfuss, G. F.: Notes on algal nomenclature. 3. Miscellaneous species of Chlorophyceae, Phaeophyceae and Rhodophyceae. Farlowia 1, 337–346 (1944)
- Provasoli, L.: Media and prospects for cultivation of marine algae. In: Cultures and collections of algae, pp 47–49. Ed. by A. Watanabe and A. Hattori. Tokyo: Japanese Society of Plant Physiology 1968
- Shannon, L. V.: The Benguela ecosystem part I. Evolution of the Benguela, physical features and processes. Oceanogr. mar. Biol. A. Rev. 23, 105–182 (1985)
- Sanbonsuga, Y. and M. Neushul: Hybridization of *Macrocystis* (Phaeophyta) with other float-bearing kelps. J. Phycol. 14, 214–224 (1978)
- Tokida, J. and H. Yabu: Some observations on *Laminaria* gametophytes and sporophytes. Proc. 9th Pacif. Sci. Congr. 4, 222–228 (1962)
- Womersley, H. B. S.: A critical survey of the marine algae of southern Australia. 2. Phaeophyta. Aust. J. Bot. 15, 189–273 (1967)

Date of final manuscript acceptance: June 23, 1987.
Communicated by O. Kinne, Oldendorf/Luhe