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An experimental assessment of the temperature responses of two sympatric seagrasses, *Amphibolis antarctica* and *Amphibolis griffithii*, in relation to their biogeography

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Abstract

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Seedlings of the viviparous seagrasses, *Amphibolis antarctica* (Labill.) Sonder & Aschers. and *Amphibolis griffithii* (Black) den Hartog, were grown in seawater cultures at temperatures of 10–30 °C. This temperature range exceeded the range of temperatures occurring in habitats where *Amphibolis* grows.

All seedlings of both species survived at 15 °C, and all *A. antarctica* at 10 and 20 °C. There was some mortality at 25 °C, but more in *A. griffithii* than in *A. antarctica.* All seedlings showed marked senescence at 30 °C within 2 weeks, and all seedlings of both species were dead at this temperature in 6 weeks. Leaf production rates were different at different temperatures for each species, but were high across the 15–25 °C temperature range for both species. Given the time of release of seedlings from parent plants (winter), these results are consistent with the observed distribution of adult plants, and so the more restricted range of *A. griffithii* can be explained partially by its' response to temperature in culture. These results do not explain the absence of both *Amphibolis* species from the east coast of Australia, which may be a consequence of habitat availability.

Introduction

The seagrasses *Amphibolis antarctica* (Labill.) Sonder et Ashers. and *Amphibolis griffithii* (Black) den Hartog are endemic to the southern Australian coast. They have an overlapping distribution (Fig. 1), although *A. antarctica* extends further north on the west coast (Walker & Prince, 1987), and further east and south on the east coast than *A. griffithii* does (Ducker *et al.*, 1977). Ducker *et al.* (1977) suggested that sea temperature 'is generally considered an important factor' in the geographical distribution of the two species. They went on to say that 'a simple relationship between sea-water temperature range and the distribution patterns of *Amphibolis* ... seems unlikely'. Productivity, biomass and nutrient responses of the two species at the same sites have been compared (Walker & McComb, (unpubl.)), showing similarities in their patterns of growth, with maximum leaf production in summer.

In studies of algal biogeography, experimental work has become a standard tool for determining the responses of particular species to temperature (van den Hoek, 1982; Breeman, 1988; Cambridge *et al.*, 1987, 1990a,b; Yarish *et al.*, 1984, 1986). These studies have determined growth and/or reproductive temperature limits as well as lethal limits, and their correspondence with distributional boundaries. Discussions of the effects of temperature on seagrasses have been confined largely to instantaneous physiological responses (Biebl & McRoy, 1971; Kerr & Strother, 1985; Bulthuis, 1983, 1987; Pirc, 1986). Walker & Prince (1987) suggested that the distribution and biogeography of West Australian seagrasses could be partially explained by water temperatures, but these were based on correlations between distributions and broad-scale sea surface temperatures, and not on any physiological or experimental basis. This experiment tested whether any differences in temperature responses (sur-

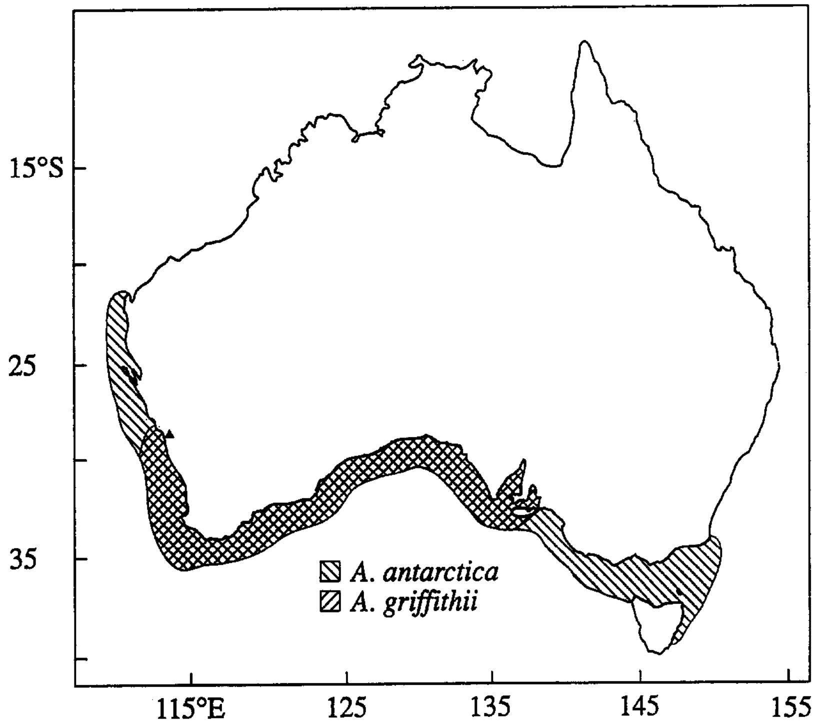

Fig. 1. Distributions of *Amphibolis antarctica* (\\\) and *Amphibolis griffithii* (///) in Australia. The location of Cliff Head is marked (▲)

vival and growth) existed between these two species under controlled conditions, and if so, whether these could account for the observed differences in distribution. As both species produce viviparous seedlings, which have been used successfully in culture (Walker & McComb, 1990), seedlings were used in this experiment.

Methods

Seedlings were collected on 30 July 1990 from the reef at Cliff Head, near Dongara, Western Australia (29 ° 18′S, 114 ° 53′E), approaching the northern limit of the distribution of *A. griffithii* (Fig. 1). They were transported in a large bin of seawater and kept aerated until the experiment was established, within 48 hours of collection. Growth rooms were maintained at 10, 15, 20, 25 and 30 °C and maximum-minimum thermometers used to check on variations in air and water temperature. Air temperatures varied by a maximum of ±2 °C weekly, and more usually ±1 °C, resulting in a <1 °C change in water temperature. Twenty seedlings of each species were grown at each temperature. At the beginning of the experiment, each seedling was wet weighed, and the number of leaves counted. Two seedlings were placed in a pot with acid-washed quartz sand and labelled. Five pots were placed in each of two aquaria, filled with 25 litres of filtered seawater, with airstones to provide aeration and circulation. No nutrients were added to the water as previous experiments have shown that serious epiphyte growth can occur. The seawater was changed completely every week for the 4 months of the experiment.

The seedlings were grown under 12:12 light:dark cycle at a light intensity of 140 μE m^{-2} s^{-1}. Light intensities varied slightly between temperatures (±10 μE m^{-2} s^{-1}), but were always above saturation irradiances for the species. Seedlings were removed from their pots every week and the leaf number counted. Observations were also made on the condition of the leaves and whether any new shoots or roots had formed at the base of the seedling. Any leaves that had been shed were 'attributed' to their parent seedling, removed and dried to constant weight. Seedlings were assessed as 'dead' when either all leaves had been shed, or when all leaves were fully blackened (Biebl & McRoy, 1971) and no new leaves were emerging at the centre of the leaf cluster. Any seedlings that had died were removed and dried and weighed.

At higher temperatures (>25 °C), seedlings were prone to attack by a fungal pathogen, probably an Oomycete of the genus *Pythium*. To eliminate this problem, seedlings were dipped in a 2% solution of a proprietary antiseptic (Milton) containing sodium hypochlorite and sodium chloride, which has been used effectively as a surface steriliser for macroalgae (Kirst, pers comm., 1988). The solution was made up with sterile seawater and the seedlings rinsed in sterile seawater before returning to the aquaria. All seedlings were treated the same way, whether or not fungal activity had been noted. This restrained fungal activity, but did not affect the high rates of mortality at higher temperatures.

Survival of seedlings was calculated by plotting the % remaining alive at each time interval. The percentage survival was calculated for each temperature (Fig. 2). Regression values were calculated for absolute seedling survival against time along the linear part of the curve (Zar, 1980).

Data for leaf production per surviving seedling were checked for normality, and found to be not normally distributed. They were then transformed ($\sqrt{(x + 0.5)}$), but this and other transformations still did not achieve normality and so a non-parametric range analysis was carried out (Zar, 1980).

Table 1. Linear regression values (Survival= $\beta \times$ time in days + α) and Student Neuman Keuls test (Zar 1980) for survival rates for *A. antarctica* seedlings cultured under different temperature regimes.

Temperature	10	15	20	25	30
r	0.612			0.893	0.930
p	0.026			0.0001	0.0024
β	−0.005	0.000	0.000	−0.171	−0.541
α	20.157	20.000	20.000	19.112	23.929
Temperature SNK test (5%)	15	20	10	25	30

Table 2. Linear regression values (Survival= $\beta \times$ time in days + α) and SNK test (Zar 1980) for survival rates for *A. griffithii* seedlings cultured under different temperature regimes.

Temperature	10	15	20	25	30
r	0.888		0.954	0.920	0.931
p	0.0001		0.0001	0.0001	0.0023
β	−0.034	0.0	−0.117	−0.302	−0.556
α	20.611	20.0	21.909	21.198	23.679
Temperature SNK test (5%)	15	10	20	25	30

Results

Observations

All seedlings survived the initial experimental establishment, but under conditions causing stress, the stress response became obvious within two weeks of culture. At 30 °C, there was a rapid change in leaf colour from green to brown and then black, no production of new leaves, and all seedlings of both species were dead within 45 days. Some *A. antarctica* seedlings survived for the duration of the experiment at 25 °C, but all *A. griffithii* seedlings turned black at 25 °C, but more slowly than at 30 °C. At 20 °C all *A. antarctica* seedlings survived and grew, whereas some *A. griffithii* seedlings started to deteriorate after 28 days. At 10 and 15 °C, seedlings of both species remained green and started to produce roots. After about 50 days, seedlings of *A. griffithii* started to deteriorate at 10 °C.

No statistically significant differences were found between the different aquaria, and so all the data for each species were pooled. *A. griffithii* leaves were heavier (0.0232 g dry weight) than *A. antarctica* leaves (0.0127 g dry weight). Within each species, average leaf weight data did not change over the course of the experiment, and so these data are not presented.

Survival

Maximum survival observed in culture for both species was at 15 °C where there was no mortality over the period of the experiment (Fig. 2). All seedlings of *Amphibolis antarctica* survived at 15 and 20 °C over 120 days. All seedlings of *A. griffithii* survived at 15 °C, but had a slight reduction in survival at both 10 and 20 °C. For *Amphibolis antarctica*, there was no significant difference in survival over the range of 10–20 °C. One seedling of *A. antarctica* died in the last week of the experiment at 10 °C, after 110 days. Survival was significantly lower at both 25 and 30 °C (Table 1). In *A. griffithii*, survival rates at 10 and 15 °C

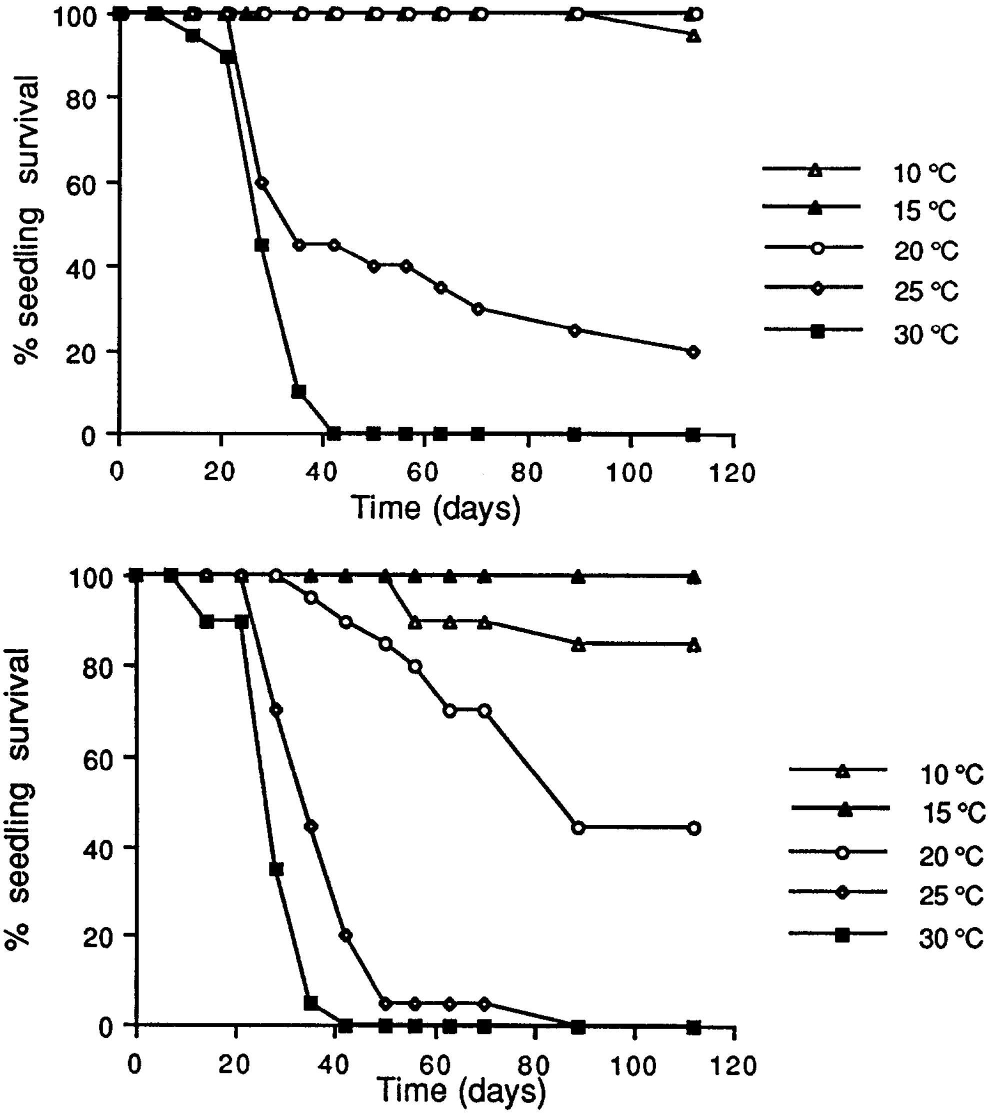

Fig. 2. Seedling survival in culture at different temperatures. a) *Amphibolis antarctica* b) *Amphibolis griffithii.*

were similar, but from 20–30 °C, survival was reduced significantly at each higher temperature (Table 2).

Leaf production rates

Leaf production rates were similar to those measured *in situ* within adult leaf clusters, and were high across the 15–25 °C temperature range for both species. These results have been expressed as production per surviving seedling, *i.e.* discounting the dead material (Fig. 3). The difference in scale between the two graphs is a consequence of the nature of the leaf clusters. In *A. antarctica* the leaves are shorter, but more numerous and have a faster turnover than in *A. griffithii.* Measured rates of biomass production *in situ* are similar between the two species (Hillman *et al.*, 1989; Walker & McComb, unpubl.).

The results for *A. antarctica* were less consistent than those of *A. griffithii.* There was highest leaf production at 25 °C, but this was not significantly different to that at 15 °C. At 20 °C, the rate of leaf production was slightly reduced, but was still higher than at either 10 °C or at 30 °C. The results for *A. griffithii* showed a typical physiological optimum curve, again with maximum leaf production at 25 °C, with no significant

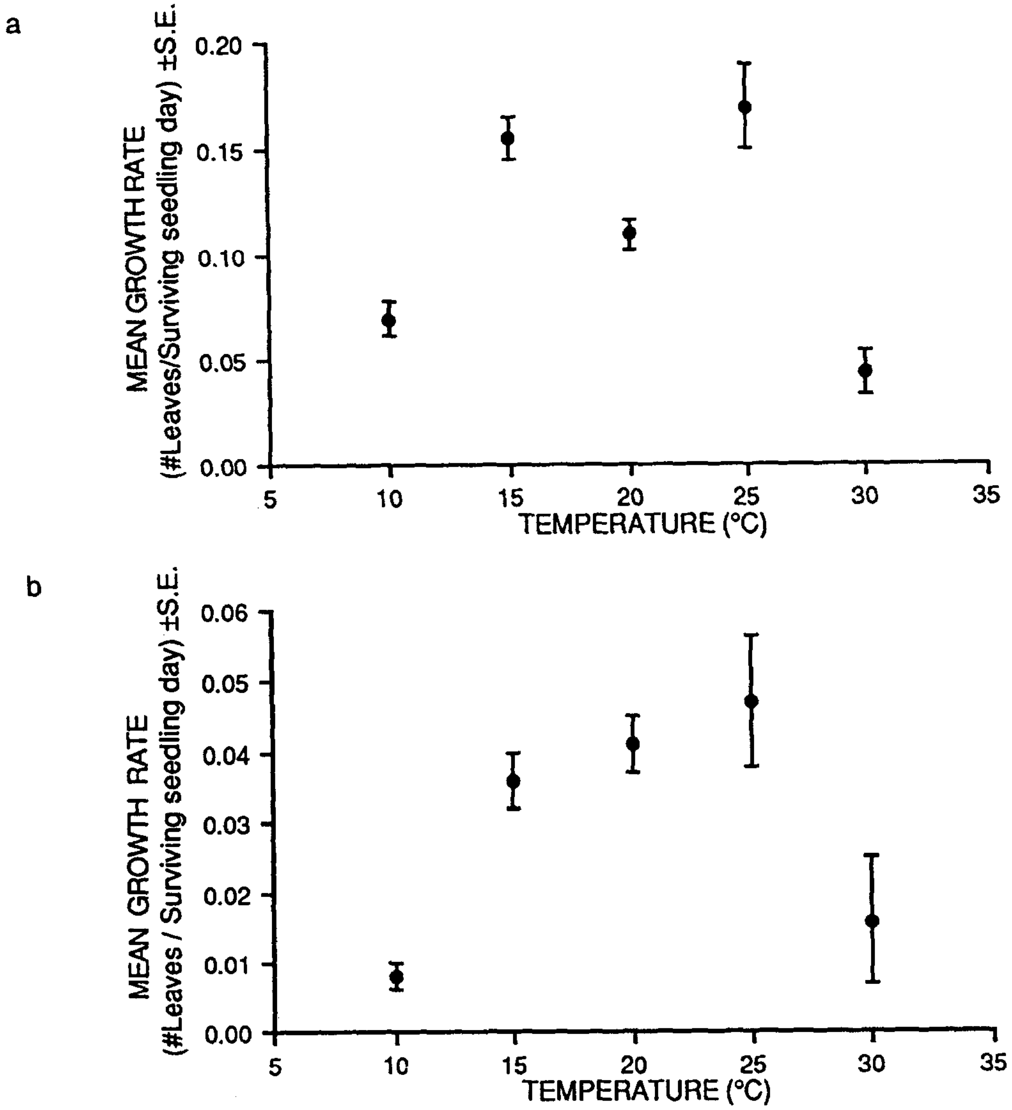

Fig. 3. Graphs of leaf production per surviving seedling in culture at different temperatures a) *Amphibolis antarctica* b) *Amphibolis griffithii.* (x±s.e.).

variation between 15–25 °C but with rapid declines above and below these values.

Discussion

Previous work on seagrass temperature tolerance has been based on short-term physiological responses of photosynthetic and respiration rates (Biebl & McRoy, 1971; Drysdale & Barbour, 1975; Kerr & Strother, 1985). In these studies most seagrasses had an optimum temperature between 28–32 °C, but increasing temperatures further caused productivity to decline abruptly. However, it has always proved difficult to relate instantaneous physiological measurements to growth responses in the field.

Increasing temperature can accelerate the rates of some enzymic reactions, but can also result in unequal enzyme reaction rates (Raven & Geider, 1988). Hence there is often an increase in productivity in response to temperature increase, but once a critical temperature is reached, decline may often be very rapid. At the highest temperature investigated (30 °C), leaf production rates had declined significantly for both species. There was a broad range over which rates of leaf production were generally high, but the higher temperatures were subject to higher mortalities, therefore dropping the leaf production rate, as resources would be unavailable for growth under conditions of stress.

This experiment has allowed interpretation of the effect of temperature as a single factor, with all other conditions controlled. The results suggest that consistent temperatures of ≥ 30 °C exceed the lethal temperature for both *Amphibolis antarctica* and *A. griffithii*. From 15–25 °C, *A. antarctica* can survive and grow very successfully, although some seedlings do die at 25 °C. At 10 °C, rates of growth are very slow, although seedlings survive. *Amphibolis griffithii* has a much more restricted tolerance, and is subject to high mortality at both 25 and 30 °C. It has a similar survival at 20 °C to that of *A. antarctica* at 25 °C. It survives best at 15 °C, where it also has moderately high leaf production rates. At 10 °C, some seedlings die, and surviving seedlings also grow very slowly.

There are potential differences between the response of adult plants, with more nonphotosynthetic tissue to support (roots and rhizomes), and the seedlings used in the experiment. However, the leaf turnover rates are similar between seedlings in culture and adult-plants measured *in situ* (Walker & McComb, 1990). In addition, the responses of seedlings will determine the extent of successful settlement and growth in a potential environment. Seedlings of both species are released in June (winter) each year, and the higher survival rates at lower temperatures may allow seedlings to establish successfully over winter, and grow faster once temperatures increase over summer (Fig. 4).

The experimental results suggest that *A. griffithii* should have a more restricted distribution than *A. antarctica* in the field. This should be most obvious in terms of the summer maximum temperatures at the northern limits and the winter minima at the southern limits. The experimental data suggest that in *A. antarctica*, winter temperatures at or above 25 °C would reduce seedling survival, with added mortality from summer maximum temperatures. For *A. griffithii*, winter temperatures of above 20 °C would reduce survival. Lower temperatures would have a greater effect on *Amphibolis* species by reducing growth rates than by inducing mortality, although temperatures <10 °C would cause mortality in *A. griffithii*.

The potential survival of seedlings in the field can be assessed in relation to sea temperature curves, suggesting times of year at particular locations which could result in mortality, and to compare the geographical limits observed in the field with the culture results. Sea surface temperatures do not provide a perfect representation of bottom temperatures (see Walker & Prince (1987) for a fuller discussion), but they do provide a relative indicator of prevailing temperatures. Sea surface temperature data are provided by Pearce (1986, pers. comm., 1992) and these have been expressed graphically for sites of particular importance to the species distributions (Fig. 4).

The distribution limits of the two species show some correspondence with sea surface temperature isotherms. The northern limit of *A. antarctica*, Exmouth, Western Australia (22 ° 47′S 113 ° 41′E), corresponds to the 25 °C January isotherm and that of *A. griffithii*, Geraldton, Western Australia (28 ° 49′S 114 ° 36′E), to the 21 °C January isotherm (Times Atlas of the Oceans, 1983). The southern limits of the two species (Maria Island, Tasmania, 42 ° 47′S, 147 ° 56′E; Encounter Bay, South Australia, 35 ° 36′S 138 ° 35′E) correspond to the 12 °C and 15 °C July isotherms (Times Atlas of the Oceans, 1983) for *A. antarctica* and *A. griffithii*, respectively.

Comparisons of the growth responses of the two species with the annual temperature curves at particular sites, allow increased interpretation of their distribution limits. Temperatures of less than 25 °C form an upper lethal (summer) limit for *Amphibolis griffithii*, which corresponds to the geographic range it occupies. *A. antarctica* has a higher summer lethal temperature, and hence extends further north. On the Tasmanian coast, *A. griffithii* would have a very restricted growing season (*ca* 3 months of the year, as opposed to 6 months at its southern limit in South Australia) suggesting a southern growth limit, rather than a lethal boundary. *Amphibolis antarctica* has a much better growth rate at 15 °C than *A. griffithii*, and can survive at 10 °C (e.g. in Tasmania in September). This suggests that *A. antarctica* has a southern growth limit between 10–15 °C.

These patterns of temperature response do not explain the absence of the two species from the east coast of Australia, where temperatures again fall between the limits of the isotherms given above (Fig. 4). Larkum & den Hartog (1989) suggested that there were a group of seagrasses restricted to the west coast and the Great Australian Bight, and in some cases Victoria, which indicated to them a radiation from west to east of relatively recent origin. They also suggested these species represent a relict distribution, with *Amphibolis*, *Posidonia* and *Heterozostera* having been

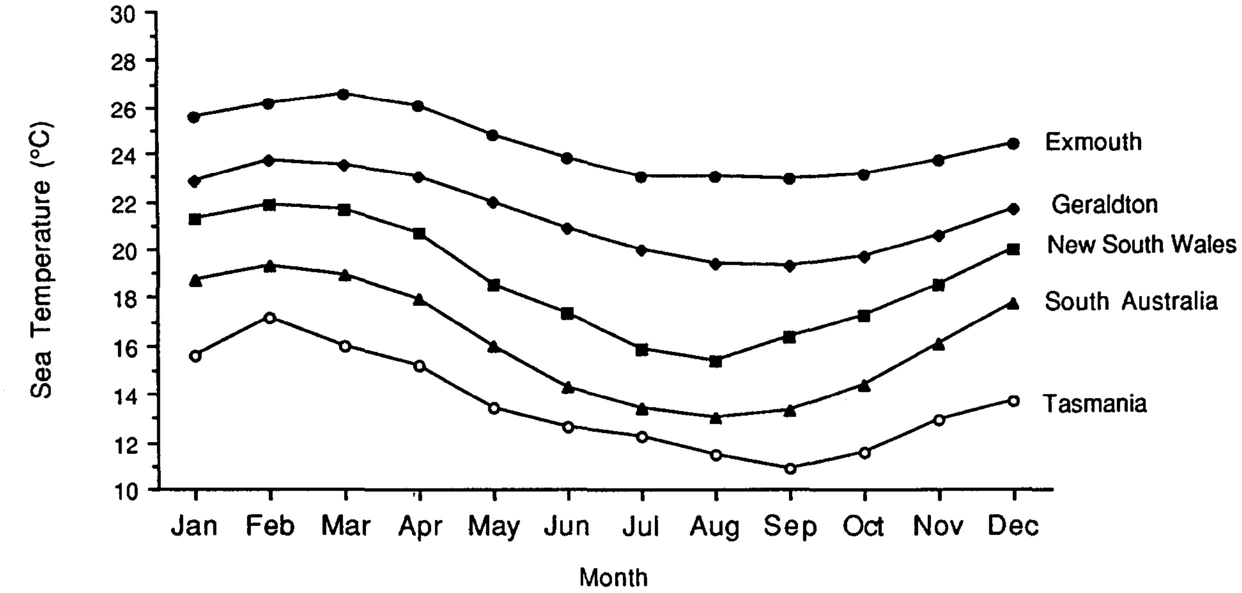

Fig. 4. Monthly mean sea surface temperatures for sites around Australia: Data for Exmouth and Geraldton from Pearce (1986). Data for Tasmania (42–43 °S, 148–149 °E), South Australia (35-36 °S, 138–139 °E) and New South Wales (34–35 °S 151–152 °E) are from Anon. (1949)

distributed right around Australia, but with no explanation for the absence of *Amphibolis* on the east coast.

Other factors influence the distribution and abundance of seagrasses. Neither of the two *Amphibolis* species is found in estuaries, within their existing geographical range, and both species occur generally within the protection of limestone reefs (Kirkman & Walker, 1989). Most seagrass on the south-eastern coast occurs in estuaries, and on the open coast is restricted to a few localities where headlands provide shelter (West *et al.*, 1989). The exposed nature of non-estuarine coastal habitat on the east coast may provide a partial explanation for the present absence of *Amphibolis* from the east coast.

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