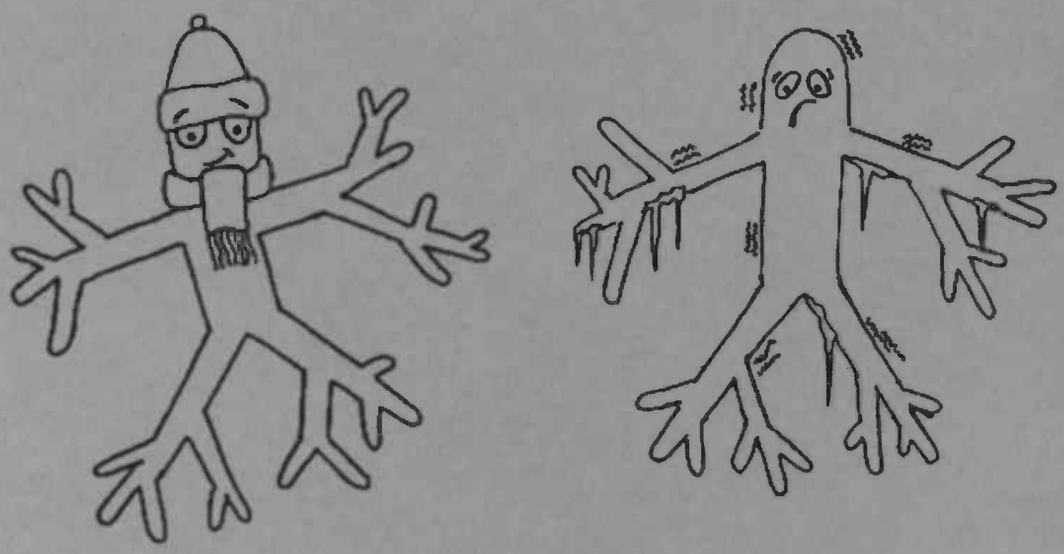
Search for ecotypic variation in *Champia parvula* (Rhodophyta) and *Cladophoropsis membranacea* (Chlorophyta).



By Angelina Y. den Besten

Search for temperature ecotypes in *Champia parvula* (Rhodophyta) and *Cladophoropsis membranacea* (Chlorophyta).

Honors Project aug.'98-may '99.
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Contents

The drawing on the cover represents two algea of which one is adapted to the cold, while the other is not.
Coutesy of J. Fitz-Jim.

Abstract

Six isolates of *Champia parvula* (C. Agardh) Harvey were cultured at different daylength and temperature combinations in order to find reproductive ecotypes. No reproduction has been found so other experiments need to be conducted.

Growth curves of 7 different *Cladophoropsis membranacea* (C. Agardh) Boergesen isolates and isolates of 3 related species (*Cladophoropsis sundanensis* Reinbold, *Chamaedoris peniculum* (Solander) Lamouroux and *Struvea anastomosans* (Harvey) Piccone & Grunow were constructed in order to examine ecotypic variation in *C. membranacea*.
No major differences in growth curves between the different isolates and species were found. When the potential growth yields per month were calculated, a decrease in the potential monthly growth rates going from the tropics towards more temperate zones was seen.
A significant correlation between the February isotherm and potential growth rate, and between the August isotherm and potential growth rate at this temperature was found. Growth curves were superimposed on the phylogenetic tree (Kooistra *et al* 1992, 1993) to compare them with the temperature survival ecotypes found by Pakker *et al* (1995) to see if the phylogentic imprint is found at the same places.
No grouping similar to that of the survival ecotypes or any other kind of grouping was found. As no clear ecotypes for growth of *C. membranacea* were found, and no adaptation to more temperate climate zones was observed, it can be conclude that a strong tropical growth imprint persists through the whole phylogenetic tree.

General introduction

Many different biotic factors, such as grazing and competition, and abiotic factors, such as temperature, salinity, nutrient levels and light, influence seaweed species. Biotic factors are important when the local distribution patterns of seaweeds are studied, as where abiotic factors are more important when global distribution patterns are considered (Lüning 1990). Experimental studies have shown that temperature and light, in particular day-length, are the most important factors to determine geographic distribution limits of seaweed species (Breeman 1988, Breeman and Pakker 1994, Lüning 1990).
Temperature is a factor that can increase heat dissipation or enhance ice crystal forming. It also has an effect, together with day-length, on enzyme activities and thus on all physiological processes, such as photosynthesis and respiration (Lobban and Wynne 1981). It follows that growth and reproduction can be expected to be temperature dependent.

The factors mentioned above, as well as other factors, must also have played a role during the geographical histories of seaweeds. Some of the other influences on the distribution patterns of seaweeds are geological events such as paleoclimatic changes and continental drift.
Seaweeds that once had a continuous distribution may now have a disjunct distribution because of the occurrence of new barriers such as large bodies of oceanic water, continents or climatic limits (such as temperature and day-length). Dispersal possibilities of species along coastlines or long-range dispersal may further have influenced distribution patterns across oceans (Pakker *et al* 1995). In disjunct populations that experience different temperature regimes and under reduced levels of gene flow, selective forces may in the long run result into genetically fixed adaptations called

ecotypes. Along continuous coastlines dispersal and uninterrupted gene flow are facilitated and therefore, the degree of thermal adaptation at these sites is expected to be small to non-existent (Lobban *et al* 1985). According to Breeman (1988) the existence of thermal ecotypes can be expressed in:

1 Differences in optimum temperatures for growth and reproduction.
2 Differences in thermal ranges over which growth and reproduction proceed.
3 Differences in tolerance to high and/or low temperatures.

Temperature ecotypes for growth, reproduction and survival have been found in seaweeds (Cunningham *et al* 1993, Molenaar 1996, Pakker *et al* 1995, Orfanidis and Breeman in review). Differences up to 10°C have been found in growth optima and differences up to 7°C have been found in reproduction limits (Lüning 1990). In addition, photoperiodic ecotypes in the control of reproduction exist (Cunningham *et al* 1993, Molenaar 1996).
It is indicated that, through adaptation in one direction, tolerance at the other end may be sacrificed. For instance as improved performance at low temperatures may be accompanied by a loss of potential at high temperatures, or a trade-off may exist between the breadth of the thermal performance range and the height of the optimum (Huey and Kingsolver 1989, 1993). By taking a phylogenetic tree of a group of taxa and looking at their ecotypes it is possible to pinpoint in which evolutionary lineage ecotypes are likely to have evolved (Pakker *et al* 1995). All organisms that have evolved from this ancestor will have this environmental adaptation unless there is a reversal in the character-state. By comparing temperature ecotypes for different processes, a general view about the historical development of a seaweed can be gained.

This paper deals with two different kinds of ecotypic variation, namely ecotypic variation in reproduction and ecotypic variation in growth. The first part (part 1) of this paper will deal with ecotypic variation in the reproduction of the red alga *Champia parvula* (C. Agardh) Harvey. In this species a strong ecotypic variation in growth and survival requirements has already been found (Orfanidis and Breeman in review). The second part (part 2) will deal with ecotypic variation in the growth of the green alga *Cladophoropsis membranacea* (C. Agardh) Boergesen . For this group, and for the 3 related species, a phylogeny based on ribosomal ITS sequences is available, which allows for an analysis of the evolution of thermal characteristics (Kooistra *et al* 1992, 1993). In this case the thermal characteristics of growth.

Part 1 The reproduction of *Champia parvula.*

Introduction:

Champia parvula is a 3-10 cm high, tropical to cold temperate red alga that is found from as far north as Roscoff and Rhode Island to as far south as Brazil (fig 1A). It is mainly found in shallow waters but has also been dredged from 37m depth (Taylor 1960). Distribution boundaries are set in the north by lethal growth and survival temperatures and in the south by a lethal winter temperature (Orfanidis and Breeman in review). The study conducted by Orfanidis and Breeman has also shown that *C. parvula* has a strong temperature ecotypic differentiation in isolates taken from within the margins of its distribution.

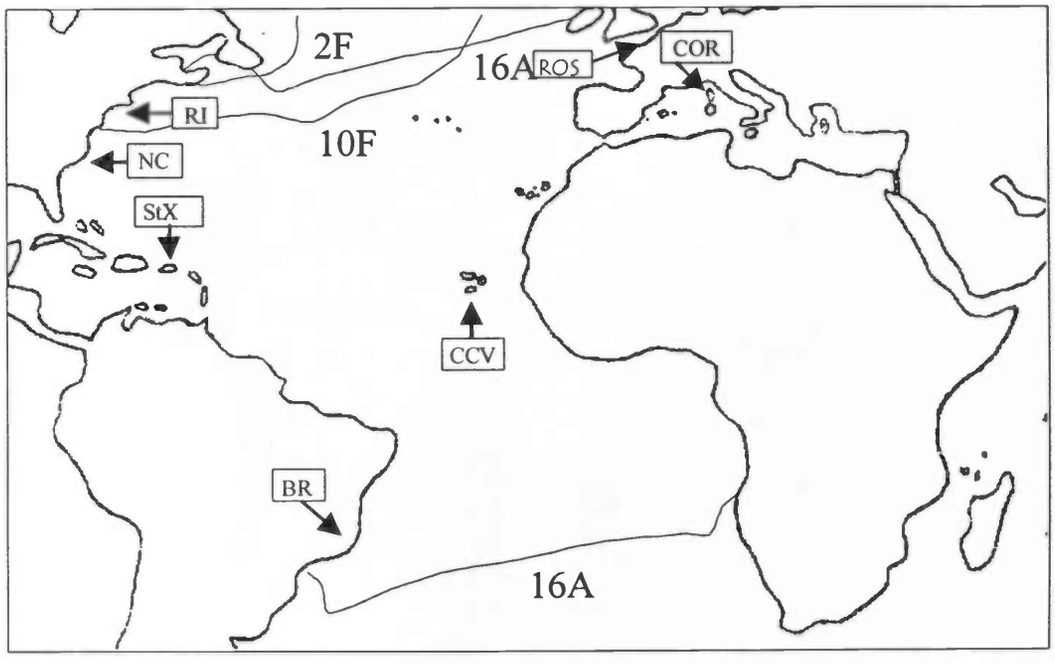


Fig 1A: Origin of the *Champia parvula* isolates used in this experiment. For explanation of the codes see table 1A. Ros represents Roscoff, France. 2F = 2°C February isotherm, 10F = 10°C February isotherm, 16A = 16°C August isotherm.

Another limiting factor for dispersion can be the ability of a species to reproduce. On the one hand the ability of a species to reproduce under certain conditions can increase or decrease the gene flow through the distribution range. On the other hand, limited gene flow can increase the forming of ecotypes and with that the ability of a species to reproduce.
Temperature and day-length are two ecological factors with a signal character that are very important in regulating reproduction (Breeman *et al* 1988, Cunningham *et al* 1993) and can thus be limiting factors. By analysing the reproduction of *C. parvula*, the gene flow through a distribution range can be studied. It can be investigated if gene flow is limited due to the reproduction possibilities or if free gene flow is possible. When gene flow is limited, a comparison can be made between the limited gene flow, and the already found temperature ecotypes by Orfanidis and Breeman (in review). So, in order to fully understand the factors that may limit the distribution of *C. parvula*, one must also examine the reproduction of this species.

C. parvula has a *Polysiphonia*-type of life history (fig 1B) which is characterised by tetrasporophytes (2n) and gamethophytes (n, male or female) of similar morphology (Lobban and Wynne, 1981).

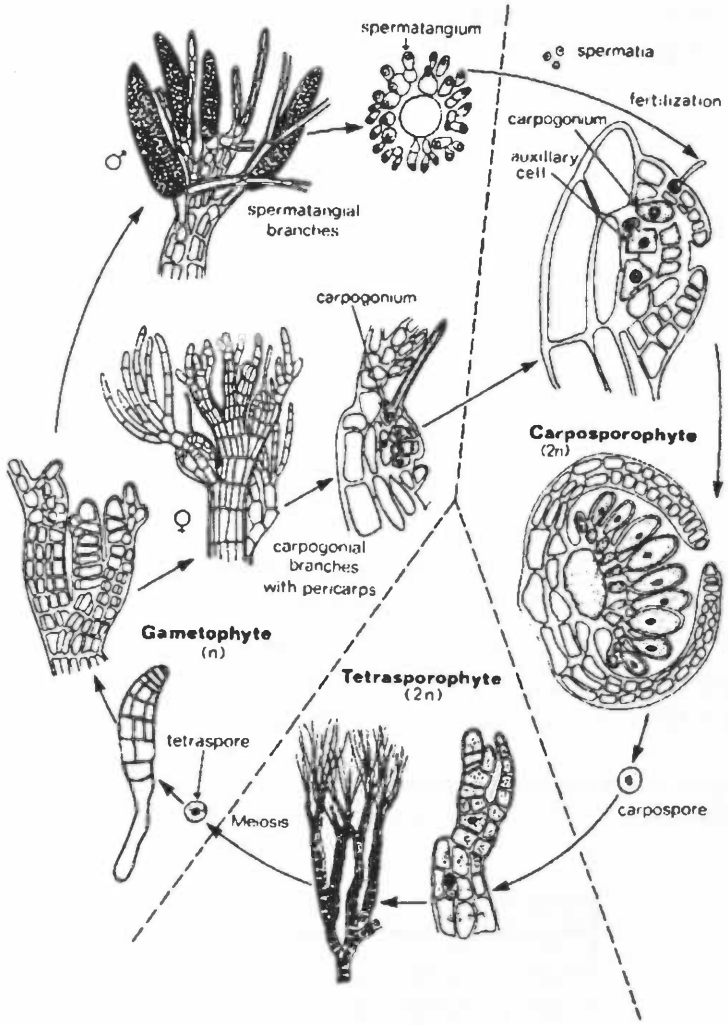


Fig 1B: The *Polysiphonia*-type of life history of *Champia parvula* (Lobban and Wynne 1981).

Questions:

What daylight and temperature requirements induce reproduction in *C. parvula*?
Is the ability to reproduce a limiting factor at the distribution boundaries of *C. parvula* ?
Does *C. parvula* have reproductive ecotypes?

Method:

Isolates from six different locations (table 1A) were grown in big glass boxes (Ø = 9,5 cm). From these plants 5 tips of 5 mm were cut off and each one was put into a small glass box (Ø = 5 cm) filled with 33‰ seawater enriched with von Stosch medium (VSES; modified after Guiry and Cunningham 1984). These glass boxes were transferred to the experimental temperature with steps no bigger than 2.5°C per day or a change in light regime from long day 16:8 to short day 8:16 conditions, or vice versa, in two days.

Table 1A: Origin and codes of the isolates used in this experiment.

Origin:	Code:
St. Criox, U.S. Virgin Islands	CStX
Sao Paolo, Brazil	Br
Santiago, Cape Verde Islands	CCV
Wrightsville Beach, North Carolina	NC
Corsica, France	Cor
Rhode Island	RI

The experimental temperatures were taken with 5°C intervals. Table 1B shows which isolates were put at 5, 10, 15, 20, 23, 25 and 30 °C and if they were exposed to longday or shortday conditions. Lowest and highest experimental temperatures were determined by using the survival temperatures found by Orfanidis (in review). If regeneration was possible at a certain temperature, isolates were put at this temperature to see if they would try to sporulate due to survival stress.

Table 1B: Isolates and their experimental temperatures, -- isolate was not put at this temperature because survival is not possible, + isolate was put at this temperature because regeneration is possible, ++ isolate was put at this temperature because survival is possible.

Isolate	Temperature	5°C	10°C	15°C	20°C	25°C	30°C
CStX	Sd	--	--	--	++	++	++
	Ld	--	--	--	++	++	++
BR	Sd	--	--	++	++	++	++
	Ld	--	--	++	++	++	++
CCV	Sd	--	--	++	++	++	--
	Ld	--	--	++	++	++	--
NC	Sd	--	++	++	++	++	++
	Ld	--	++	++	++	++	++
COR	Sd	--	++	++	++	++	++
	Ld	--	++	++	++	++	++
RI	Sd	+	++	++	++	++	+
	Ld	+	++	++	++	++	+

All isolates were exposed to a photon fluence rate of 20 $mol.m^{-2}.s^{-1}$. If the isolates got too big they were transferred to larger glass boxes (Ø = 9.5 cm) by carefully scraping them lose and putting them with the right side up again. Observations were made every week and included the recording of colour, growth, attachment, forming of spores, sporulation and in some cases death. The seawater was replaced every two weeks for small glass boxes and every week for large glass boxes, to prevent lack of minerals. The experiments lasted for 4 months.

Results:

After 4 months still no spore formation was found in any isolate at any temperature/ daylight combination. Some isolates died at their extreme survival limits and some were thrown away because of an infection. The remaining isolates showed good growth at their optimum growth temperatures and lesser growth towards their lower and upper survival temperature limits. Most isolates attached themselves to the glass, grew hairs and appeared healthy.

Discussion:

Clearly the factors that influence the reproduction of *C. parvula* are a little more complicated than thought. More experiments need to be conducted to answer the questions, stated in the introduction. In the introduction it mentioned that the tetrasporophyte and the gamethophyte are of similar morphology. As stated before, one tip was placed in each glass box. It would be possible that only a male or female gametophyte was placed in each glass box since there is a similar morphology between sporophytes and gametophytes and hence no reproduction was found. This is not likely to have happened for all tips since some reproduction was observed in the cultures in a pilot-experiment.
Follow-up experiments could involve changing temperature from warm to cold and vice versa or changing of day-length from short to long days and vice versa. Experiments involving adaptation to temperatures could also be a possible.
Another possible explanation could be that reproduction is not timed by a certain temperature/day-length combination but by an untested factor such as nutrient level or increased light quantity. Reports of a red alga with a *Polysiphonia*-type of life history that starts reproducing after increasing the amount of light quantity are known (Dring 1984). As far as the nutrient levels are concerned, both elevated as well as reduced nutrient levels can induce spore formation (Dring 1984).

Conclusion:

Further experiments need to be conducted before any conclusion can be drawn on which factors induce reproduction in *C. parvula*, and whether ecotypic variation occurs.

Part 2 The growth of *Cladophoropsis membranacea* and related species.

Introduction:

Cladophoropsis membranacea (C.Agardh) Boergesen is a lower intertidal and upper sublittoral green alga of about 2-5 cm in height, with a pantropical distribution pattern (fig 2A) (Pakker *et al* 1995). It can be found growing on stones and woodwork in the intertidal zone, especially in calm water (Taylor 1960). It is also known to form extensive mats, which may become infiltrated with fine sand. A disjunct distribution can be found on the Atlantic and Pacific coasts. *C. membranacea* is also known as *C. modonensis* (Kützing) Boergesen (Pakker *et al* 1995), in the Mediterranean.

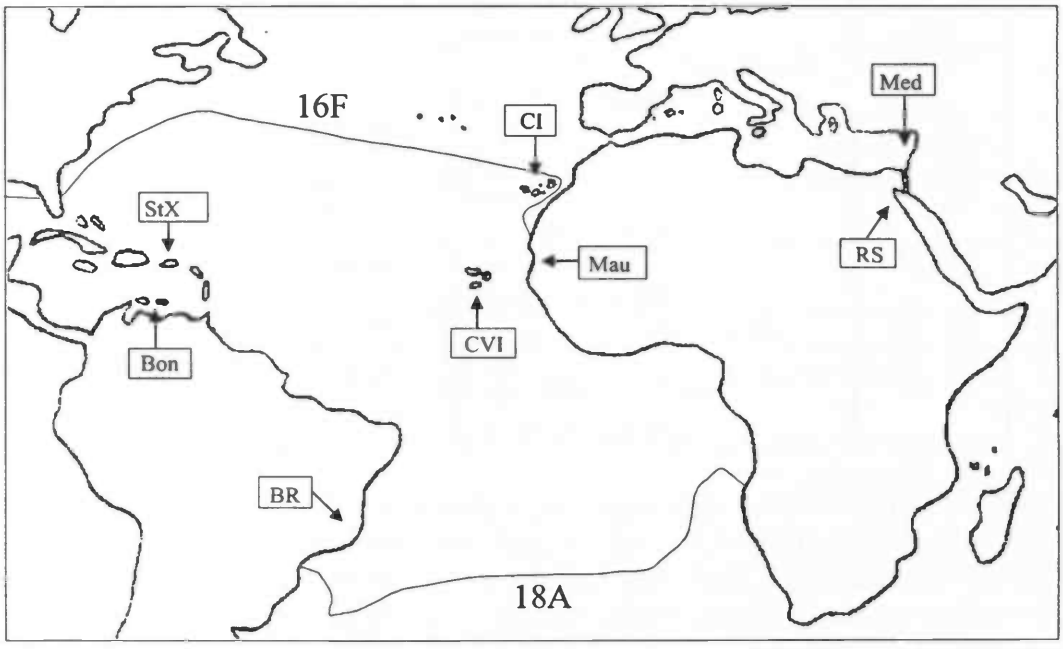


Fig 2A: The origin of the isolates used in this experiment. Codes are explained in table 2A. CI = Canary Islands. The locality of Hawaii is not shown on the map. 18F = 18°C February isotherm, 18A = 18°C August isotherm.

Related species to *C. membranacea* are *Struvea anastomosans* (Harvey) Piccone & Grunow, *Cladophoropsis sundanensis* Reinbold and *Chamaedoris peniculum* (Solander) Lamouroux . The distribution of *S. anastomosans* is approximately the same as that of *C. membranacea* but it has not been found in the Mediterranean. *S. anastomosans* is a 3-5 cm tall plant that can be found densely entangled or attached to each other. It is usually found of off reefs or other rather exposed places such as crevices of rocks or among coarse algae (Taylor 1960). Very little is known about *C. sundanensis*. It has mainly been reported from the Indo-West Pacific but Pakker *et al* (1995) reports an isolate from the Caribbean, which will be used in this study. *C. peniculum* is found on the east as well as the west coasts of the Atlantic. It is a 1-2 dm tall plant that is generally washed ashore from deep water. It has been dredged from a depth of 55m. Occasionally it is found in shallow water, growing under rock ledges (Taylor 1960). The related species are used as an outgroup in this experiments to be able to compare growth curves of different *C. membranacea* isolates with those of the three related species.

The place of origin, code and the annual temperature range at the collection localities of each isolate can be found in table 2A.

Table 2A: The place of origin, used code and the annual temperature range for each used species (Lipkin and Safriel 1971, U.S.Navy 1981).

Species	Code	Locality	Annual temperature range (°C)
Cladophoropsis membranacea	CmCIF	Tarajalejo, Fuerteventura, Canary Islands	18 - 22
	CmCIT	Punta del Hidalgo, Tenerife, Canary Islands	18 - 22
	CmCVI1	Praia, Santiago Islands, Cape Verde Islands	22 - 26
	CmCVI4	Cicade Velha, Santiago Islands, Cape Verde Islands	22 - 26
	CmHaw	Oahu, Hawaii	24 - 27
	CmMed	Lattakia, Syria	17 - 28
	CmStX	Bioler Bay, St.Croix, U.S. Vrigin Islands	26 - 28
Cladophoropsis sundanensis	CsBon	Hato, Bonaire, Netherlands Antilles	26 - 28
Chamaedoris peniculum	CpStX	St.Croix, U.S. Virgin Islands	26 - 28
Struvea anastomosans	SaBra	Brazil	24 - 26
	SaStX	Maltha Baths, St.Croix, U.S. Virgin Islands	26 - 28

Pakker *et al* (1995) already conducted growth experiments with several other *C. membranacea* isolates from other localities (one from Cane Bay, St Croix, U.S Virgin Islands (CmStX), one from Lac Bay, Bonaire, Netherlands Antilles (CmBon), one from Banc d'Arguin, Mauritania (CmMau) and one from Bur Safâga, Egypt (Red Sea) (CmRS)) as well as with two isolates (CmStX and CmCIT) that will be used again. This is done to see if a valid comparison can be made between results reported in this study and growth experiments done by Pakker *et al* (1995) and thus if it is possible to use his data in comparisons.

All isolates used are genetically linked in a phylogenetic tree. Therefore, it is possible to see if any similarities or differences have genetically evolved from a common ancestor. Pakker *et al* (1995) already showed the phylogenetic background of temperature tolerances in comparison with field temperatures during the coldest month (fig 2B). A comparison between the findings of Pakker *et al* (1995) and the data gathered in this experiment will be made, to see if a similar phylogenetic background can also be found in growth curves.

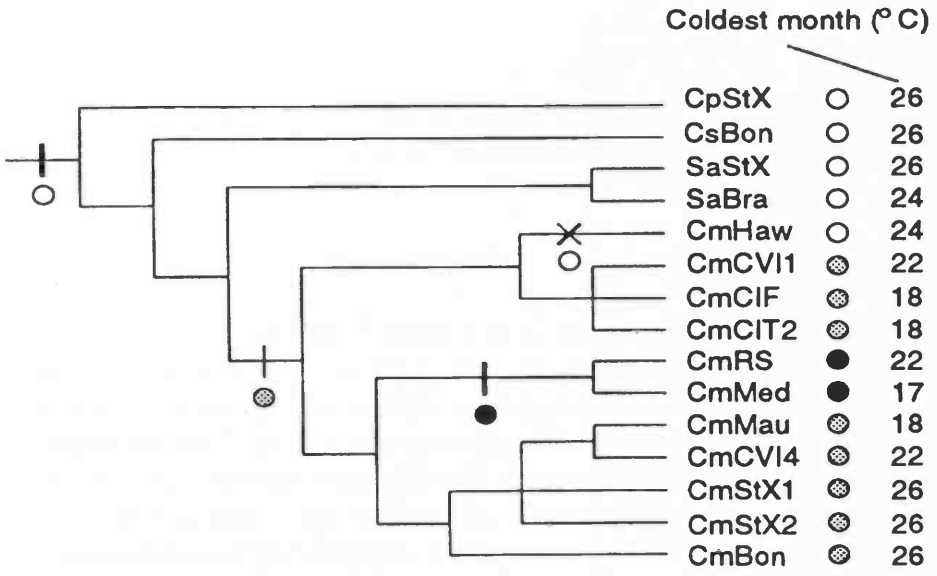


Fig 2B: Phylogenetic hypothesis based on parsimony analysis of ITS sequences from Kooistra *et al* (1992, 1993) with temperature tolerances superimposed. Length of branches has no significance. For isolate codes see table 2A. Dots represent character state changes for temperature tolerance; open dots = damage occurs at 18°C; shaded dots = no damage occurs at 18°C; black dots = no damage at 18°C and additional tolerance for low and high temperatures. Cross depicts reversal of character states, with the new character state shown. Seawater temperatures for the coldest months from Lipkin and Safriel (1971) and U.S.Navy (1981). Taken from Pakker *et al* (1995).

As an arbitrary definition for good growth an isolate should be able to reach 80% of the possible growth yield for at least one month during the year and should not drop below the limit of a 20% yield during the rest of the year. These are, of course, no absolute limits but are only used to compare the potential performances of the different isolates.

Expected responses for temperature ecotypes and adaptation are shown below:

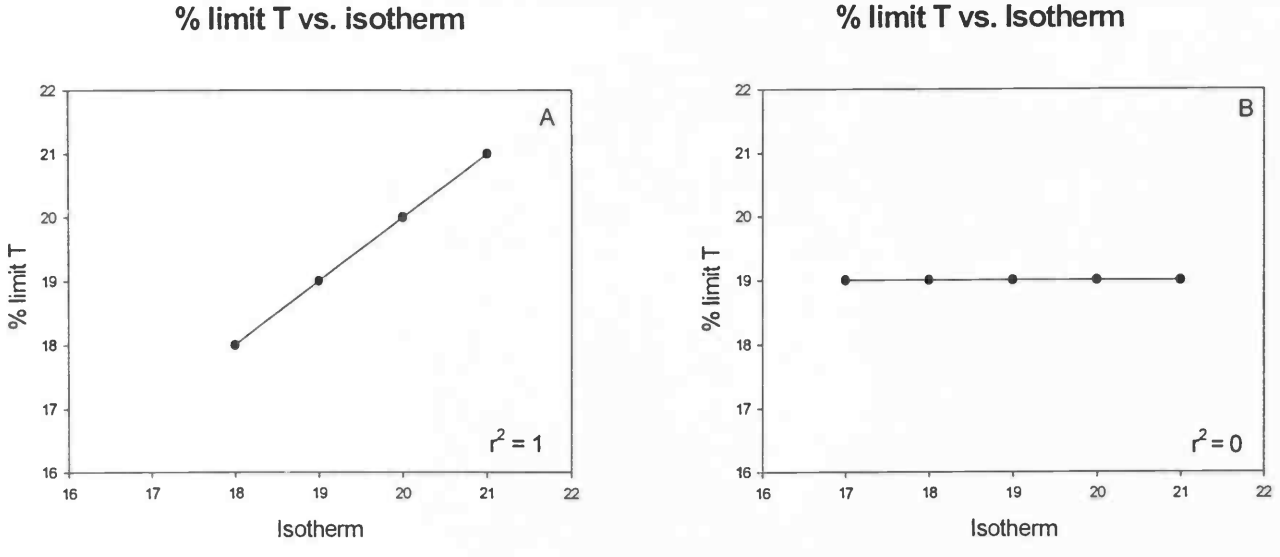


Fig 2C: Expected responses. A shows the expected response for ecotype foriming and/or adaptation. B shows the expected response for no ecotype froming or adaptation.

Questions:

Does *C. membranacea* have temperature ecotypes for growth?
Do these temperature ecotypes occur in the same isolates as the survival ecotypes (Pakker *et al*, 1995)?

Method:

Growth experiments were conducted with 7 isolates of *C. membranacea* and 4 isolates of related species (*Cladophoropsis sundanensis* (1 isolate), *Struvea anastomosans* (2 isolates) and *Chamaedoris peniculum* (1 isolate)). The isolates were grown at 25°C and long day conditions before starting the experiments. Tips of 5 mm were cut off and put in small petridishes filled with approximately 10 ml of 33‰ seawater enriched with ½ strength Provasoli (prepared according to Starr and Zeikus 1987). Per isolate, 5 tips were taken. The petridishes were sealed with strips of parafilm to prevent evaporation of the seawater during the experiments. The experimental temperatures were 15, 18, 20, 23, 25, 30, 32 and 35°C, under long day conditions with a photon fluence rate of 30 $\mu mol.m^{-2}.s^{-1}$. Measurements took place every 2-5 days, depending on the rate of growth, with an OPTIMAS 5.2 image analyser. Length was taken as a representative of growth. Experiments lasted for 1-2 weeks at optimum growth conditions and for 2-3 weeks at sub-optimum growth conditions. After this period the data were analysed with Sigma-plot. The lag-phase was eliminated from the graph and a regression line was constructed for each tip. The growth rate was calculated by use of the following equation:

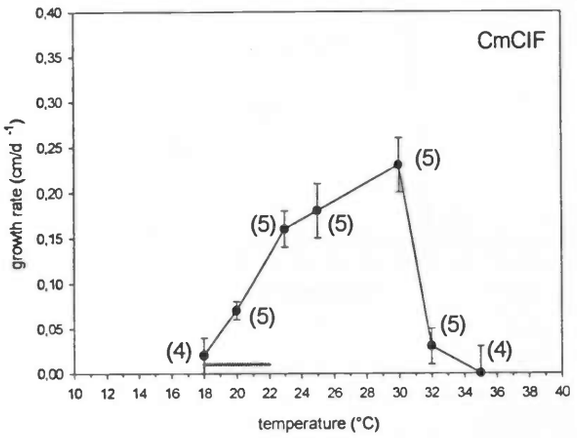
$$\text{Relative growth rate} = \frac{\ln l_2 - \ln l_1}{t_2 - t_1}$$

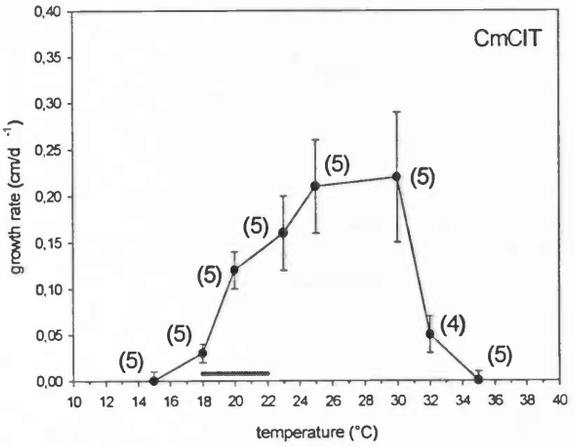
l_1 = length at day t_1
l_2 = length at day t_2

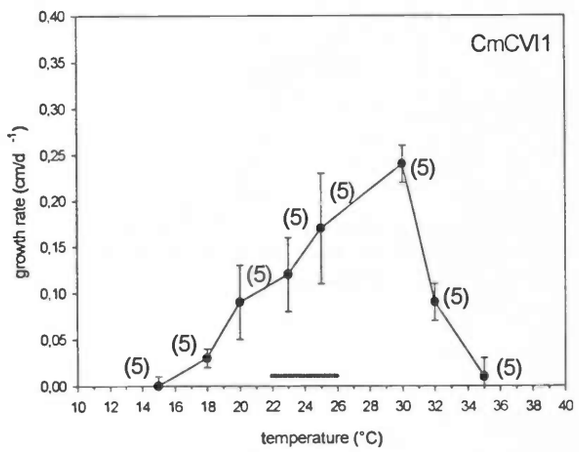
Per isolate the average growth rate, based on 5 tips, and the standard deviation for each temperature was calculated. The growth rates at different temperatures were used to construct a growth curve for each of the isolates.

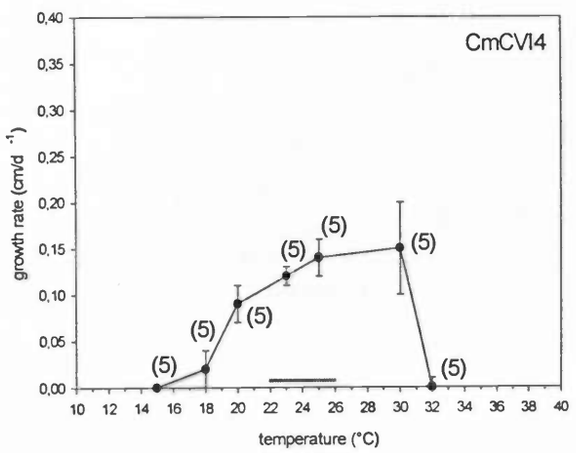
Results:

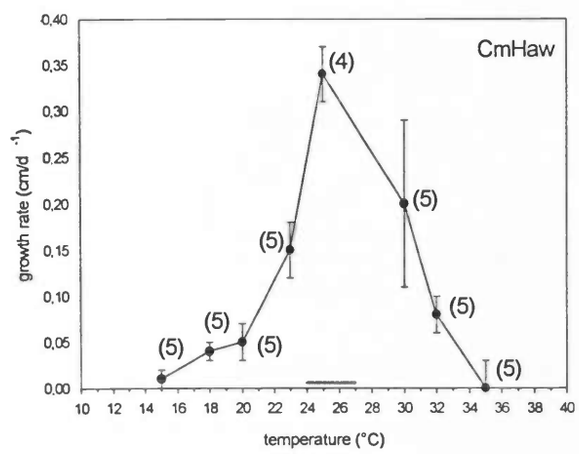
Growth curves were constructed from the found data. These are shown in figure 2D.

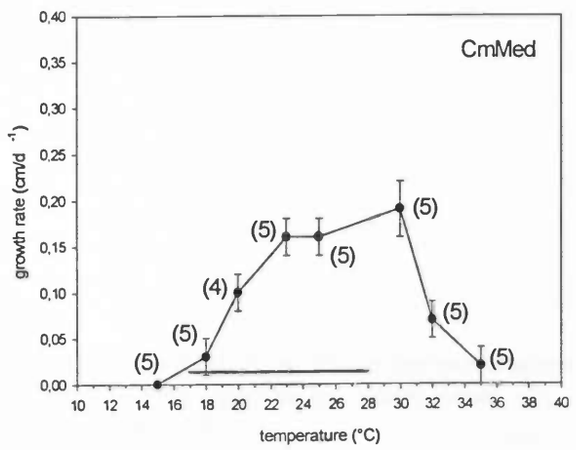












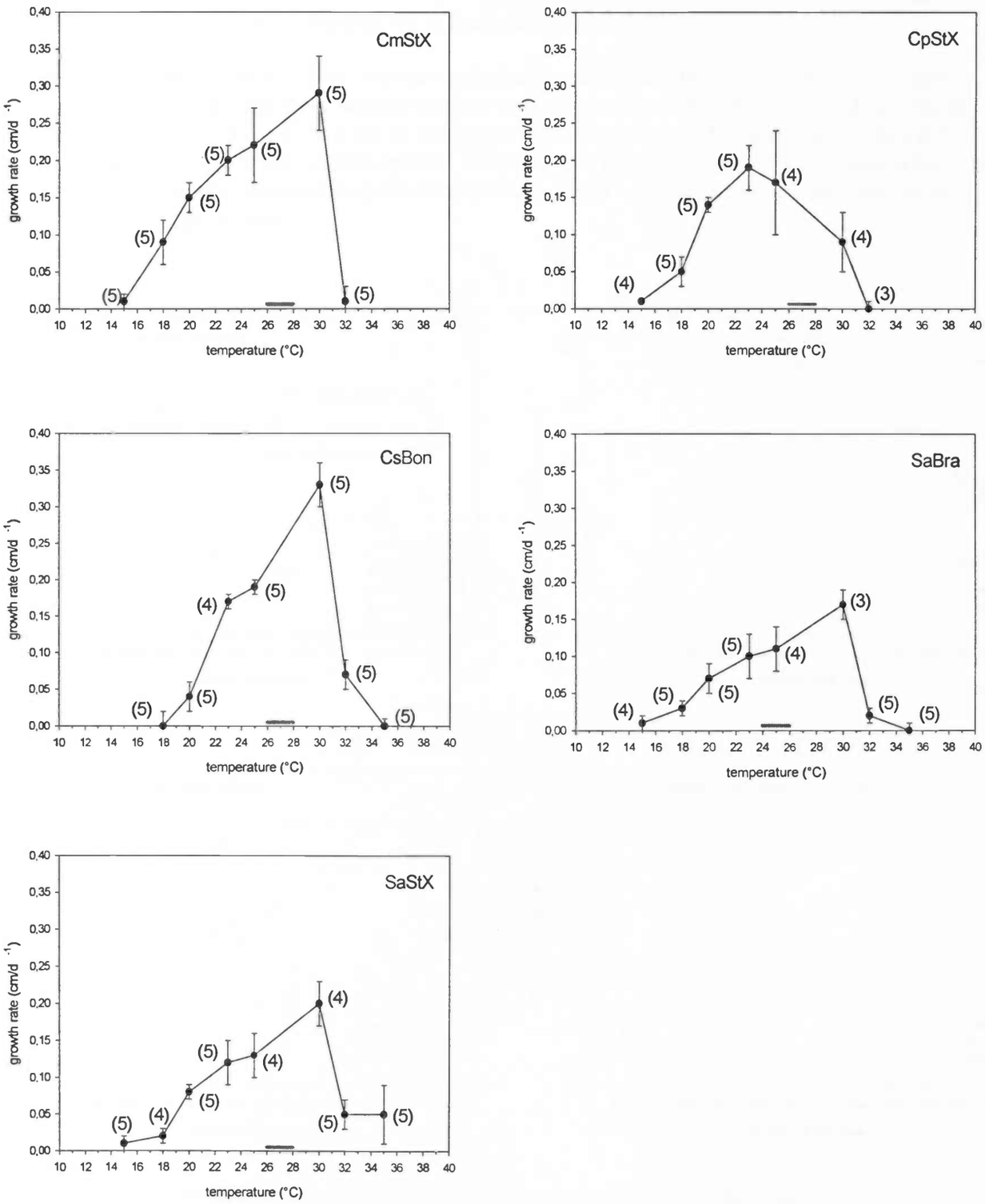


Fig 2D: Growth curves for each tested isolate. Points are means with standard deviations. Shown between brackets are the number of growth rates used to calculate the mean. The grey strip represents the natural annual temperature range at collection localities. See for isolate codes table 2A.

There was not much difference between the growth curves of the different isolates. The only major difference was the ability of the isolates to grow at optimal (for the tropical isolates) or at sub-optimal (temperate isolates) temperatures at their collection sites

The potential growth yields per month were calculated and a clear difference between the curves was observed (fig 2E). The more temperate growing isolates (CmCIF, CmCIT, CmMed, CmMau, and more or less CmCVI1) have a dip in their growth yield in winter. The tropical isolates show an almost constant growth potential through the year, except CmRS. CmHaw is an exception because it shows a dip in winter due to sub-optimal temperatures and a dip at the end of summer due to supra-optimal temperatures.

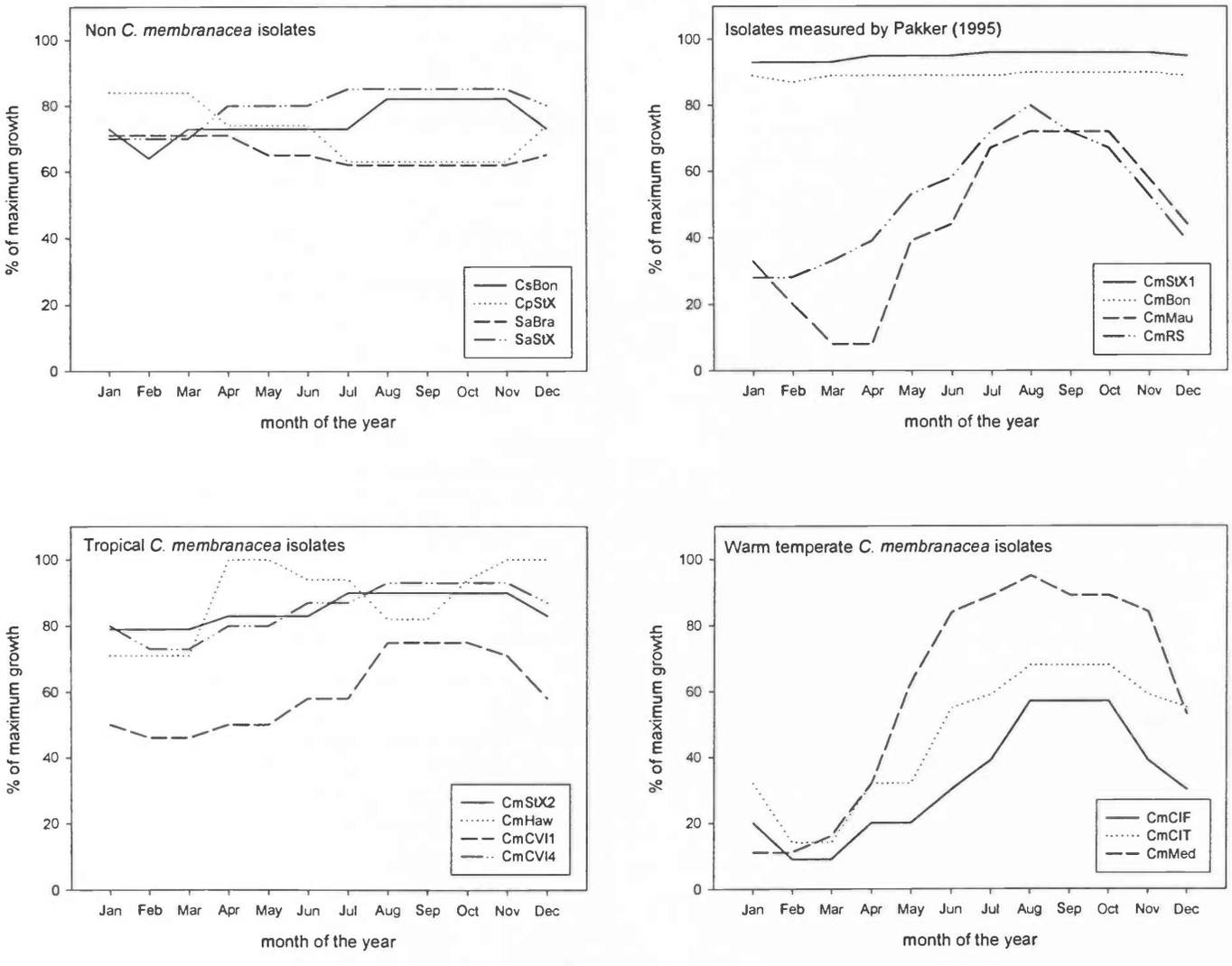


Fig 2E: Potential growth yield per isolate per month. For isolate codes, see table 2A. Graphs are divided in non *C. membranacea* isolates (being *C. sundanensis*, *C. peniculum* and *S. anastomosans*) ; data on *C. membranacea* isolates taken from Pakker *et al* (1995); tropical *C. membranacea* isolates and temperate *C. membranacea* isolates.

All tropical isolates, except one from the Cape Verde Islands (CVI1), reach 80% of their possible growth yield and all of them stay above the 20% growth yield in winter. The temperate isolates have a growth yield of less than 20% in the winter during at least one month. In summer only the Mediterranean isolate sustains an 80% growth yield. All isolates not belonging to *Cladophoropsis membranacea* have a winter growth yield of more than 60%. In summer only the isolate from Brazil does not reach the 80% growth yield. The tropical isolates of *C. membranacea* from St.Croix and Bonaire sustain a growth yield of over 80% during the whole year. The more temperate isolate from Mauritania reaches a yield of 80% during the summer. The tropical Red Sea isolate shows seasonality that stays above the 20% limit in winter.

When the temperatures for 20% growth at the lower limit are plotted against the February isotherm or when the 80% lower and upper limit temperatures were plotted against the August isotherm (fig 2F) no significant relation was found.

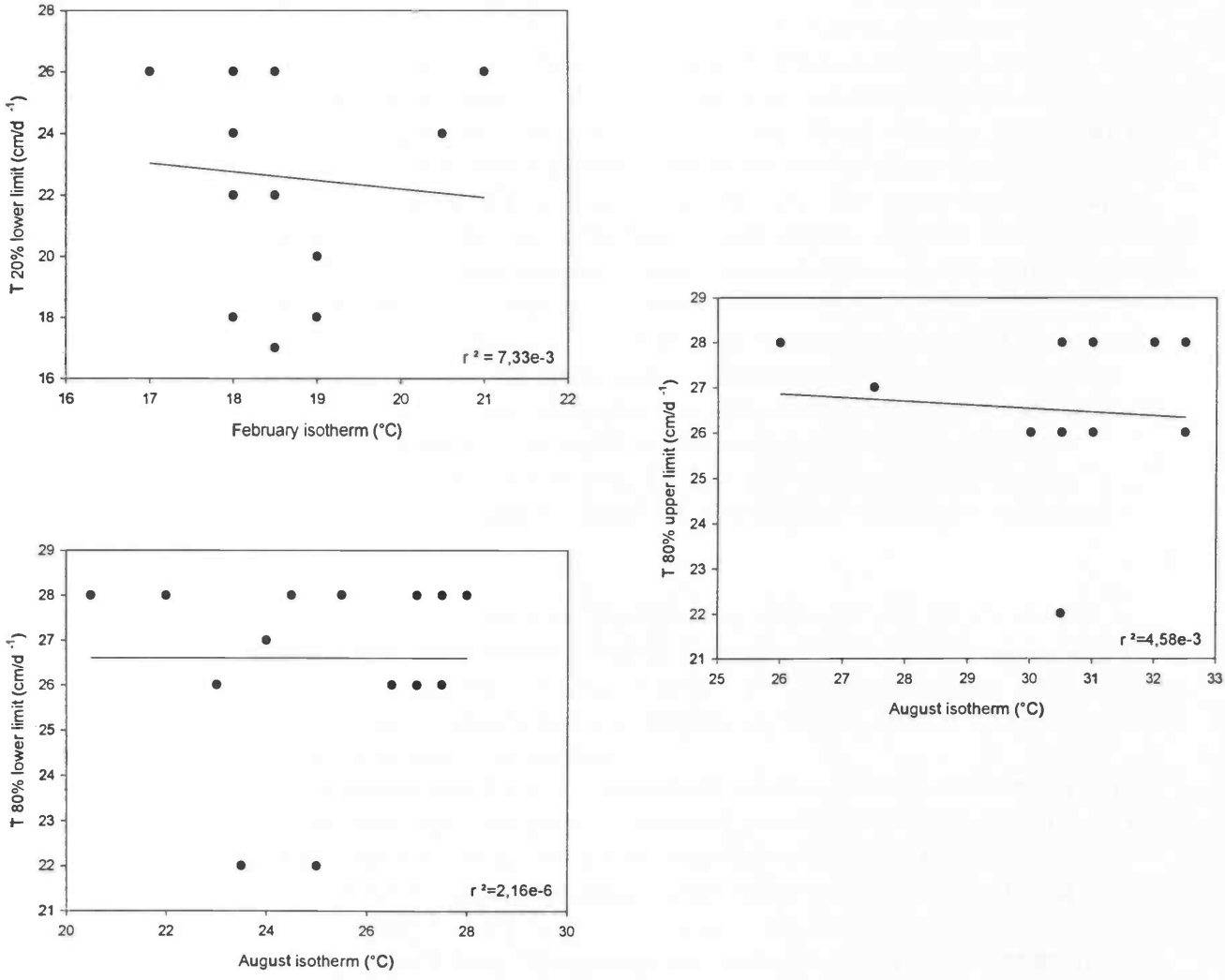


Fig 2F Correlation between 20% lower limit and the February isotherm and 80% lower and upper limit against the August isotherm.

Discussion:

When the growth curves from this study are compared with the data found by Pakker *et al* (1995), no big differences are observed in cases where the same isolates were tested. Thus, the data found by Pakker *et al* (1995) were included in the further analysis.
When the growth curves of different *C. membranacea* isolates and a few related species are compared, it appears that there are no major differences between the curves, although the shape of the curve may vary some what. Only the possibility of an isolate to grow at optimal or sub-optimal temperatures differs greatly.

As stated in the introduction, as an arbitrary definition for good growth, an isolate should be able to reach 80% of the possible growth yield for at least one month during the year and should not drop below the limit of a 20% yield during the rest of the year. These are, of course, no absolute limits but are only used to compare the potential performances of the different isolates. Taken these definitions into account one can say that the tropical Bonaire, Cape Verde Islands 4, St.Criox, and Hawaii isolates sustain good growth except the Cape Verde Islands 1 and the Red Sea isolates. The isolates from tropical to sub-tropical areas (Brazil and Mauritania) sustain reasonable growth whereas the other temperate isolates (Canary Islands and the Mediterranean) are not able to reach a 20% growth yield in winter and from these, only the Mediterranean isolate sustains a 80% growth yield in summer. This is according to the scheme made by Lüning. When only the curves are taken into account, the isolate from the Cape Verde Islands 1 has more similarities to the tropical to sub tropical zone and the isolate from the Red Sea to the temperate zone. The curve from the Cape Verde Islands 1 isolate does resemble the one of the Cape Verde Islands 4 isolate, it is only situated a little lower in the graph. The Cape Verde Islands 1 isolate sustains less potential growth yield per month when compared to the Cape Verde Islands 4 isolate. This is caused by the higher growth peak that the Cape Verde Islands 1 isolates has. This higher growth peak causes that not so much the actual growth rate is more or less but the growth rate in percentage decreases for the Cape Verde Islands 1 isolate. The Red Sea isolate resembles the curve from a more temperate algae due to the lower temperatures at the collection site which decreases the potential growth rate.
It is apparent that growth yields, during a whole year, get less when going from tropical to temperate regions (fig 2E). This analysis shows that there is no adaptation to more temperate climates.

The correlation between the 20% lower limit and the February isotherm, and the correlation between the 80% lower and upper limits and the August isotherm are not significant (fig 2F). If these are compared with the expected responses stated in the introduction they show a similarity to the expected response for no temperature ecotypes or adaptation. Hence, there is no evidence for a consistent pattern of adaptation to local circumstances.
If this is the case than it is expected that there is a significant relation between the February and August isotherms on the one hand and a decrease in potential growth yield on the other hand. When the February and August temperatures at the collection localities of each isolate were plotted against the potential growth yield at that temperature, significant correlation's were found (fig 2G). For the February isotherm $r^2 = 0.90$ ($v = 13$, $\alpha_{0.05} = 0.514$, $\alpha_{0.01} = 0.614$), for the August isotherm $r^2 = 0.42$ ($v = 13$, $\alpha_{0.05} = 0.514$, $\alpha_{0.01} = 0.641$). This means that potential yields decreased from the tropics towards the temperate zone.

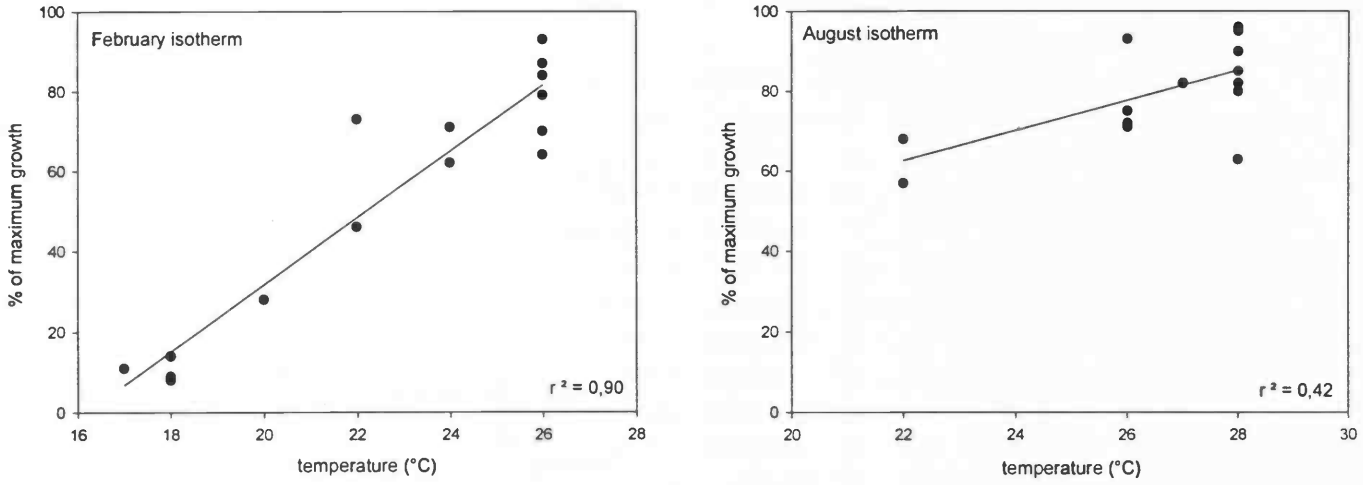
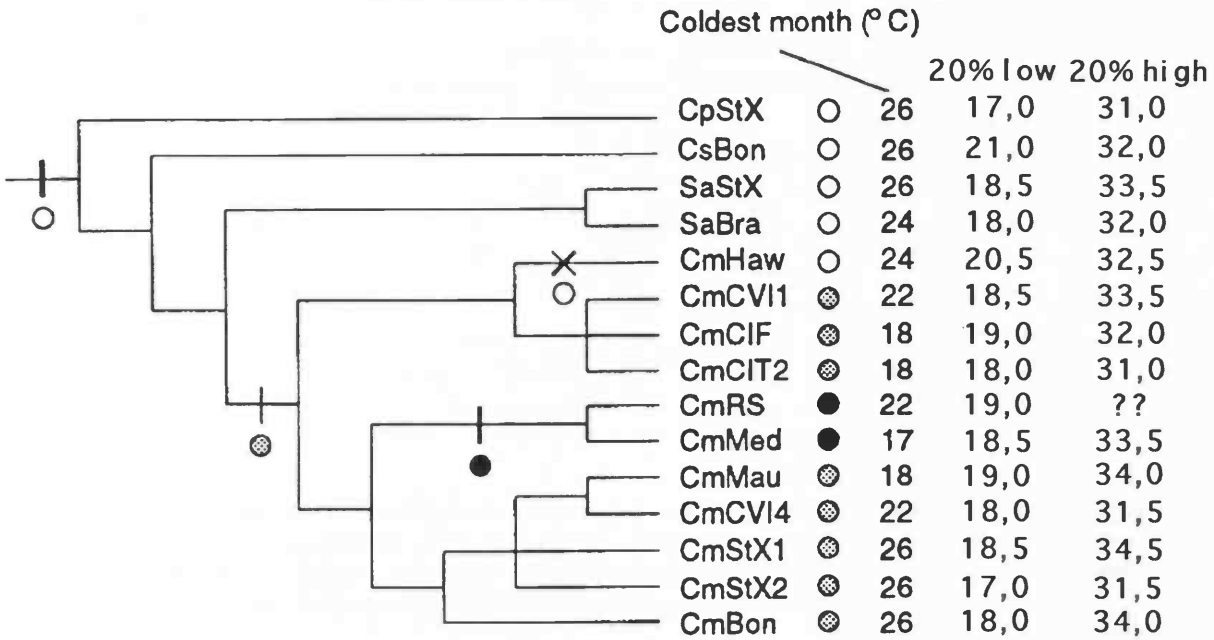


Fig 2G: Potential growth yield at the February and August isotherms for the natural situation for each isolate.

Pakker *et al* (1995) found a grouping in the phylogenetic tree for cold resistance. When the temperatures at which 20% and 80% growth occurs and the temperature ranges are superimposed on the phylogenetic tree (fig 2H), one can see no grouping similar to that found by Pakker *et al* (1995) or any other kind of grouping in these data.



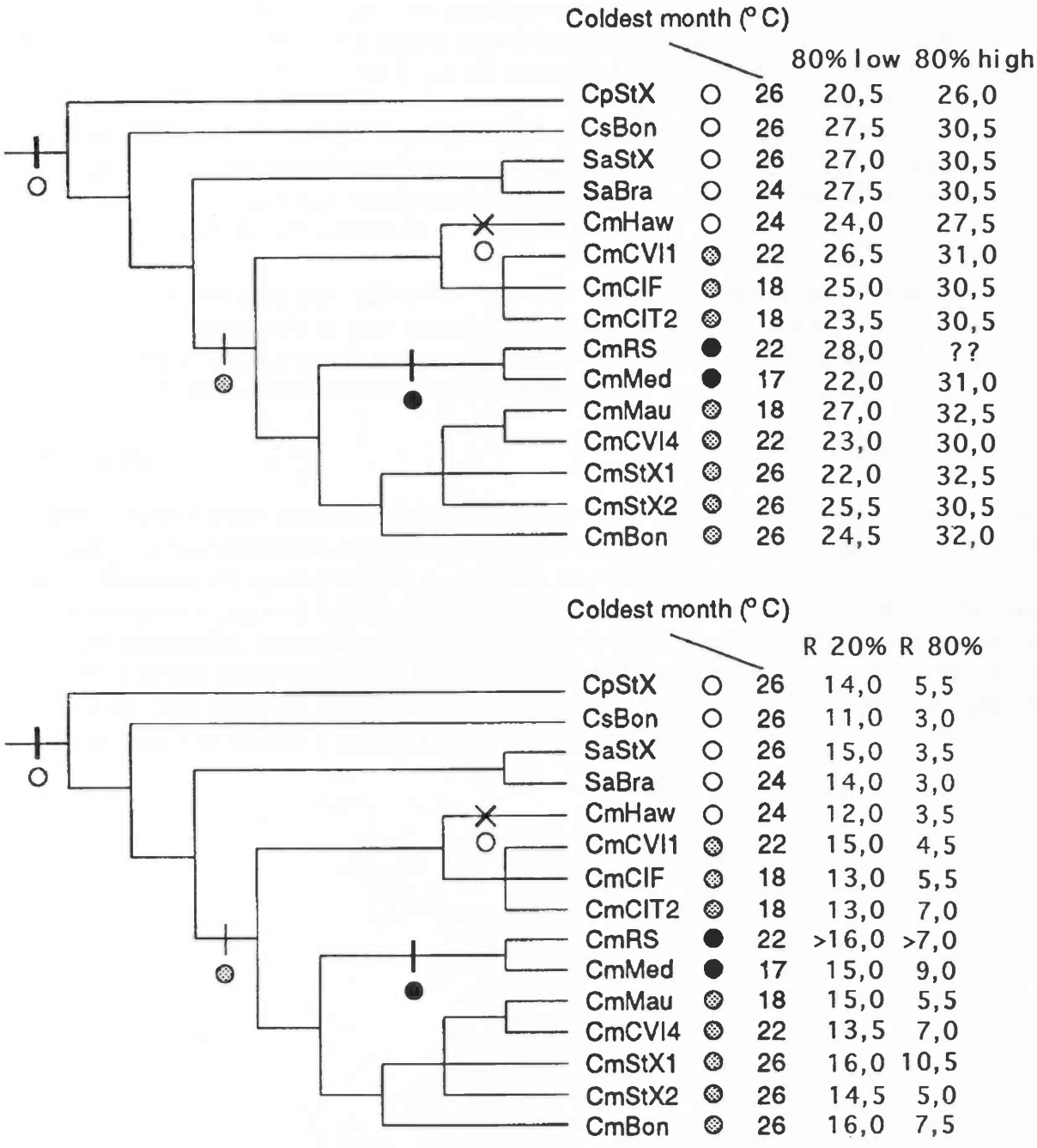


Fig 2H: Phylogenetic tree of *C. membranacea* and related species according to Kooistra *et al* (1992, 1993). Length of branches has no significance. 20%, 80% growth limits and growth ranges are superimposed. Low stands for % of growth before optimum temperature, high stands for % of growth after optimum growth temperature is reached. R stands for range. For RS the high % are not known so ?? is given.

Both the 20% low and high temperatures as the 80% low and high temperatures show little variation. This can be seen again in the 80% growth ranges and the 20% growth ranges, although the 80% values vary a little more than the 20% values. It can be said that some isolates have a wider temperature range in which they are able to sustain good growth. In general no grouping can be seen and so there is no evidence for evolutionary evolved ecotypes.

The tropical Red Sea isolate is grouped together with the temperate Mediterranean isolate in the phylogenetic tree (fig 2B) so one would expect that the Red Sea isolate would be adjusted to lower temperatures during the winter but figure 2F shows that this is not the case. Something similar goes for the Cape Verde Islands 1 isolate which is grouped together with the Canary Islands isolates. The Cape Verde Islands 4 isolate is grouped with tropical isolates. Expected would be that the Cape Verde Islands 1 isolate would grow better at low temperatures than the Cape Verde Islands 4 isolate but figure 2F shows again that this is not the case. Again this supports the conclusion that there are no temperature ecotypes for growth in *C. membranacea*.

Survival ecotype forming is an adjustment which is more important than growth ecotype forming since it is no use being able to grow better at a temperature or place, at which you are not able to survive. Possibly the adjustment to form ecotypic variation in growth is a process that is still at work and due to this no evidence can be found yet.

Conclusion:

It can be concluded that the tropical character of *C. membranacea* and related species is very clear since no growth adaptation to temperate regions or ecotypes have been found. The phylogenetic imprint for survival found by Pakker *et al* (1995) can not be found in growth.
Further research could look into the adaptation of reproductive cycles to see if an adaptation to temperate regions has occurred after a way is found to stimulate reproduction of *C. membranacea* and related species under controlled condotions. These data could also be superimposed on the phylogenetic tree (Kooistra 1992, 1993) to see if there is a link between the character state changes found by Pakker *et al* (1995) and the reproduction requirements.

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Appendix:

The original measured data, the modified data and the graphs, including the regression lines, can be found on the disk at the end of this paper. Filenames are listed in the appendix 1. The data for the growth curves can be found in appendix 2. Figure 2C shows the growth curves, for each isolate, that was constructed from these values.

Appendix 1:

Original data can be found on disc 1, Excel 5.0/7.0 file, org data.
Modified data can be found on disc 1, *Cladophoropsis membranacea* isolates, on disc 2, non-*Cladophoropsis membranacea* isolates, Sigma plot 4.0 files by isolate codes.
Regression line graphs can be found on disc 2, Sigma plot 4.0, groei.

Appendix 2:

Data for construction of the growthcurves given per isolate:

CmCIF			CmCIT			CmCVI1			CmCVI4		
	avg	sd		avg	sd		avg	sd		avg	sd
15°C	--	--	15°C	0.00	0.01	15°C	0.00	0.01	15°C	0.00	0.00
18°C	0.02	0.02	18°C	0.03	0.01	18°C	0.03	0.01	18°C	0.02	0.02
20°C	0.07	0.01	20°C	0.12	0.02	20°C	0.09	0.04	20°C	0.09	0.02
23°C	0.16	0.02	23°C	0.16	0.04	23°C	0.12	0.04	23°C	0.12	0.01
25°C	0.18	0.03	25°C	0.21	0.05	25°C	0.17	0.06	25°C	0.14	0.02
30°C	0.23	0.03	30°C	0.22	0.07	30°C	0.24	0.02	30°C	0.15	0.05
32°C	0.03	0.02	32°C	0.05	0.02	32°C	0.09	0.02	32°C	0.00	0.01
35°C	0.00	0.03	35°C	0.00	0.01	35°C	0.01	0.02	35°C	--	--

CmHaw			CmMed			CmStX			CpStX		
	avg	sd		avg	sd		avg	sd		avg	sd
15°C	0.01	0.01	15°C	0.00	0.00	15°C	0.01	0.01	15°C	0.01	0.00
18°C	0.04	0.01	18°C	0.03	0.02	18°C	0.09	0.03	18°C	0.05	0.02
20°C	0.05	0.02	20°C	0.10	0.02	20°C	0.15	0.02	20°C	0.14	0.01
23°C	0.15	0.03	23°C	0.16	0.02	23°C	0.20	0.02	23°C	0.19	0.03
25°C	0.34	0.03	25°C	0.16	0.02	25°C	0.22	0.05	25°C	0.17	0.07
30°C	0.20	0.09	30°C	0.19	0.03	30°C	0.29	0.05	30°C	0.09	0.04
32°C	0.08	0.02	32°C	0.07	0.02	32°C	0.01	0.02	32°C	0.00	0.01
35°C	0.00	0.03	35°C	0.02	0.02	35°C	--	--	35°C	--	--

CsBon			SaBra			SaStX		
	avg	sd		avg	sd		avg	sd
15°C	0.00	0.02	15°C	0.01	0.01	15°C	0.01	0.01
18°C	0.04	0.02	18°C	0.03	0.03	18°C	0.02	0.01
20°C	0.17	0.01	20°C	0.07	0.07	20°C	0.08	0.01
23°C	0.19	0.01	23°C	0.10	0.10	23°C	0.12	0.03
25°C	0.33	0.03	25°C	0.11	0.11	25°C	0.13	0.03
30°C	0.07	0.02	30°C	0.17	0.17	30°C	0.20	0.03
32°C	0.00	0.01	32°C	0.02	0.02	32°C	0.05	0.02
35°C	--	--	35°C	0.00	0.00	35°C	0.05	0.04

Avg is the average of the regression lines, sd is the standard deviation, -- means that this isolate was not tested at this experimental temperature. For isolate codes see table 2A. For regression graphs see appendix 1.