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# Studies on Tanzanian Hypneaceae: Seasonal Variation in Content and Quality of Kappa-Carrageenan from *Hypnea musciformis* (Gigartinales : Rhodophyta)

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***Key words:*** Hypneaceae, *Hypnea musciformis,* kappa-carrageenan, seasonal variation

***Abstract—***Seasonal effects on yield and quality of kappa-carrageenan from the red alga *Hypnea musciformis* were investigated in samples collected from natural populations in Oyster Bay, Dar es Salaam during June 1996–May 1997. The mean annual carrageenan yield, gel strength (after treatment with 0.1 M KCl) gelling and melting temperature (± standard deviation) were 25.24 ± 4.44 % dry weight, 171.72 ± 41.42 g/cm$^2$, 54.66 ± 3.12 ºC and 68.62 ± 0.60 ºC, respectively. Carrageenan yield and quality (gel strength) during the SE and NE monsoon seasons were not significantly different ($t = 0.55$, $p > 0.05$) and ($t = 1.91$, $p > 0.05$), respectively. The reported carrageenan yield and gel strength values were, respectively, about 50% and 40% those of carrageenan from *Kappaphycus alvarezii*. Although the carrageenan properties from *H. musciformis* were promising, its natural populations are generally insufficient to sustain the pressure of economic harvesting. Moreover, the extent to which its carrageenan yield and properties could be improved is not known. Suitable methods for mariculture are therefore needed before the resource can be exploited economically.

## INTRODUCTION

The family Solieriaceae has the greatest number of commercially exploited carrageenophytes (Doty, 1988, 1995) of any family in the red algal order Gigartinales. Most members consistently display a predominance of either the kappa or iota types through all phases of their life histories. In 1991, the annual world commercial harvest of seaweeds for production of carrageenans was estimated to be 250,000 tonnes (FW)/y. More than half of this harvest is from cultivated seaweeds, predominantly of the genera *Kappaphycus* and *Eucheuma* (Jensen, 1993). The annual value of the crop is estimated to be about US$ 200 million (Bixler, 1995).

Current demand for kappa carrageenan are greater than that for iota carrageenan; a situation partly caused by a relatively slower growth rate of the kappa carrageenophyte *K. alvarezii* as compared to the iota carrageenophyte *E. denticulatum* (Mtolera et al., 1995). In places like Zanzibar, where *E. denticulatum* grows best and farmers are reluctant to farm the slower-growing *K. alvarezii*, harvests of *E. denticulatum* have accumulated for several years. Mariculture of *K. alvarezii* in Zanzibar is also negatively affected in some areas by epiphytism. Faced with such a situation, development of alternative kappa-carrageenophytes e.g. *Kappaphycus striatum* and *Hypnea* species (Hypneaceae) (Semesi, 1979) is critical, and seaweed agronomy stakeholders (including marine biologists, scientists and the government) have a role to play to encourage the development and implementation of suitable cultivation technologies (Kapraun, 1999).

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The seaweed genus *Hypnea* is one of the most widespread on tropical and subtropical shores (Mshigeni, 1974a). In tropical regions, *Hypnea* species are abundantly distributed (Rao, 1970) throughout the year. In Brazil, for example, *Hypnea musciformis* is harvested (Mshigeni, 1974b) all year round.

In Tanzania, Jaasund (1976) listed six species of *Hypnea,* namely *H. pannosa, H. nidulans, H. musciformis, H. cornuta, H. hamulosa* and *H. nidifica. Hypnea musciformis* occurs mostly as an epiphyte on other algae, particularly *Laurencia* species. A study by Semesi (1979) revealed that *H. musciformis* may contain kappa-carrageenan with percentage yield of 43.5 ± 1.8. Attempts to domesticate *Hypnea* species and make it amenable to mariculture are starting to produce encouraging results (Berchez et al., 1993; Camaro Neto, 1987). Here we provide information on the seasonal variation of the carrageenan yield and quality of *Hypnea musciformis* from naturally growing Tanzanian stocks.

## MATERIALS AND METHODS

### Study area

Samples of *Hypnea musciformis* were collected monthly during low tide at the lower littoral zone of the Oyster Bay rocky shore, Dar es Salaam (39°16–39°17.5’E and 6°46.5’S), from June 1996 to May 1997. The annual migration of the Inter-Tropical Convergence Zone (ITCZ) creates the two seasons commonly known in East Africa as the northeast (NE) and southeast (SE) monsoons. The NE monsoon extends from December to April, and the SE from June to October, with periods of rainfall in between the monsoons (Bryceson 1982, McClanahan, 1988). Low air temperatures, high wind speeds, high cloud cover, low light intensity and rough seas characterise the SE monsoon period, while the NE monsoon has higher air temperatures, lower wind speeds, lower cloud cover, higher light intensities and calmer seas, by contrast.

### Environmental variables

Environmental factors were measured during each field visit, including light intensity (LI-COR model LI-189 Photometer), temperature (mercury thermometer), salinity (ATAGO S-10 refractometer), pH (Metrohm 632 pH meter), seawater and thallus nitrogen and phosphorus levels (colorimetry according to Allen, 1989). Sampling for nutrient, salinity and pH analysis and direct measurements of light and temperature were taken 5–10 cm below the water surface at the established sampling site (see below). A mean of three estimations of randomly collected samples was recorded as a representative value for the measurement. Field visits were made between 10:00 and 14:00 hours during lowest tides, with samples for estimating environmental factors, or direct measurement accomplished within 30 minutes of the lowest tide. This was to ensure that the measured factors represented the situation in the outgoing waters. Samples for nutrient analysis were filtered (0.2 mm), stored in acid-washed bottles (twice rinsed with filtered seawater) and transported to the laboratory in an icebox. The nutrient samples were kept at –21 ºC until analysis. Meteorological data (monthly total rainfall and mean maximum and minimum air temperatures and sunshine hours per day) were obtained from the Directorate of Meteorology.

### Biomass estimation

Biomass was estimated for four months covering the wet and dry SE and NE monsoon periods. It was estimated during lowest tides from a 50 x 50 m permanent quadrat using three, randomly thrown, 0.25 x 0.25 m quadrats. Samples within the 0.25 x 0.25 m quadrats were collected, sorted, washed (in seawater), rinsed (in distilled water to remove salts), mildly shaken and blotted to remove excess water, before drying at 105°C to constant weight.

### Carrageenan extraction and determination of properties

Carrageenan was extracted using essentially the method described by Mshigeni and Semesi (1977) and Semesi (1979). This should allow comparison with the data presented in these reports collected from the same sites.

Samples for carrageenan extraction were collected, by hand, each month, between June 1996 and May 1997. They were placed in plastic buckets and transported to the laboratory where they were sorted (to remove debris, epiphytes and other algae), cleaned with seawater and rinsed briefly with tap water to remove surface salts. These samples were then hand-centrifuged using cheesecloth to remove excess water and blotted dry before spreading out to sun-dry.

For quantitative determination of carrageenan yields, 20 g of dried seaweeds were soaked in 700 ml distilled water for two hours before being transferred into boiling water and boiling for 30 minutes. Boiled samples were thereafter chopped in the blender, mixed with 3 g sodium hydroxide pellets and left to continue boiling with constant stirring for four hours. The boiled samples were mixed with 10.0 g NaCl and filtered through a nylon cloth held over a strainer. Residues were washed using 300 ml of hot, distilled water until the carrageenan was removed. The resultant mixture was added slowly to 700 ml of 85% isopropanol while continuously stirring to precipitate the carrageenan. The precipitate was rinsed three times with 300 ml of isopropanol, squeezed to remove the excess, oven-dried to constant weight at 60 °C and thereafter ground for storage. The mean dry weight of three extractions was recorded as yield (dry carrageenan weight/dry algal weight).

To determine carrageenan gel strength, aliquots of 4 ml of (1% w/v) carrageenan solution in 0.1 M KCl were gelled in plastic vials at room temperature (25 °C) and kept at 4 °C overnight. Thereafter, gels were left to warm up for 1 hour at room temperature prior to determination of their gel strength and gelling temperature, using the apparatus designed by O. Sandren (Innovest AB, Ronnang, Sweden) (Buriyo et al., 2001). This apparatus automatically increases the load on the gel surface until the point of gel breakage.

Melting temperature was determined with glass beads suspended on the gel. The gels were heated stepwise at increments of 0.5 °C with equilibration for 20 minutes at each stage. The melting temperature was taken as the stage at which the beads sank into the gel.

The apparatus for determining gelling temperature had a rotating paddle inside the hot gel. At gel set paddle rotations stopped and the corresponding gelling temperature was displayed.

Carrageenan from *Kappaphycus alvarezii* was prepared in the same manner and its quality was studied for comparison.

### Statistics

The Pearson's product-moment correlation coefficient (r) was calculated to find out if the yield and quality of phycocolloids co-varied with the studied environmental parameters. The variations in yield and quality of phycocolloids between the monsoons were analyzed by a paired sample t-test. It was assumed that $p < 0.05$ signifies a difference (Zar, 1984).

## RESULTS

### Algal biomass

The mean algal biomass was higher during the NE monsoon (8.67 ± 1.1 g/m$^2$) than the SE monsoon period (3.10 ± 0.5 g/m$^2$) (Table 1). Higher biomass was observed during November to February (NE monsoon), when the sea was relatively calm, and nitrogen and phosphorous in the water column and solar radiation were at their highest (Table 2). The lowest mean biomass (2.4 ± 0.4) was recorded during the long rains (May).

### Carrageenan yield

Higher carrageenan content was measured between September and December (except November) (Table 3) while low carrageenan content was measured in August and November. Statistical analysis, however, showed that carrageenan yield was not significantly higher in any of the seasons

**Table 1. Comparison of mean biomass and carrageenan yield and quality of *Hypnea musciformis* between the SE and NE monsoon seasons**

| Season (monsoon) | Biomass (mean ± SD) | Mean carrageenan yield (% DW) | Mean gel strength (g/m$^2$) |
|---|---|---|---|
| SE | 3.1 ± 0.5 | 25.1 | 194.72 |
| NE | 8.67 ± 1.1 | 26 | 168.89 |

($p > 0.05$) (Table 4a). The environmental factors associated with high carrageenan content were the short rains (Table 2) and temperature (see some positive correlation with temperature, nitrogen and phosphorous, Table 5).

## Carrageenan quality

The gel strength of carrageenan was highest during the cool SE monsoon (June to August, Table 3; gel strength was inversely correlated with temperature, Table 5), soon after the long rains (Table 2). However, the difference between monsoons was not statistically significant (Table 4b). There was a

**Table 2. Environmental parameters recorded during the study period**

| | Water conditions+ | | | | | Air conditions | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Month (1996–97) | Temp. (ºC) | Salinity (‰) | pH | N (µg. at.N./l) | P (µg. at.N./l) | Light+ (mmol/m²/s) | Sunshine (hrs/day) | Temp. (ºC) | Rainfall (mm) Min | Max |
| Jun | 29.8 | 34.8 | 7.8 | 2.9 | 0.5 | | 8.8 | 18.8 | 29.4 | 5.7 |
| Jul | 29.5 | 35.3 | 8.0 | 2.8 | 0.4 | | 7.6 | 18.3 | 29.0 | 27.1 |
| Aug | 27.5 | 35.9 | 8.3 | 1.7 | 0.3 | 1785.5 | 9.0 | 17.4 | 29.4 | 9.5 |
| Sep | 28.8 | 37.0 | 8.3 | 0 | 0.7 | 1592.1 | 8.2 | 18.3 | 30.1 | 28.0 |
| Oct | 30.0 | 37.0 | 8.2 | 0.2 | 0.7 | 1992.8 | 7.6 | 19.4 | 29.7 | 87.5 |
| Nov | 31.1 | 36.3 | 8.0 | 5.4 | 0.2 | 2010.6 | 9.6 | 20.8 | 30.9 | 9.9 |
| Dec | 30.6 | 35.5 | 8.0 | 2.2 | 0.6 | 1984.7 | 9.8 | 24.5 | 32.0 | 0 |
| Jan | 31.0 | 34.8 | 8.5 | 2.4 | 0.7 | 2122.0 | 9.2 | 24.8 | 32.3 | 0.5 |
| Feb | 32.0 | 35.0 | 8.2 | 4.3 | 0.7 | 1857.0 | 9.1 | 23.7 | 32.1 | 0.3 |
| Mar | 26.5 | 30.0 | 8.3 | 1.6 | 0.0 | 312.1 | 6.1 | 23.8 | 30.9 | 222.5 |
| Apr | 28.0 | 30.8 | 8.5 | 2.2 | 0.3 | 436.2 | 5.5 | 23.0 | 30.5 | 187.0 |
| May | 28.0 | 29.9 | 8.4 | 4.0 | 0.7 | 1806.8 | 5.9 | 21.4 | 29.4 | 115.1 |

+Mean values of at least three determinations as detailed in the Materials and Methods section

**Table 3. Seasonality in carrageenan yield, gel strength, gelling temperature, melting temperature and thallus dry weight percentage (% DW) nitrogen (N) and phosphorous (P) in *Hypnea musciformis***

| Month (1996–97) | Yield (% DW) | Gel strength (g/cm²) | Gelling temp. (°C) | Melting temp. (°C) | % DW content (x10⁻²) N | P |
|---|---|---|---|---|---|---|
| Jun | 24.92 ± 0.4 | 197.22 ± 1.1 | 57.00 ± 0.1 | 69.75 ± 0.4 | | |
| Jul | 25.32 ± 0.2 | 243.06 ± 1.3 | 58.75 ± 0.2 | 68.68 ± 0.2 | | |
| Aug | 20.56 ± 0.8 | 194.44 ± 0.9 | 57.50 ± 0.1 | 67.94 ± 0.1 | 35.71 | 0.48 |
| Sep | 29.68 ± 0.3 | 138.89 ± 0.5 | 58.00 ± 0.2 | 68.47 ± 0.2 | | |
| Oct | 35.63 ± 0.7 | 162.50 ± 1.4 | 51.50 ± 0.5 | 68.26 ± 0.5 | | |
| Nov | 20.56 ± 1.4 | 77.78 ± 3.1 | 50.50 ± 0.9 | 67.47 ± 0.1 | 392.85 | 0.57 |
| Dec | 27.30 ± 0.7 | 169.44 ± 3.2 | 51.25 ± 1.0 | 69.00 ± 0.1 | | |
| Jan | 21.35 ± 1.5 | 169.44 ± 1.9 | 53.25 ± 0.3 | 69.00 ± 0.0 | | |
| Feb | 22.94 ± 0.5 | 194.44 ± 7.1 | 55.25 ± 0.5 | 68.68 ± 0.0 | 78.57 | 1.90 |
| Mar | | | | | | |
| Apr | 24.92 ± 0.2 | 156.94 ± 5.2 | 51.50 ± 0.1 | 68.89 ± 0.7 | | |
| May | 24.52 ± 0.3 | 184.72 ± 3.2 | 56.75 ± 0.8 | 68.74 ± 0.2 | 264.29 | 3.48 |

Mean values for at least three determinations a month are shown. Mean annual yield and gel strength (after treatment with 0.1M KCl) of carrageenan from *Kappaphycus alvarezii* collected monthly from June 1994 to May 1995 and extracted and tested using similar procedures were 50.20 ± 0.2 % DW and 442.5±3.2 g/cm², respectively.

**Table 4.** Statistical comparison of (a) mean yield and (b) mean gel strength of carrageenan from *H. musciformis* between SE and NE monsoon seasons

| Season | n | df | Mean | t | p |
|---|---|---|---|---|---|
| **a (mean yield)** | | | | | |
| SE | 18 | 17 | 25.1 | 0.55 | >0.05 |
| NE | 18 | 17 | 26 | | |
| **b (mean gel strength)** | | | | | |
| SE | 18 | 17 | 194.72 | 1.91 | >0.05 |
| NE | 18 | 17 | 168.89 | | |

sudden fall in gel strength in November (Table 3) when carrageenan yield was also low.

Mean annual yield and gel strength (after treatment with 0.1 KCl) of carrageenan from *Kappaphycus alvarezii* (collected monthly from June 1994 to May 1995 and put through the same procedures of extraction and determination of gel properties) were 50.20 ± 0.2 % DW and 442.5±3.2 g/cm$^2$, respectively.

## DISCUSSION

In this study, *Hypnea musciformis* was most abundant between November and January and carrageenan yields (although they did not follow the monsoon pattern) were slightly higher between September and December (with the exception of November). High plant abundance and carrageenan yield were recorded at a time of the year when the sea was relatively calm, and light intensity and concentrations of nitrogen and phosphorus nutrients were also high. In the iota carrageenophyte *Eucheuma denticulatum* (Buriyo et al., 2001), periods of seaweed abundance (November–February) and high carrageenan yield (March–August) did not coincide. The latter species had its highest carrageenan content during a cooler season of the year. In the kappa-carrageenophyte *Kappaphycus alvarezii* that was grown in nutrient-enriched water, carrageenan content was lower compared to that from plants grown in natural seawater without ammonia additions (Rui et al., (1990).

Although the growth rate of *Hypnea musciformis* was not investigated in this study, studies by Guist et al. (1982) reported a growth rate of 20%/day. The reported growth rate of *H. musciformis* is twice that of *E. denticulatum* and thee times that of *K. alvarezii* (Mtolera et al., 1995). It appears that the three carrageenophytes differ in their optimum growth conditions and capacities. The filamentous *H. musciformis* required higher light intensities for growth. As *H. musciformis* also grows as an epiphyte to other algae (Semesi, 1979), calm waters facilitated higher biomass accumulation. The observed lack of seasonal patterns for carrageenan content may be explained by the alga's high growth rate. The samples collected may have belonged to differing age groups. Although this study did not investigate the variation of carrageenan content with age, studies such as that of Lignell and Pedersén (1989)

**Table 5. Correlation coefficients between carrageenan yield, gel strength and the recorded environmental parameters**

| | | Yield | | Gel strength | |
|---|---|---|---|---|---|
| Parameters | n | r | P | r | P |
| Light | 31 | -0.622 | $p < 0.05$ | 0.317 | $p > 0.05$ |
| Water temperature | 31 | 0.285 | $p > 0.05$ | -0.326 | $p > 0.05$ |
| Air temperature | 31 | 0.617 | $p < 0.05$ | -0.603 | $p < 0.05$ |
| Salinity | 31 | 0.132 | $p > 0.05$ | 0.001 | $p > 0.05$ |
| Nitrogen in seawater | 31 | -0.083 | $p > 0.05$ | -0.452 | $p < 0.05$ |
| Phosphorous in seawater | 31 | 0.007 | $p > 0.05$ | -0.066 | $p > 0.05$ |
| Thallus nitrogen | 31 | 0.183 | $p > 0.05$ | -0.827 | $p < 0.05$ |
| Thallus phosphorus | 31 | 0.813 | $p < 0.05$ | 0.412 | $p < 0.05$ |
| Biomass | 31 | -0.044 | $p > 0.05$ | 0.08 | $p > 0.05$ |
| Ash content | 31 | -0.305 | $p > 0.05$ | -0.704 | $p < 0.05$ |

have elegantly shown that phycocolloid content in algae may vary significantly with age. Modification of agar chemistry with age was proposed to arise from a type of 'secondarization' of the cell wall (Lahaye & Yaphe, 1988).

Abrupt changes in environmental parameters such as light, temperature and salinity may affect significantly the field biomass of *Hypnea musciformis*. For example, when the sampling site was visited on 11 and 12 March 1997 (a week before the long rains locally known as *masika*) a high biomass of *H. musciformis* was observed. However, after a week of rains, *H. musciformis* was no longer found. Factors such as low salinity, high light intensity and temperature induce 'ice-ice' conditions in both agarophytes and carrageenophytes (Collen et al., 1995; Mtolera et al., 1995).

Seasonal studies in *H. musciformis* growing in India and in Florida, USA revealed that higher carrageenan yield and gel strength occurred in January to April (Rao & Krishnamurthy, 1978; Solimabi et al., 1980) and March to May (Durako & Dawes, 1980), respectively. The two carrageenan properties seem to vary with *H. musciformis* growth conditions. Rao (1970) was of the opinion that the peak levels in carrageenan content followed the period of most rapid growth while Guist et al. (1982) suggested that high biomass and carrageenan yield in *H. musciformis* are obtained about two weeks after the plants have been nutrient-'starved'.

In a survey done by Semesi (1979), carrageenan yields from *H. musciformis* growing in different parts of the world seem to vary from 4 to 53%. In a non-annual assessment of carrageenan yield from *H. musciformis*, Semesi (1979) reported a yield of around 43%. In the present study, the highest yield was around 35%, obtained in October, and the mean annual yield was around 25%. In *K. alvarezii*, carrageenan yield fall to below 30% during the unfavorable growth season (long rains) and rise to more than 60% during the favorable season in October (data not shown).

The annual mean gel strength of the carrageenan from *H. musciformis* was around 170 g/cm$^2$, with a maximum of 240 g/cm$^2$ measured in July. The annual mean yield and gel strength of the carrageenan from *H. musciformis* are about 50% and 40% (Table 3), respectively, values for carrageenan from *K. alvarezii*.

Although the properties of carrageenan from *H. musciformis* found in this study are promising, natural populations of *Hypnea* species are generally insufficient to sustain economic harvest pressure (Mshigeni & Chapman, 1994; Schenkman, 1989) and the extent to which its carrageenan yield and properties could be improved is not yet clear. Attempts such as those of Berchez et al. (1993) and Camaro Neto (1987) to domesticate *Hypnea musciformis*, and of Wallner et al. (1992) to improve its carrageenan yield, provide encouragement that the resource could be utilised as a supplement to the existing kappa carrageenophytes.

More studies are needed to establish optimal conditions for growth and improved carrageenan yield and properties.

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