

a very short time. To confirm whether this sensitivity is primarily due to external change in ionic concentrations of the salt or due to the change in the osmotic pressure of the medium, the following experiment was conducted.

Taking distilled water as base Na^+ concentration was raised to that of normal sea water (33‰) by adding Na_2SO_4 . Another solution was prepared taking the same base and increasing Cl^- concentration to that of normal sea water adding MgCl_2 . Ten selected clams were introduced in these solutions to observe opening of shell valves. In none of these solutions the clams opened, revealing that the 2 ionic concentrations were not responsible to open the shell valves. Hence it was felt necessary to study whether the osmotic pressure is required to open the shell valves. For this, distilled water was chosen as base and its osmotic pressure increased by adding a nonelectrolyte. A stock solution of glycerine isotonic to full strength sea water (34.8‰) was prepared in distilled water and further isotonic sea water solutions (50, 40, 30, 20, 10, and 5%) were prepared from this stock solution in full strength sea water. These solutions were taken separately in glass troughs along with normal sea water, 50% normal sea water and pure isotonic glycerine solution separately in glass troughs. In each trough 10 selected clams were introduced and the time taken to open (starting to open and fully open) shell valves in each was noted. Because of the individual variation in the time of opening of the shell valves, mean values were considered. Osmotic pressure of the outside medium is the main stimulus to open the shell valves though some amount of salt is necessary to fully open the shell valves of the clams (Table 1).

Marine molluscs are osmoconformers with varying degree of stenohalinity. Some marine molluscs (*Patella vulgata*, *Cardium edule*, *Mytilus edulis*, etc.) acclimated to fresh water by very gradual lowering of salinity

within 6 months could live in healthy state with fresh water forms like *Lymnaea* and *Planorbis*². *Crassostrea virginica* was found to remain with shell valves open till the changes from higher to lower salinity of the surrounding water stopped^{3,4}. *Katelysia opima* could tolerate salinity as low as 10.5‰, with the shell valves remaining closed in very low salinities¹. In the present study *P. laterisulca* was sensitive to environmental changes even when apparently closed. The powerful adductor muscles in this clam are capable to close the shell valves hermetically to withstand the periodically changing and generally unstable estuarine condition. Milne⁵ stated that in the Aberdeenshire Dee estuary, the mussels with shell valves closed lived in low salinity (3.45‰) and isolated their mantle water concentration from outside medium.

P. laterisulca could tolerate salinity of 19.2‰ in summer whereas in monsoon by gradual acclimation to low salinities (12.2‰). During considerable inflow of river water in Kalbadevi estuary the clams close shell valves till they develop suitable hydrostatic pressure on the mantle edges by slight shell valve movement and a slight increase in the salinity of the external medium. Thus the nature of adjustment in *P. laterisulca* vary with environmental changes—the changes in salinity fluctuations and exposure during low tide, which develop the compensatory alternations in the clam by gradual acclimation and also due to the nature of valve closing mechanism.

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Seasonal Changes in Carrageenan & Other Biochemical Constituents of *Hypnea musciformis*

SOLIMABI, B DASS, S Y KAMAT, L FERNANDES & C V G REDDY

National Institute of Oceanography, Dona Paula, Goa 403004

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Carrageenan content was maximum in winter. Near inverse relationship was observed between the total carbohydrate and carrageenan. Protein levels were highest when the nitrogenous nutrients were high.

An understanding of seasonal changes in seaweeds such as *Hypnea* sp., a carrageenan producer¹, is desirable for deciding the suitable time for commercial harvesting. With this in view an attempt has been made

Table 1—Time Taken to Part Shell Valves in Various Isotonic Solutions of Sea Water

[Temp. $30 \pm 0.5^\circ\text{C}$, S - starting to open, F - fully open]

Type of solution	The time taken to part shell valves min	
	S	F
Normal sea water	0.56	1.67
Normal 50% sea water	4.12	5.84
Isotonic sea water %		
50	1.38	2.54
40	1.73	3.36
30	1.84	3.41
20	4.33	6.29
10	7.8	—
5	—	—
Isotonic glycerine sol.	—	—

SHORT COMMUNICATIONS

to study the variations in carrageenan content and other biochemical properties of *Hypnea musciformis* from Goa coast. Distribution of this alga in the Goa region is not very common².

Monthly specimens (from Nov. 1977 to Oct. 1978) of *Hypnea musciformis* were collected from the intertidal zone at Anjuna beach (15°27'N; 73°48'E) during low tide. These were washed quickly with freshwater to remove surface salt and then carefully sorted to remove any adhering solids and other algal growth. They were dried at room temperature for 4 days followed by oven drying at 40°C for another 12 hr. Prior to analysis, the dried plant material was powdered and a 20 mesh screen was used for carrageenan extraction and a 80 mesh screen for all other analyses. Total carbohydrate was determined by phenol-H₂SO₄ method³ and protein by the method of Lowry *et al.*⁴ after homogenizing with Na₂CO₃.

Carrageenan was estimated by the addition of 600 ml hot water to 10 g dried milled plant material followed by 2 g of CaO as a paste. Samples were heated for 18-22 hr on a boiling water bath. The solution was filtered and the filtrate adjusted to pH 7. Carrageenan, being strongly sulphated was easily precipitated from the solution by the addition of 2 volumes of 85% isopropyl alcohol/volume of extract. Phycocolloid precipitates out as long fibres. After 15 min, the coagulum was washed thrice with 85% isopropyl alcohol, collected on a weighed filter paper and dried in an oven to constant weight. Yields were determined as percentage extract from the dried plant.

Total sulphate was determined colorimetrically following digestion of the polysaccharide with HNO₃ for 12 hr at 100°C. After evaporation to dryness the residual solid was treated with conc. HCl (1 ml) and evaporated to dryness. The solid was treated with water, evaporated to dryness and the tube placed in an oven at 105°C for 2 hr. The following modification in the method of Jones and Letham⁵ was used for sulphate determination. To the sulphate solution, 4-chloro-4' amino diphenyl and a trace of solid hexadecyltrimethyl ammonium bromide were added.

After mixing, this solution and a blank were kept for 2 hr and then centrifuged. Aliquots (0.2 ml) of supernatant were removed and diluted to 25 ml with 0.1 N HCl. The optical densities were read at 254 nm on a Beckman spectrophotometer. From a standard graph the differences over the blank reading gave the sulphate content.

Monthly surface water temperature was recorded, at the site of collection. Water samples were also collected from the site for the determination of salinity and nutrients (phosphate, nitrite, nitrate and ammonia) using standard methods.

Qualitative study on the relative abundance of *Hypnea* populations indicated that the seaweed starts growing in October-November and becomes abundant in December to February (Table 1). Thereafter it showed a decline and became scarce. High light intensity, increasing temperature and to some extent high salinity in hot season and severe wave action during SW monsoon seem to be the factors responsible for its destruction.

Carrageenan content increases from October to December and then shows a decline (Table 2) after which the plant apparently dies away. It is interesting to note that minimum values of phycocolloid recorded from February to May coincide with the decline in the vegetative growth of *H. musciformis* as well as with the agar-agar content⁶.

Carbohydrate concentration varied from 31 to 50% with maximum values in October, November and May and minimum in January and February. Intermediate levels occurred during the intervening period. Comparison of carbohydrate and carrageenan values reveals that they are inversely related suggesting that monosaccharides are converted into polysaccharides. Protein levels show a gradual increase from October to May (Table 2).

Comparison of abundance and biochemical properties of the seaweed with environmental factors indicates that during its growth period from October to February, temperature and salinity remain nearly stable. During the maximum growth period,

Table 1—Surface Water Temperature, Salinity and Nutrients at the Site of Collection

Month of collection*	Temp. °C	NO ₂ -N µg-at/l	NO ₃ -N µg-at/l	NH ₄ -N µg-at/l	PO ₄ -P µg-at/l	Salinity ‰
Nov. ^c	28.5	0.13	1.78	3.57	0.26	33.95
Dec. ^a	27	0.16	0.67	4.2	0.26	34.7
Jan. ^a	27	0.17	1.14	ND	1.38	35.3
Feb. ^b	31	0.31	1.36	5.5	1.58	35.62
March ^c	31	0.43	2	2.5	0.5	36.46
April ^d	30	0.9	4	2.7	0.42	35.98
May ^d	29	0.96	3.9	7.7	1.5	34.69

**Hypnea* not available from June to Sept. and starts growing during Oct.

^a, Most abundant; ^b, abundant; ^c, less abundant; ^d, scarce.

ND—Not detectable

Table 2—Biochemical Properties of *H. musciformis*
(Values represent per cent dry wt)

	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May
Carrageenan	30.5	32.8	51.6	47.7	36	34.04	32.01	29.23
Carbohydrates	50	42	36	31	31	33.5	33.6	46
Protein	9.92	10	12.5	12.5	14.5	16.55	15	17
Sulphate	12.48	16	16.7	20.8	20.4	15.8	15	13.5

carbohydrates are comparatively higher than proteins. Carrageenan also attains maximum concentrations at this time. The nitrogen and phosphorus in the water do not show any relationship with the biochemical composition of the seaweed except that the high protein content is associated with high nitrogen in the sea. Temperature and to some extent salinity seem to have significant effect on the growth and abundance of the organisms and this feature is reflected on its biochemical composition of the seaweed.

K-carrageenan is the gelling component of carrageenan⁷ and enzymatic studies⁸ with K-carrageenase from *Pseudomonas carrageenovora* revealed a higher content of K-carrageenan (87%) in *H. musciformis*. Therefore, the best period for harvesting the alga based on yield is from December to February.

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Early Life History of *Anadontostoma chacunda* (Ham.-Buch.) from Porto Novo

M THANGARAJA & K RAMAMOORTHY
Centre of Advanced Study in Marine Biology
Annamalai University, Parangipettai 608 502

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Eggs and larvae of *A. chacunda* are described. Of the larvae (2.8-15 mm) described those up to 5 mm are grown in the laboratory from hatching, and the rest collected from the plankton. Occurrence of the early stages in the Vellar estuary commences in January (sal. 29‰) and attains a peak in March-April (sal. 33-35‰).

Information on the life history of *Anadontostoma chacunda* (Clupeidae: Pisces) is fragmentary¹⁻⁶. The present report provides information on 6 developmental stages of this species from egg through 56 hr old larva reared in the laboratory and on subsequent 6 stages collected in the plankton from Vellar estuary (lat. 11°29' N; long. 79°49' E).

Plankton was collected with a net (bolting silk No. 10). Eggs of *A. chacunda* in the samples were separated and kept in culture troughs up to 56 hr after hatching. The post larvae from plankton were preserved in 5% neutralised formalin immediately after collection. Stages of development are shown in Figs 1 and 2. Morphometric measurements are given in Table 1. The terminology given by Jones⁷ was adopted for different stages of development. Identification of eggs and larvae was made according to Delsman⁴ Bensam² and Venkataramanujam⁶.

In vitro development—Egg (Fig. 1A): Spherical (1.05 to 1.16 mm diam.); yolk spherical, colourless, segmented (0.95 diam.); oil globules 5 to 10, uneven (0.0035-0.0058 mm diam.). Embryo 2 mm length (oral end to caudal tip) with 27 distinct myotomes, pigmented and uniformly distributed punctate black chromatophores on dorsal side. Optic vesicles and auditory sacs feebly visible. Developing brain and pulsating tubular heart distinct.

Newly hatched larva (Fig. 1B): Length 2.8 mm; 8 oil globules at the base of yolk mass. Anus 2.17 mm from snout, in between 34th and 35th myotomes. Postanal myotomes (11) distinguishable with some difficulty. Number of myotomes (45) higher than vertebral count of 41 in adult. Larva transparent; eyes not pigmented and mouth not opened. Pigments in punctate black chromatophores present on dorsal side from head to tail. Formation of the caudal rays noticed.

Prolarva - 8 hr (Fig. 1C): Growth rapid, prolarva 4.4 mm in length. Out of 8 initial oil globules 1 remains. Mouth not yet open. Auditory vesicle becomes large (0.18 mm). Number of myotomes increases to 46; anus located just behind 34th myotome and eyes slightly pigmented. About 18 stellate black chromatophores arranged throughout the length of the alimentary canal. All punctate chromatophores on dorsal aspect disappear. Caudal rays increase in number and in length.