- Constable, J., Packer, C., Collins, D. and Pusey, A., Nuclear DNA from primate dung. *Nature*, 1995, **373**, 393.
- Gerloff, U., Schlötterer, C., Rassmann, K., Rambold, I., Hohmann, G., Fruth, B. and Tautz, D., Amplification of hypervariable simple sequence repeats (microsatellites) from excremental DNA of wild living bonobos (*Pan paniscus*). *Mol. Ecol.*, 1995, 4, 515– 518.
- Taberlet, P. *et al.*, Noninvasive genetic tracking of the endangered Pyrenean brown bear population. *Mol. Ecol.*, 1997, 6, 869–876.
- Reed, J. Z., Tollit, D. J., Thompson, P. M. and Amos, W., Molecular scatology: The use of molecular genetic analysis to assign species, sex and individual identity to seal faeces. *Mol. Ecol.*, 1997, 6, 225–234.
- Kohn, M. H., York, E. C., Kamradt, D. A., Haught, G., Sauvajot, R. M. and Wayne, R. K., Estimating population size by genotyping faeces. *Proc. R. Soc. London, Ser. B*, 1999, **266**, 657–663.
- Eggert, L. S., Rasner, C. A. and Woodruff, D. S., The evolution and phylogeography of the African elephant inferred from mitochondrial DNA sequence and nuclear microsatellite markers. *Proc. R. Soc. London, Ser. B*, 2002, 269, 1993–2006.
- Taberlet, P., Waits, L. and Luikart, G., Noninvasive genetic sampling: Look before you leap. *TREE*, 1999, 14, 323–327.
- Sukumar, R., The Living Elephants. Evolutionary Ecology, Behavior, and Conservation, Oxford University Press, New York, 2003.
- Nozawa, K. and Shotake, T., Genetic differentiation among local populations of Asian elephants. J. Zool. Syst. Evol. Res., 1990, 28, 40–47.
- Hartl, G. *et al.*, Population genetics and systematics of Asian elephants (*Elephas maximus*): A study based on sequence variation at the *Cytb* gene of PCR-amplified mitochondrial DNA from hair bulbs. Z. Saeugetierkd., 1996, 61, 285–294.
- Fernando, P., Pfrender, M., Encalada, S. and Lande, R., Mitochondrial DNA variation, phylogeography and population structure of the Asian elephant. *Heredity*, 2000, 84, 362–372.
- Fleischer, R., Perry, E., Muralidharan, K., Stevens, E. and Wemmer, C., Phylogeography of the Asian elephant (*Elephas maximus*) based on mitochondrial DNA. *Evolution*, 2001, 55, 1882–1892.
- Fernando, P., Vidya, T. N. C., Rajapakse, C., Dangolla, A. and Melnick, D. J., Reliable non-invasive genotyping: Fantasy or reality? *J. Hered.*, 2003, 94, 115–123.
- Fernando, P., Vidya, T. N. C. and Melnick, D. J., Isolation and characterization of tri- and tetranucleotide microsatellite loci in the Asian elephant, *Elephas maximus. Mol. Ecol. Notes*, 2001, 1, 232– 234.
- Nyakaana, S. and Arctander, P., Isolation and characterization of microsatellite loci in the African elephant, *Loxodonta africana*. *Mol. Ecol.*, 1998, 7, 1436–1437.
- Raymond, M. and Rousset, F., GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. J. Hered., 1995, 86, 248–249.
- 22. Rice, W., Analysing tables of statistical tests. *Evolution*, 1989, **43**, 223–225.
- Mills, L. S., Citta, J. J., Lair, K. P., Schwartz, M. K. and Tallmon, D. A., Estimating animal abundance using noninvasive DNA sampling: Promise and pitfalls. *Ecol. Appl.*, 2000, **10**, 283–294.
- Waits, J. L., Luikart, G. and Taberlet, P., Estimating the probability of identity among genotypes in natural populations: Cautions and guidelines. *Mol. Ecol.*, 2001, **10**, 249–256.
- Paetkau, D., Waits, L. P., Clarkson, P. L., Craighead, L., Vyse, E., Ward, R. and Strobeck, C., Variation in genetic diversity across the range of North American brown bears. *Conserv. Biol.*, 1998, 12, 418–429.
- Morin, P. A., Chambers, K. E., Boesch, C. and Vigilant, L., Quantitative polymerase chain reaction analysis of DNA from noninvasive samples for accurate microsatellite genotyping of wild chimpanzees (*Pan troglodytes verus*). *Mol. Ecol.*, 2001, **10**, 1835–1844.

27. Comstock, K. *et al.*, Patterns of molecular genetic variation among African elephant populations. *Mol. Ecol.*, 2002, **11**, 2489–2498.

ACKNOWLEDGEMENTS. The molecular work was supported by the US Fish and Wildlife Service – Asian Elephant Conservation Fund, and the Centre for Environmental Research and Conservation Small Grants Program. We thank our collaborators Dr P. Fernando and Prof. D. J. Melnick at the Center for Environment Research and Conservation, Columbia University, New York for support. Field work in southern India was funded by the Ministry of Environment and Forests, Government of India. Samples were collected with research permissions from the state forest departments of Tamil Nadu, Karnataka, Kerala, Orissa, Jharkhand, West Bengal, Assam, Arunachal Pradesh, Meghalaya, and Uttaranchal. We thank Mr C. Arivazhagan and Dr T. R. Shankar Raman for help in obtaining samples, and Mr K. Krishna, Mr. R. Mohan, and several forest department trackers who provided field assistance.

Received 23 February 2004; revised accepted 11 October 2004

Factors affecting the occurrence of *Porphyra vietnamensis* Tanaka & Pham-Hoang Ho along the Goa coast

N. Pereira^{1,*}, J. Kakode¹ and V. K. Dhargalkar²

¹National Institute of Oceanography, Dona Paula, Goa 403 004, India ²Marine Conservation Society of India, c/o National Institute of Oceanography, Dona Paula, Goa 403 004, India

Porphyra vietnamensis Tanaka & Pham-Hoang Ho occurs in the intertidal region of the Goa coast, during monsoon months from June to September. The growth and distribution patterns of P. vietnamensis populations along the Goa coast were studied quantitatively. Data on algal distribution, abundance, biomass and hydrological parameters were collected at three sites (Dona Paula, Reis Magos estuarine sites and Vagator open coast), every fortnight for a period of 4 months (June-September 2002). Highest biomass was recorded at Vagator (1.3 kg m⁻²) followed by Dona Paula (0.9 kg m⁻²) and Reis Magos (0.5 kg m^{-2}). The study reflected that nutrients (nitrate, nitrite and phosphate) and temperature were the factors responsible for the growth of this alga. Among them, phosphate was the main growthpromoting factor at Vagator; this was supported by its positive and significant correlation with the algal biomass. Low temperature was found to be conducive for algal growth, since it exhibited negative and significant correlation with algal biomass. Morphological observation of this alga indicated that thalli growth was relatively larger and well developed at Vagator compared to the other two sites.

THE marine red alga *Porphyra* (Rhodophyta, Bangiales), popularly known as 'Nori' in Japan, has an annual value of over US \$18 billion, and occurs widely on temperate and tropical rocky shores throughout the world^{1,2}. Nearly 133

^{*}For correspondence. (e-mail: neelam@darya.nio.org)

species of *Porphyra* have been reported from all over the world and they occupy diverse spatial and temporal habitats^{1,3}. Its life cycle consists of alternation of macroscopic gametophytic stage with microscopic conchocelis stage^{1,4}. It is not only delicious, but also contains high levels of protein (25–50%), vitamins (vitamin C is higher than that in oranges), trace minerals and dietary fibres⁵. *Porphyra* has been cultivated for the past 100 years in Japan, Korea and China⁶. Because of its economic importance and other health benefits, *Porphyra* cultivation is now being expanded to other countries⁷. Seven species have been recorded from the Indian coast. However, these are not being exploited commercially³.

The occurrence of *Porphyra* on the Indian peninsular region was first reported from Karachi⁸ as *Porphyra vulgaris* C.Ag., followed by two species, *Porphyra lanciata* (Lightf.) Ag. and *P. vulgaris* C.Ag., recorded from Ceylon⁹. Along the Indian coast, leafy phase of *Porphyra vietnamensis* reported from Vishakapatnam coast, is a summer annual (April/May), while the same species occurs on the Goa coast during active monsoon (July/October), showing peak growth in August. *Porphyra okhaensis* sp.nov. from Okha is a winter annual occurring during November–January¹⁰. *Porphyra kanyakumarensis* Krishnamurthy *et* Baluswamy grows during the southwest monsoon, July–September, along the Kerala coast¹¹.

Growth of *P. vietnamensis* during monsoon is one of the most conspicuous features along the west coast of India. This alga grows profusely in magenta coloured mats over the entire rocky surfaces in the upper littoral zone¹². The thalli are membranous, undulate with spinulose processes along the margins¹³. A number of studies reveal that each stage in the life cycle of *Porphyra* is species-specific and dependent on light, temperature and photoperiod¹⁴.

The aim of this study was to identify the causal factors that lead to the variation in growth patterns of this alga. The growth and development of alga was monitored over a four-month period to note changes in distribution, abundance, biomass and other characters for better understanding of the seasonal and differential growth patterns of *Porphyra*.

Fortnightly collections of littoral seaweeds were carried out at three sites along the Goa coast (Figure 1). Two sites (Dona Paula and Reis Magos) with estuarine influence of Zuari and Mandovi river respectively, and (Vagator) open coast were selected for seaweed collection. The distribution and relative abundance of P. vietnamensis were recorded at each station. For qualitative studies, a line transect was laid perpendicular to the coast and seaweeds were sampled in triplicate using 0.25 m² quadrant. The collected seaweeds were carefully sorted, cleaned to remove extraneous matter such as epiphytes, shells, sand particles, etc., weighed and expressed as kg m^{-2} (wet weight). In order to find the relation between length and biomass of P. vietnamensis, lengths of 20 thalli of this alga were measured at each sampling at all the three sites. The biomass and thallus measurements of alga (length) were taken as indicators of growth.



Figure 1. Map showing sampling sites.

Data on sea water nutrients such as NO₃, NO₂ and PO₄ at three different sites were analysed according to the Strickland and Parsons method¹⁵ and are presented in Figure 2. Temperature, pH, dissolved oxygen and salinity records are shown in Figure 3. Rainfall data were obtained from the Panaji observatory of the India Meteorology Department, Goa (Table 1). The correlation coefficient (r) between physicochemical parameters and biomass was calculated using Microsoft excel software program and the significance level (P) was ascertained according to the Fischer's statistics book¹⁶.

Studies undertaken on the monsoon annual of *P. viet-namensis* along the west coast of India and Sonkhla Province, southern Thailand, indicate that this species occurs during rainy season and cooler parts of the year, when the sea temperature and salinity are $low^{12,17}$. It has been pointed out in culture studies that low temperature and short days are suitable factors for growth of *Porphyra* thalli; this response corresponded with the observed seasonal behaviour of plants in nature^{4,18}.

Dhargalkar *et al.*¹² observed that high rainfall influenced the growth of *Porphyra* (peak biomass was 1.6 kg m⁻²), due to reduced salinity (3.5‰) and low temperature (25°C). Decline in rainfall levels led to increased salinity and temperature, and hence a marked decline in biomass. We observed that the three sites showed different growth levels of this alga. Maximum biomass of *Porphyra* recorded at Vagator was 1.3 kg m⁻² in the first fortnight of August followed by Dona Paula (0.9 kg m⁻²) and Reis Magos (0.5 kg m⁻²) in the second fortnight of August (Figure 4). Highest biomass coincided with the lowest temperature and shorter sunshine hours (Table 1). Also, maximum species of seaweeds associated with *Porphyra* were found at Vagator. The thalli of *Porphyra* were relatively well developed at Vagator. This is supported by positive as well as significant correlation between the length of the thallus and its biomass (r = 0.839, P < 0.01; r = 0.785, P < 0.02 at Vagator and Dona Paula respectively). A similar relationship was observed between length and biomass of *Asparagopsis delilei* Montagne at Putty island (r = 0.812, P < 0.001) and Valai island (r = 0.699, P < 0.01), located in the Gulf of Mannar¹⁹. There have been published generalizations regarding the connection of the mass with linear body size and of the mass with intensity of certain biological functions²⁰.

At Vagator, highest phosphate concentration (5.88 μ mol l⁻¹) corresponded with highest biomass of the season (1.3 kg m⁻²),

whereas at Dona Paula and Reis Magos, rainfall, temperature and salinity were found to affect algal growth. Moreover, the difference in salinities at these estuarine systems due to some geological features, like the width of the river mouth and sand bar formation at the mouth of the Mandovi estuary during the monsoon months could be a cause for lower salinity at Reis Magos (2.78–33.62‰), compared to the slightly higher salinity at Dona Paula (3.06–34.17‰). Lower temperature was found to be conducive for the growth of *P. vietnamensis*. This is supported by a negative and significant correlation with biomass at Reis Magos (r = -0.83, P < 0.01), Vagator (r = -0.81, P < 0.01) and Dona Paula (r = -0.6, P < 0.1). A similar relationship was observed in



Figure 2. Nutrient concentration at three sites during the study period.



Figure 3. Physico-chemical parameters of water during the study period. CURRENT SCIENCE, VOL. 88, NO. 3, 10 FEBRUARY 2005

Table 1. Meteorological data of the locality during the study period							
Date	Temperature (°C)				Wind speed		
	Maximum	Minimum	Mean	Rainfall (mm)	KMPH	Knot	Sunshine (h/day)
13/6/02	31.9	26.2	29	7.5	7	4	3.1
27/6/02	29.7	25.4	27.5	18.4	19	10	5.5
12/7/02	30.2	26.6	28.4	0.6	15	8	7.6
26/7/02	29.1	25.5	27.3	1.7	10	6	5.9
12/8/02	27.8	25	26.4	1.4	15	8	2.2
26/8/02	28.1	24.1	26.1	10.5	11	6	7.4
9/9/02	29.7	24	26.9	1.5	6	3	7.6
25/9/02	30.9	24.5	27.7	0	7	3	69





Figure 5. N : P ratio at three different sites.

Figure 4. Biomass of Porphyra vietnamensis at different sites during the study period.

the intertidal populations (r = -0.536) of A. delilei from the Mandapam region²¹. These differences in salinities could be the causative factor for the differences in biomass.

Various studies conducted on this alga indicate that the parameters responsible for its growth remain more or less constant. A study made on Porphyra leucosticta Thuret in Le Jolis, from southern Mediterranean coast of Spain, showed that leafy thallus occurs only during winter (mid-December to mid-March), with peak cover in February 22 . Thus its disappearance coincides with increasing day length, high irradiance and sea water temperature. The conchospore formation and release in Porphyra spiralis var. amplifolia Olievera Filho et Coll. from warm, temperate coast of southern Brazil, is controlled by a combination of short days along with varying range of temperatures²³.

Variation in nitrate and phosphate concentration of the Mandovi-Zuari estuarine system from that of the open ocean may be due to addition of nitrogenous material in the former from land and river run-off²⁴. The seasonality in nutrient limitation of marine macro algal growth is a well-known phenomenon²⁵. The positive correlation observed between the biomass (fresh weight) of Porphyra and phosphate concentration (r = 0.772, P < 0.01) at Vagator, indicates that phosphate rather than nitrate is a growth-limiting factor. Vagator exhibited higher level of phosphate compared to nitrate. Hence, lower ratio of nitrate and phosphate corresponded with higher biomass at Vagator (Figure 5).

Even though nitrogen is believed to be the primary nutrient limiting factor, evidence suggests that phosphorus may commonly be the primary limiting nutrient to algal growth in certain waters. The results of in situ macro algal bioassays in Florida reveal that inorganic phosphorus was quantitatively more important than nitrogen in limiting both photosynthetic capacity and growth of *Gracilaria tikvahiae* Mac Lachlan²⁶. These studies, however, contrast with temperate North American shore waters where nitrogen is considered the primary nutrient limiting growth factor for macro algae²⁷.

The decline in biomass of P. vietnamensis towards the end of September is marked by the discoloration of its thalli. This could be due to decrease of nutrient, and increase in salinity and water temperature²⁸. This progressive disappearance of thalli is linked with increased temperature and day length. Although senescence is largely unknown in algae, it is probably an important physiological process in those algae with alternations of generations. Thus, it can be said that there exists a relationship between senescence and phosphorus limited growth, as seen at the end of the life cycle of $P. leucosticta^{29}.$

Umamaheshwara Rao and Sreeramulu³⁰ pointed out that the seasonal growth period in which Porphyra flourishes

RESEARCH COMMUNICATIONS

luxuriantly on the coast is around the period when most of the algal forms growing in that region are in a degenerating condition. However, at Vagator, *Porphyra* and other seaweeds, *Enteromorpha flexuosa*, *Gelidium pusillum* (Stackh.) Le Jolis and *Ulva fasciata* Delile showed a more or less equal proportion. The abundant growth of seaweeds at Vagator could be due to the fact that open shore plants have higher biomass than those growing in an inlet²⁰.

Field observations showed that *Porphyra* entities were restricted in their distribution to particular parts of the intertidal and upper littoral zones. The relative abundance of substrata suitable for *Porphyra* attachment varied among the sites. The sites also differed in degree of water movement, tidal emersion characteristic and topography³¹. Therefore, these factors are presumably important in recruitment, survival, growth and development of *P. vietnamensis*, and hence population structure at each site differs. It appears that the dominant parameters influencing the growth of *Porphyra* are nutrients (particularly phosphate at Vagator, r = 0.77, P < 0.01) and temperature (r = -0.81, P < 0.01at Vagator; r = -0.63, P < 0.01 at Reis Magos, and r = -0.6, P < 0.1 at Dona Paula). A more detailed study needs to be made in order to pinpoint the precise causative factor.

- 1. Burzychi, G. M. and Waaland, J. R., On position and meiosis in life history of *Porphyra torta*. *Bot. Mar.*, 1987, **30**, 5–10.
- 2. Hanisak, M. D., World Aquacult., 1998, 29, 18-21.
- Sahoo, Dinabandhu, Nivedita and Debashish, Seaweeds of Indian Coast, APH Publishing Corporation, New Delhi, 2001, pp. 1–283.
- Kurogi, M., Study of life history of *Porphyra*. I. Germination and development of carpospores. *Bull. Tohoku Res. Fish. Res. Lab.* (In Japanese, English summary), 1953, 2, 67–103.
- Noda, H., Effect of nitrogenous factor on recovery of discolored fronds of *Porphyra yezeonsis. J. Appl. Phycol.*, 1993, 5, 255–258.
- Mumford, T. F. and Miura, A., In *Algae and Human Affairs* (eds Lemby, C. A. and Walland, J. R.), Cambridge Univ. Press, Cambridge, 1988, pp. 87–117.
- 7. Levine, I. A., Bull. Aquacult. Assoc. Can., 1999, 1, 31-33.
- Murray, J. A., *The Plants and Drugs of Sind*, London, 1881, pp. 1–219.
- Murray, G., A catalogue of Ceylon algae in herbarium of British Museum. Ann. Mag. Nat. Hist. Ser., 1887, 5, 20–26.
- Joshi, H. V., Moza, K. and Tewari, A., Life cycle and growth of *Porphyra okhaensis* sp. nov. in culture. *Indian J. Mar. Sci.*, 1992, 22, 262–267.
- Chennubotla, V. S. K., Matthew, S. and Joseph, I., A note on *Porphyra kanyakumarensis* (Bangiales, Rhodophyta) along the Kerala coast. *Sea Res. Util.*, 1990, **13**, 1–4.
- Dhargalkar, V. K., Agadi, V. V. and Untawale, A. G., Occurrence of *Porphyra vietnamensis* (Bangiales, Rhodophyta) along the Goa coast. *Mahasagar–Bull. Natl. Inst. Oceanogr.*, 1981, 14, 75– 77.
- 13. Krishnamurthy, V. and Baluswami, M., The species of *Porphyra* from Indian region. *Seaweed Res. Util.*, 1984, **7**, 31–38.
- Orfandis, S., Culture studies of *Porphyra leucosticta* (Bangiales, Rhodophyta) from Gulf of Thessaloniki, Greece. *Bot. Mar.*, 2001, 44, 533–539.
- Strickland, J. D. H. and Parsons, T. R., A Practical Handbook of Seawater Analysis, Alger Press Limited, Ottawa, Canada, 1972, pp 1–310.

- Fischer, R. A. and Frank, Y., Statistical Tables for Biological, Agricultural and Medical Research, Oliver Boyd, Edinburg, 1963, pp. 1–146.
- Lewmanomont, K. and Chittpoolkusol, O., Life cycle of *Porphyra* vietnamnesis, Tanaka and Pham-Hoang Ho from Thailand. *Hydro*biology, 1993, 260/261, 397–400.
- Rao, G. M. N., Seasonality in growth, reproduction and spore liberation of *Porphyra vietnamensis* Tanaka et Ho (Rhodophyta, Bangiales). *Mahasagar–Bull. Natl. Inst. Oceanogr.*, 1992, 25, 11–17.
- Vasuki, S., Ganesan, M. and Subba Rao, P. V., Seasonal variation in growth of marine red alga *Asparogopsis delilie* (Rhodophyceae/ Ronnemaisoriales) from Mandapam region, southeast coast of India. *Indian J. Mar. Sci.*, 2000, 29, 61–64.
- Khailov, K. M., Kholodov, V. I., Firsov, Yu K. and Prazukin, A. V., Thallus of *Fucus vesiculosis* in ontogenesis: Changes in morpho–physiological parameters. *Bot. Mar.*, 1978, 28, 289–311.
- Thomas, P. C. and Subbaramaih, S., Seasonal variations in growth, reproduction, alginic acid, mannitol, iodine and ash contents of brown alga Sargassum wightii. Indian J. Mar. Sci., 1990, 19, 148– 150.
- Hernandez, I., Coezo, A., Gordillo, F. J., Robles, M. D., Saez, E., Hernandez, J. A. and Niels, F. X., Seasonal cycle of the gametophytic form of *Porphyra umbilicalis*: Nitrogen and carbon. *Mar. Ecol. Prog. Ser.*, 1993, **99**, 301–311.
- Kapraun, D. F. and Lemus, A. J., Field and culture studies of *Porphyra spiralis* var. *amplifolia* Olieveira Filho et Coll (Bangiales, Rhodophyta) from Isla de Margarita, Venezuela. *Bot. Mar.*, 1987, 30, 483–490.
- De Souza, S. N., Sen Gupta, R., Sanzgiri, S. and Rajagopal, M. D., Studies on nutrients of Mandovi and Zuari river systems. *Indian J. Mar. Sci.*, 1981, **10**, 314–321.
- Lobban, C. S. and Harrison, P. J., *Ecology and Physiology of Seaweeds*, Cambridge University Press, 1994, pp. 1–366.
- Lapointe, B. E., A comparison of nutrient limited productivity in macroalgae from a Caribbean barrier reef and from a mangrove ecosystem. *Mar. Biol.*, 1987, 93, 561–567.
- Chapman, A. R. O. and Craigie, J. S., Seasonal growth in *Laminaria longicruris:* Relations with dissolved inorganic nutrients and internal reserves of nitrogen. *Mar. Biol.*, 1977, 40, 197–205.
- Sano, T., Studies on colour change of cultured lavers II. On the change of chlorophyll, carotenoid and other hydrochromes. *Bull. Tohoku Region. Fish. Res. Lab.*, 1955, 5, 64–78.
- Flores Moya, A., Altamiriano, M., Cordero, M., Gonzalez, M. E. and Perez, M. G., Phosphorus limited growth in winter red alga *Porphyra leucosticta* Thuret in Le jolis. *Bot. Mar.*, 1997, **40**, 187–191.
- Umamaheshwara Rao, M. and Sreeramulu, T., Vertical zonation and seasonal variation in growth of *Porphyra* on Vishakhapatnam coast. *Curr. Sci.*, 1963, **32**, 173–174.
- Vuki, V. C. and Price, I. R., Sea changes in *Sargassum* populations on a fringing coral reef, Magnetic Island, Great Barrier Reef, Australia. *Aquat. Bot.*, 1994, 4, 153–166.

ACKNOWLEDGEMENTS. We thank the Director, National Institute of Oceanography, Goa for encouragement and the Ministry of Environment and Forests, New Delhi for financial support.

Received 17 August 2004; revised accepted 20 October 2004