NOTE

RECOVERY AND GENETIC DIVERSITY OF THE INTERTIDAL KELP LESSONIA NIGRESCENS (PHAEOPHYCEAE) 20 YEARS AFTER EL NIÑO 1982/83¹

Enrique A. Martínez,² Leyla Cárdenas

Center for Advanced Studies in Ecology and Biodiversity, Departmento de Ecología, Pontificia Universidad Católica de Chile, Casilla 114 D, Santiago, Chile

Raquel Pinto

Equipo de estudios de ecosistemas de niebla, Dalmacia 3251, Iquique, Chile

Massive mortality in kelp beds of the Pacific coasts of North and South America was caused by the rise in surface seawater temperature during the El Niño Southern Oscillation (ENSO) event of 1982/83, the strongest in the four and half previous centuries. In northern Chile a stretch of 600 km of coastline showed massive mortality of the intertidal kelp species Lessonia nigrescens Bory, of which only a few individuals managed to survive. Kelps and their associated biodiversity recovered but kelp beds recolonization in general was variable in time and space seemingly very slow along northern Chilean coasts. Here we show, effectively, that northward recolonization advanced less than 60 km in 20 years. Conversely, kelp beds of the Northern Hemisphere recovered 300 km in only six months after the same ENSO event. Genetic diversity in the two most affected populations of L. nigrescens shows half of the heterozygosity and polymorphism with respect to that observed in six non affected populations. In addition, geographically separated populations seem highly isolated as evidenced by high and significant fixation indices (all F_{ST} values over 0.4).

Strong natural bottlenecks occurred long ago in the past, well studied in terrestrial populations affected by the Pleistocene ice age during the glacial-interglacial periods (Stewart and Lister 2001). In these cases the ecological scenario under which the events occurred varied due to the dramatic climate changes of the ice ages (Hewitt 2000). Massive mortality due to ENSO events is a much more recent phenomenon affecting marine environments (Fig. 1, a and b) with one of the strongest events of the last 4 and half centuries occurring in 1982/83 (Quinn et al. 1987). In addition, the frequency of ENSO events of the last century has doubled in the last 50 years (Holmgren et al. 2001, Jaksic 2001) reducing the biodiversity of coastal habitats at large spatial scales (Camus et al. 1994). The novelty of these changes makes it difficult for the scientific community to make accurate predictions for the whole system but the recovery of some key ecosystem engineer or habitat-forming species (Jones et al. 1994, Coleman and Williams 2002) may help to understand the magnitude of the impact. Kelp beds of the north and south Pacific are engineer species in terms of biomass and habitat. In both hemispheres kelp beds were killed by the 1982/83 ENSO event (Gunnill 1985, Castilla and Camus 1992, Tegner and Dayton 1987). Recovery of kelp beds has been in general variable in time and space (Foster and VanBlaricom 2001) seemingly very slow along northern Chilean coasts (Camus 1994).

In Chile the 1982/83 El Niño event killed kelp beds along 600 hundreds kilometres of coastline (Castilla and Camus 1992). Kelp gatherers collect these seaweeds from cast-ashore individuals so that the landings' statistics of these species is an estimation of their abundance in nature. Landings recorded during the last 20 years showed a steep decrease in three northern localities of Chile after the ENSO event, and natural recovery has occurred, but mainly on the southernmost and less impacted areas (Figs. 1c and 2) where coastal fishermen moved to collect stranded algae. At sites like Punta Patache (21°S) only a few patches of individuals of the intertidal kelp species Lessonia nigrescens survived in 1982/83. This survival was probably due to the protection provided by local geographical conditions (coastal points) with stronger coastal upwelling (Fig. 1, a and b), resulting in water masses richer in nutrients and of colder temperatures closer to the coast (Martínez 1999). Such upwelling zones are stable at many points along the coast as described by Vásquez et al. (1998) and they were present during most of the 1997/98 ENSO event in northern Chile (Lagos et al. 2002) probably avoiding massive kelp mortality as it occurred in the ENSO event of 1982/83. The intertidal at only 60 km North of Punta Patache was densely covered by kelps in 1978 (Fig. 1d) but it has not yet been re-colonized, and 20 years later rocks are still covered mainly by crusts of calcareous red algae (Fig. 1e). The ENSO events of 1992, 1997/98 although not causing massive mortality in Lessonia nigrescens might have contributed to less kelp reproduction and lower recruitment. Landings decreased in 1999 probably due to mortality rise in the subtidal species

¹Received 10 December 2002. Accepted 7 April 2003.

²Author for correspondence: eamartin@bio.puc.cl, fax 56-2-6862621



FIG. 1. (a,b) NOAA satellite images of superficial seawater temperatures (coloured scale in $^{\circ}$ C) off the coast between 19 $^{\circ}$ S and 21 $^{\circ}$ S in September 1997(a), and September 1998(b). Arrows: Upwelling sites with colder waters. DE-arrow: site of pictures d and e. 1-arrow: sampling site 1. (c) Position of all sampling sites. (d,e) DE-site view in 1978 with *Lessonia nigrescens* (d) and in February 2002 without kelps (e).

Lessonia trabeculata. Thus, on average, the leading recruitment edge of *L. nigrescens* did not advance more than 3 km per year. This is a much slower rate than the one observed in kelp beds of the northern hemisphere where they recovered 300 km in only six months after the same ENSO event (Foster and Schiel 1985, Hernández-Carmona et al. 1989, Dayton et al. 1999). Subtidal kelps may have escaped mortality due to the presence of individuals in deeper colder habitats not reached by the warm surface waters of ENSO events.

The genetic consequences of recovery after massive mortality events have not been studied in macroalgae. This is particularly important in the kelp *Lessonia nigrescens* a species seemingly very sensitive to environmental changes as it was also affected by human-induced massive mortality in other areas of northern Chile, also followed by low recovery and unknown genetic impact (Correa et al. 2000). Population genetic diversity was evaluated by multilocus Random Amplified Polymorphic DNA (RAPD) molecular data from samples of *Lessonia nigrescens* along 3,000 km of the species distribution (Fig. 1c, Table 1). The methodology of sampling and genetic analysis included:

Sampling sites and collection of tissues. Individuals of Lessonia nigrescens (n=10-20) scattered along 500 m of the intertidal were sampled during 1999/2000 in eight localities (Table 1) distributed along 3000 km of coastline, covering 3/4th of the length of the species

distribution that reaches to Cape Horn in the south. Only the two northernmost localities were highly impacted by the 1982/83 ENSO event (Castilla and Camus 1992). The samples at site 1 (Punta Patache) recovered from a few surviving individuals and now the colonising northward leading edge is at a locality called Huaque, only 55 km north of that site. At this edge point there was only 1 individual m^{-2} (SD = 1.6, n = 45) while in populations of central Chile not af-



FIG. 2. Landings of two *Lessonia* species between 1980-2000 in sites 1, 2 and in Caldera (27°S) between sites 2-3. Arrow: 1982/83 ENSO event. Source: National Fisheries Service at www.sernapesca.cl

TABLE 1. Geographical position of each sampling locality (main cities in parentheses) and number of individuals used for molecular analysis.

Abbreviation	Locality	Latitude south	Individuals sampled
1	Punta Patache (Iquique)	20°50′	16
2	Antofagasta	23°40′	20
3	Coquimbo	28°50′	13
4	Las Cruces	33°30′	12
5	Constitución	35°20′	10
6	Dichato (Concepción)	36°30′	16
7	Niebla (Valdivia)	39°45′	20
8	Guabún (Chiloé Island)	$41^{\circ}50'$	14

fected by ENSO events or massive mortality there are normally between 4 and 30 adult individuals $\cdot m^{-2}$ (Santelices and Ojeda 1984). Vegetative tissues were excised from the base of fronds, where meristematic areas are normally clean of epi/endophytes. Tissues were brushed in distilled water, blotted on paper towel and dried in tubes with Silica gel in beads until DNA extraction.

DNA extractions, PCR with RAPD primers and electrophoretic conditions. DNA was extracted from 50 µL of finely ground tissues. This volume was mixed with equal volume of Poly Vinyl Pyrrolidona (PVP) and then extracted with 700 µL of CTAB buffer (3%) in Tris-HCL 1M, pH 8.0, Na Cl 1.4 M, EDTA 20mM. This method combines a standard CTAB extraction (Richards 1987) with the addition of PVP to extract polyphenols, very abundant in phaeophycean algae. Addition of PVP is a similar solution to that used by Hong et al. (1992) also to discard impurities. The mix was agitated every 20 min for one h at 60° C, then centrifuged at 12,000 rpm for 5 min. Supernatant was pipeted and mixed with 700 µL of Chlorophorm: Isoamilic Alcohol (24:1). The supernatant was precipitated in 0.6 volumes of 100% isopropanol at -20° C for 30 min. Then the solution was centrifuged for 4 min at 14,000 rpm and the DNA pellet washed in alcohol 70% prior to drying. The DNA pellet was diluted in MilliQ water and quantified in a spectrophotometer under 260/280 nm. The four chosen primers gave 30 informative bands of a size range between 250 and 1450 pb (Table 2). The PCR were run using 20 ng of DNA using standard RAPD protocols (Martínez et al. 1999) with higher $MgCl_2$ concentration (4 mM). The PCR cycle started with 5 min at 94° C followed by 35 cycles of 1 minute at 94° C, 1 min at 38° C and 1.5 min

at 72° C. A final extension period of 10 min at 72° C was added.

Data analysis. RAPD banding patterns were transformed to a matrix of 1 (band presence) and 2 (band absence) and the expected Polymorphism and Heterozygosity were estimated using the Taylor's expansion as suggested by Lynch and Milligan (1994) for dominant genetic data. This modality was incorporated in the TFPGA software (Miller 1997) used for this analysis and for the estimation of paired F_{ST} values. The Mantel test was used to correlate the matrix of geographical and genetic ($F_{ST}/(1-F_{ST})$) distances.

Results showed that genetic diversity was highly reduced in the two northern and most impacted populations recovering in northern Chile, with estimated heterozygosities being less than half of those found in the six other localities (Fig. 3). There was also a significant pattern of genetic isolation by distance (with all F_{ST} values higher than 0.4) where paired genetic distances ($F_{ST}/(1-F_{ST})$) significantly increased from spatial scales of less than 500 kilometres to longer paired distances (Fig. 4).

The low recovery is probably due to the intertidal habitat of the species and to a varied set of single and complementary inhibitory factors affecting kelp recruitment (Martínez and Santelices 1998). Namely short dispersion capacity, presence of red algal crusts, strong waves, desiccation and herbivores. Thus, it is very likely that within a time scale of a few decades genetic diversity in northern populations will not completely recover before new strong ENSO events impact those low latitudes. New events will probably come sooner than large-scale climate changes (Jaksic 1998) further decreasing the kelp genetic variability or completely inhibiting its recovery at its actual northern distribution limit. Comparisons with other kelps are difficult to establish as genetic structure has been evaluated in the North Atlantic kelp Laminaria digitata (Valero et al. 2001) but in populations not submitted to any known recent event of massive mortality. However, genetic variability in the intertidal kelp Postelsia palmaeformis has revealed that a strong genetic structure occurs at spatial scales as short as 25 m, with genetic distances not being stronger than those observed at 250 km (Coyer et al. 1997). Consequently, genetic data seem to indicate very short dispersion in intertidal kelps. Similarly, in the winged kelp Alaria marginata genetic heterogeneity was also strong at short local scales (patches and within-stand individuals), as detected by dominant AFLP data (Kusumo and

TABLE 2. RAPD primers used, sequence and number and size of informative bands (30 loci in total).

RAPD primer (number of informative bands)	Sequence $5' \rightarrow 3'$	Band sizes (bp)
C2 (8) C8 (6) C11 (8) L5 (8)	GTGAGGCGTC TGGACCGGTG AAAGCTGCGG ACGCAGGCAC	$\begin{array}{c} 1165,1026,885,813,672,604,458,379\\ 1109,829,620,431,372,265\\ 1451,1281,1062,978,607,485,396,316\\ 1027,868,765,674,594,501,442,350 \end{array}$



FIG. 3. Heterozygosity and Polymorphism at each locality.

Druehl 2000). Long dispersion seem to be more the case in subtidal kelps such as *Macrocystis pyrifera*, where spores have been detected at great distances far from potential parental plants (Reed et al. 1988). In addition, drifting thalli of *Macrocystis pyrifera* have pneumocysts that allow better buoyancy and dispersion (Dayton 1985) while those of *Lessonia* do not have such structures and they sink. Furthermore recruitment might result from dormant microscopic stages in *M. pyrifera* (Ladah et al. 1999). Such set of strategies differ from intertidal species and it might favour the rapid re-colonisation of *Macrocystis* post ENSO events described in the northern Pacific in California and Baja California.

ENSO events may become biological disasters affecting biodiversity at lower latitude coasts in both hemispheres. Their study hold lessons of great value not only in giving clues about the magnitude of such mortality events but also on the variability of ecosystem recovery. Human-induced disasters of similar magnitude are occurring on *Lessonia nigrescens* populations affected by mining activities in northern Chile (Correa et al. 2000). Recovery rates and genetic impact can now be compared with this case of ENSOinduced massive mortality. However, the ENSO effects are still in progress, as are the lessons.

Comments by Fabián Jaksic and two anonymous reviewers are deeply appreciated as the improvement to the English by Rose Mary Cotter and Cecilia Contreras. Help of Pedro Paolini with preparation of satellite images is also appreciated. Field sampling in different localities along the country was greatly facilitated by Sandra Silva, María Inés Hormazábal, Jack Daniels, Mauricio Cerda, Krisler Alveal and Claudio Bombero. This work was supported by Grants DIPUC, Fondecyt 1990235, IAI-SGP and Fondecyt-FONDAP 1501-0001.

- Camus, P. A. 1994. Recruitment of the intertidal kelp *Lessonia nigrescens* Bory in northern Chile: successional constraints and opportunities. *J. Exp. Mar. Biol. Ecol.* 184:171–81.
- Camus, P. A., Vásquez, E. O., González, E. O. & Galaz, L. E. 1994. Fenología espacial de la diversidad intermareal en el norte de Chile: patrones comunitarios de variación geográfica e impacto de los procesos de extinción-recolonización post El Niño 82/83. Medio Ambiente 12:57–68.



FIG. 4. Paired genetic and geographical distances among localities (Slope P = 0.02).

- Castilla, J. C. & Camus, P. A. 1992. The Humboldt-El Niño scenario: Coastal benthic resources and anthropogenic influences, with particular reference to the 1982/83 ENSO. *In*: A.I.L. Payne, K.H. Brink, K.H. Mann and R. Hilborn (eds.). Benguela trophic functioning, *S. Afr. J. Mar. Sci.* 12:703–12.
- Coleman, F. C. & Williams, S. L. 2002. Overexploiting marine ecosystem engineers: potential consequences for biodiversity. *Trends in Ecology and Evolution* 17:40–4.
- Correa, J.A, Ramírez, M. A., De la Harpe J-P., Roman, D. & Rivera, L. 2000. Copper mining effluents and grazing as potential determinants of algal abundance and diversity in northern Chile. *Environmental Monitoring and Assessment* 61:265–81
- Coyer, J. A., Olsen, J. L. & Stam, W. T. 1997. Genetic variability and spatial separation in the seapalm kelp *Postelsia palmaeformis* (Phaeophyceae) as assessed with M13 fingerprints and RAPDs. *J. Phycol.* 33:561–68.
- Dayton, P. K. 1985. Ecology of kelp communities. Ann. Rev. Ecol. Syst. 16:215–45.
- Dayton, P. K., Tegner, M. J., Edwards, P. B. & Riser K. L. 1999. Temporal and spatial scales of kelp demography: the role of oceanographic climate. *Ecological Monographs* 69:219–50.
- Foster, M. & Schiel, D. R. 1985. The Ecology of Giant Kelp Forests in California: A Community Profile. U.S. Fish and Wildlife Service, *Biological Report* 85 (7.2). 152 pp.
- Foster, M. S. & VanBlaricom, G. R. 2001. Spatial variation in kelp forest communities along the Big Sur Coast of central California, USA. *Cryptogamie, Algologie* 22:173–86.
- Gunnill, F. C. 1985. Population fluctuations of seven macroalgae in Southern California during 1981–1983 including effects of severe storms and El Niño. J. Exp. Mar. Biol. Ecol. 85:149–64.
- Hernández-Carmona, G., Rodríguez-Montesinos, Y. E., Torres-Villegas, J. R., Sánchez-Rodríguez, I & Vilchis, M. A. 1989. Evaluación de los mantos de *Macrocystis pyrifera* (Phaeophyta, Laminariales) en Baja California, Mexico, I. Invierno 1985–1986. *Ciencias Marinas* 15:1–27.
- Hewitt, G. 2000. The genetic legacy of the quaternary ice ages. *Nature* 405:907–13.
- Holmgren, M., Scheffer, M., Ezcurra, E., Gutiérrez, J. R. & Mohren, G. M. J. 2001. El Niño effects on the dynamics of terrestrial ecosystems. *Trends in Ecology and Evolution* 16:89–94.
- Hong, Y-K., Coury, D. A., Polne-Fuler, M., & Gibor, A. 1992. Lithium Chloride extraction of DNA from the seaweed *Porphyra perforata* (Rhodophyta). *J. Phycol.* 28:717–20.
- Jaksic, F. M. 1998. The multiple facets of El Niño/Southern Oscillation in Chile. Revista Chilena de Historia Natural 71:121–31.
- Jaksic, F. 2001. Ecological effects of El Niño in terrestrial ecosystems of western South America. *Ecography* 24:241–50.
- Jones, C. G., Lawton, J. H. & Shachak, M. 1994. Organisms as ecosystem engineers. *Oikos* 69:373–86.
- Kusumo, H. T. & Druehl, L. D. 2000. Variability over space and time in the genetic structure of the winged kelp *Alaria marginata. Mar. Biol.* 136:397–409.

- Ladah, L., Zertuche-González, J. A., Hernández-Carmona, G. (1999) Giant kelp (*Macrocystis pyrifera*) recruitment near its southern limit in baja California after mass disappearance during ENSO 1997–1998. J. Phycol. 35:1106–12.
- Lagos, N. A., Barría, I. D. & Paolini, P. 2002. Upwelling ecosystem of northern Chile: Integrating benthic ecology and coastal oceanography through remote sensing. In: The oceanography and ecology of the nearshore and bays in Chile. Proceedings of the international symposium on linkages and dynamics of coastal systems: open coasts and embayments, Santiago, Chile 2000. J.C. Castilla & J.L. Largier (eds.). Ediciones Universidad Católica de Chile, Santiago, Chile, pp. 117–41.
- Lynch, M. & Milligan B. G. 1994. Analysis of population genetic structure with RAPD markers. *Molecular Ecology* 3:91–9.
- Martínez, E. A. 1999. Latitudinal differences in thermal tolerance among microscopic sporophytes of the kelp *Lessonia nigrescens* (Phaeophyta: Laminariales). *Pacific Science* 53:74–81.
- Martínez, E. Á. & B. Santelices. 1998. Selective mortality on haploid and diploid microscopic stages of *Lessonia nigrescens* Bory (Phaeophyta, Laminariales). J. Exp. Mar. Biol. Ecol. 229: 219–39.
- Martínez, E. A., Destombe, C., Quillet M. C. & Valero, M. 1999. Identification of random amplified polymorphic DNA (RAPD) markers highly linked to sex determination in the red alga *Gracilaria gracilis. Molecular Ecology* 8:1533–8.
- Miller, M. P. 1997. Tools for population genetic analysis (TFPGA) 1.3. A Windows program for the analysis of allozyme and mo-

lecular population genetic data. Computer software distributed by author.

- Quinn, W. H., Neal, V. T., & Antunez de Mayolo, S. E. 1987. El Niño occurrences over the past four and half centuries. J. Geoph. Res. 92:14449–61.
- Reed, D. C., Laur, D. R. & Ebeling, A. W. 1988. Variation in algal dispersal and recruitment: the importance of episodic events. *Ecol. Monogr.* 58:321–35.
- Richards, E. 1987. Preparation of genomic DNA from plant tissue. In: Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., & Struhl, K. (eds.) Current Protocols in Molecular Biology. Greene Publishing Associates and Wiley-Interscience, John Wiley and Sons, New York pp. 2.3.1–3
- Santelices, B. & Ojeda, F. P. 1984. Recruitment, growth and survival of *Lessonia nigrescens* (Phaeophyta) at various tidal levels in exposed habitats of Central Chile. *Mar. Ecol. Progr. Ser.* 19:73–82.
- Stewart, J. R. & Lister, A. M. 2001. Cryptic northern refugia and the origins of modern biota. *Trends in Ecology and Evolution* 16: 608–13.
- Tegner, M. J. & Dayton, P. K. 1987. El Niño effects on southern California kelp forest communities. Adv. Ecol. Res. 17:243–77.
- Valero, M., Engel, C., Billot, C., Kloareg, B. & Destombe, C. 2001. Concepts and issues of population genetics in seaweeds. *Cahiers de Biologie Marine* 42:53–62.
- Vásquez, J. A., Camus, P. A. & Ojeda, F. P. 1998. Diversidad, estructura y funcionamiento de ecosistemas costeros rocosos del norte de Chile. *Rev. Chil. Hist. Nat.* 71:479–99.