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Experimental transplants of the large kelp *Lessonia nigrescens* (Phaeophyceae) in high-energy wave exposed rocky intertidal habitats of northern Chile: Experimental, restoration and management applications

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Abstract

Potential for addressing ecological and physiological issues becomes severely limited when the organisms required to experimentally test specific hypotheses are absent from the study areas. This report describes a simple and inexpensive device for re-planting kelps into the lower intertidal zone of wave-swept rocky habitats, using *Lessonia nigrescens* Bory as a model organism. The device allows a wide range of plant sizes to be anchored by the holdfast. Transplanted kelps regenerated, re-attached to the substratum and overgrew the transplanting device. These results confirm the feasibility of using this technique to tackle a variety of highly relevant questions involving ecological, physiological, conservation, restoration and management issues.

Keywords: Experimental ecology; Intertidal; Kelps; *Lessonia nigrescens*; Rocky shore; Restoration; Transplant

1. Introduction

Experimental manipulation of organisms in the field has been the key to unravel the processes determining the structure and dynamics of rocky intertidal benthic communities (i.e. Underwood, 1981; Paine, 1994; Castilla, 2000). An important contribution of these experimental studies is the demonstration that certain species are crucial in shaping the structure of intertidal communities (Jones et al., 1994). There are instances however, where natural phenomena or human-related activities result in losses of entire assemblages of benthic organisms, including those species required for experimental purposes.

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Lessonia nigrescens is one of the most ecologically and economically important species dominating the lower wave-swept intertidal rocky habitats of the eastern southern Pacific (Santelices et al., 1981). Its ecological importance as an ecosystem engineer (sensu Jones et al., 1994) along this zone has been demonstrated in studies where its presence affords refuge from desiccation stress and wave impact (Vásquez and Santelices, 1984), food for grazers (Rodríguez, 2003) and habitat for several invertebrate taxa (Cancino and Santelices, 1981). However, *L. nigrescens* appears to have a limited tolerance to abiotic changes. For example, this kelp has been decimated from large expanses of coastline in northern Chile due to inter-annual variability induced by the arrival of subtropical warm waters during the El Niño-Southern Oscillation (ENSO) (Camus, 1990; Martínez et al., 2003). Similarly, the chronic disposal of copper-mine wastes to coastal environments, also in northern Chile, has resulted in the loss of most of the biota, including *L. nigrescens* (Castilla, 1983; Medina et al., 2005).

The absence of *L. nigrescens* from these extensive coastal areas highlights the urgent need to assess its capacity for re-colonization and, eventually, design strategies for assisted restoration. In this study, we report the design and testing of a transplanting device that allows experimental manipulation of the kelp in its natural habitat. We expect the device to facilitate meaningful testing of hypotheses in the areas of ecology, physiology, conservation, restoration, and ecosystem management in wave-swept intertidal rocky habitats.

2. Material and methods

The transplanting device was designed to hold plants of *L. nigrescens* in place at the lower wave-swept rocky intertidal zones, after detached from the natural substratum. The device consisted of 20×20 cm pieces of Vexar net, 1-cm mesh, reinforced along the borders with plastic clips secured with 3 mm plastic cable ties (Fig. 1). Two perpendicular cuts (5 cm) intersecting at the center of the net were made to allow fronds and stipes of different sizes to slip through, retaining the holdfast (Fig. 1A, B). Plastic cable ties of different lengths and passed over the holdfast and stipes, were used to secure the kelp to the net. Transplant units were then anchored to the substratum by four 5.5-cm stainless steel bolts screwed to the rocky platforms after perforating the rocks with a hammer drill.

In July 2003, 50 immature plants of *L. nigrescens*, 3 to 10 cm in holdfast diameter and 128 cm of maximum frond length were collected in two different sites where natural populations of the kelp flourish. The sites, 107 km apart, were Guanillo (G) (25° 53.774′ S; 70° 41.552′ W) and Zenteno (Z) (26° 51.082′ S; 70° 48.550′ W), both located in open coastal areas continuously affected by strong wave action.

Removal of plants from the platforms was done by detaching the holdfast with iron chisels. After removal, frond length and holdfast diameter were recorded. All plants, uniquely identified by numbered plastic tags fastened to the Vexar net, were then anchored to rocks within the same site of collection using the device described above (Fig. 2A). In each site five additional undisturbed plants of similar characteristics to those detached for transplants were selected and tagged as controls.

Survivorship, frond length and holdfast diameter were recorded every two or three months during a year. Special attention was paid to the possible natural re-attachment of holdfasts and onset of reproduction.

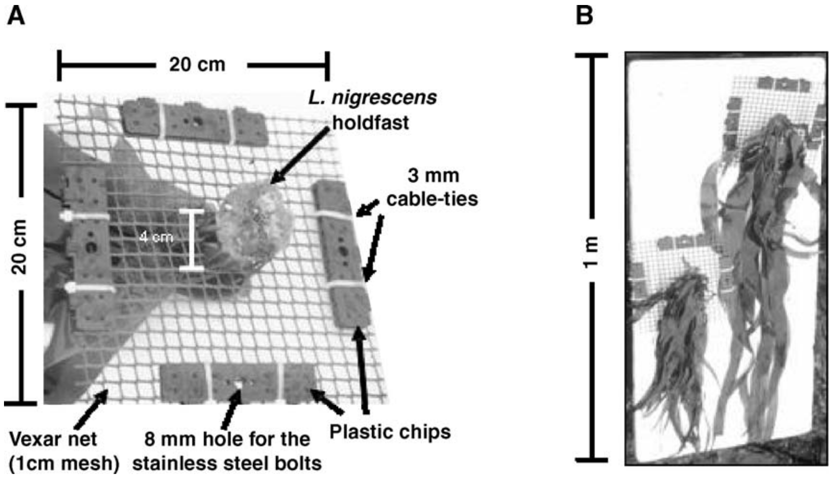

Fig. 1. (A) Structure, dimensions and materials used in the transplanting device and (B) example of the kelp size range being held by the same device.

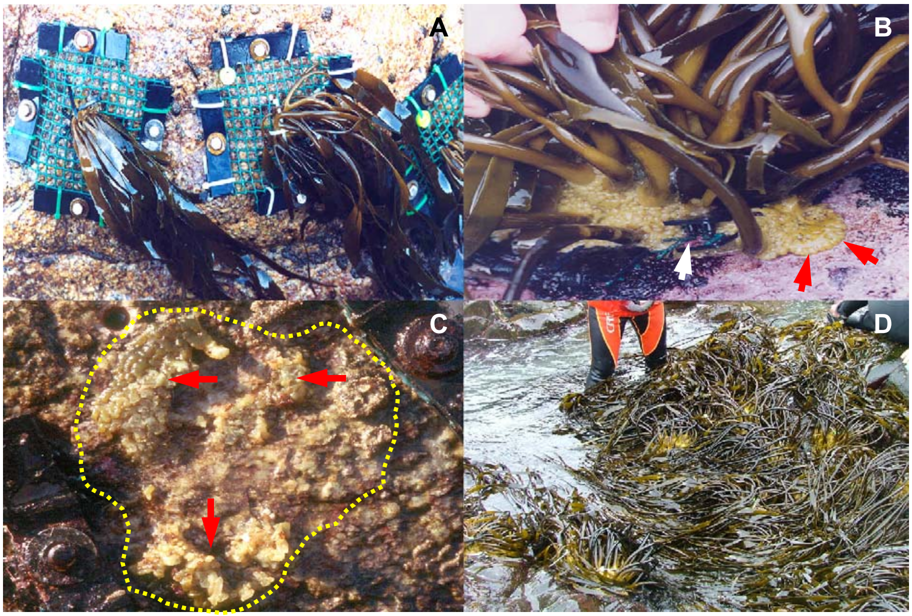

Fig. 2. Transplanted *Lessonia nigrescens*. (A) General view of new transplant units. (B) In a more advanced stage, a holdfast appears overgrowing the net and the plastic frame (white arrow), colonizing the rocky substratum outside the transplanting device (red arrows). (C) Holdfasts re-attach to the rocks, as indicated by the remains of tissue (arrows) still adhered to the rock after holdfast removal. Dotted line indicates the area occupied by the re-attached individual. Finally, healthy stands of *L. nigrescens* (D) were established after 7 months of re-planting.

Survivorship was estimated as the number of surviving plants over the total number of plants at the beginning of the experiment. Frond and holdfast size were compared with their respective values at the beginning of the experiment to calculate the mean net growth of surviving plants. Data did not violate the assumptions of normality and homogeneity of variances and therefore raw data were used for calculations of differences in net growth between the control and the transplanted group (Zar, 1999).

3. Results

Transplanted *L. nigrescens* rapidly developed holdfasts and within 2 months the net of the transplanting device appeared overgrown by holdfast tissue. Later, the progressive development of the holdfast resulted in a protrusion of the tissue and a generalized overgrowth of the net. In many cases, new holdfast tissue infiltrated and overgrew the plastic frame and colonized the rocky surface outside the transplanting device (Fig. 2B). At this stage, plants were firmly re-attached to the substratum by massive development of the holdfast, as demonstrated by tissue still adhered to the rock after mechanical removal of some individuals (Fig. 2C). By months 7–9, healthy stands of fully developed *L. nigrescens* were established (Fig. 2D). In spite of a constant strong wave action, particularly during winter storms, and the beginning of an illegal harvesting rampage of natural kelp stands (Correa pers. obs.), more than 70% of the plants survived after three months of experimentation. Six months after re-planting, more than 60% of transplanted plants survived, even though plants continued to be lost due to natural causes and sporadic and random human harvesting. It is important to mention that no control plants were lost during the first six months of the experiment. By April 2004 survivorship of transplanted and control plants was 43% and 60% respectively. Losses in the control group occurred mainly during the maximum activity of the illegal harvesting rampage. The experiment was discontinued in July 2004, when all control plants were lost. However, 28% of transplanted individuals were still healthy and firmly in place. During the trial, 18% and 40% of transplanted and control individuals respectively became reproductive, displaying well developed sori.

After nine months of re-planting, fronds of control and transplanted individuals increased an average of 134.33 cm (±SD 43.5 cm) and 63.39 cm (±SD 69.7 cm) in length respectively (Fig. 3A). This apparently large difference between control and transplants was, however, only marginally significant ($t_{43}=2.409$, $P=0.02$). Changes in holdfast diameter showed a trend similar to that observed in frond length. After a period of nine months, control individuals displayed a significantly higher ($t_{43}=2.346$, $P=0.024$) net growth in diameter

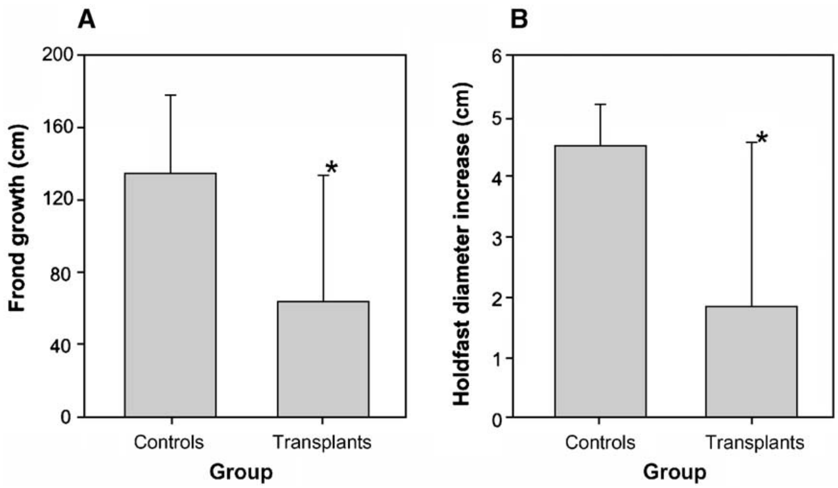

Fig. 3. (A) Net frond growth after nine months of re-planting. (B) Net holdfast diameter increase after nine months of re-planting. Mean and ±SD represent the values of surviving plants only. Asterisk indicates significant differences ($P<0.05$).

(4.48 cm, ±SD 0.7 cm) than transplanted individuals (1.84 cm, ±SD 2.7 cm) (Fig. 3B).

4. Discussion

This report describes a simple and inexpensive device for re-planting kelp into the lower intertidal zone of wave-swept rocky habitats, using *L. nigrescens* as the model organism. There has been at least one previous attempt at re-planting juvenile *L. nigrescens* using non-toxic epoxy cement to glue plants to the substratum (Vásquez and Tala, 1995). However, results were discouraging as 100% of the plants were lost in only two weeks. Numerous other studies in the northern hemisphere have used transplanted Laminariales, including large-sized *Macrocystis*, *Fucus*, *Laminaria* and *Sachorriza* to i) assess the relative importance of genetic and habitat influences on morphological features of the plants (Norton, 1969; Chapman, 1974; Druehl and Kemp, 1982; Brostoff, 1988) or ii) restore areas where kelps have disappeared by biotic or abiotic causes (McPeak, 1977; Rice et al., 1989; Hernández-Carmona et al., 2000). The technique used in the above cases, however, cannot be applied to *L. nigrescens*, as it attaches directly on the rock or on coralline crusts and not on other living organisms. Furthermore, *L. nigrescens* lives on steep platforms of fairly smooth rocks, where organisms similar to those used as anchoring subjects for transplanting kelps in the northern hemisphere (Brostoff, 1988; Hernández-Carmona et al., 2000) are absent. Other studies have reported mechanical removal of rockweed-covered rocks or rock fragments with the plant attached to them (Ho, 1984; Buschmann et al., 1997; Blanchette et al., 2002; Marsden et al., 2003). These fragments were removed from one site and transported to the place of interest, where they were glued to the substratum using quick-drying cement or submarine epoxy resins. Again, although useful for certain purposes and species of small size, this approach cannot be applied to large kelp inhabiting open shores exposed to a heavy wave impact. For example, the position of *L. nigrescens* at the low intertidal, where waves continuously beat the rocks even during good low tides, leaves no time for polymerization of epoxy resins. Furthermore, incoming waves exert such a strong drag on plants and on the cementing resin, that only small organisms can be successfully anchored during low tide (i.e. Buschmann et al., 1997). Another advantage of our system is that it allows anchoring a large number of transplant units during a single low-tide period, with a maximum of up to 35 units per low tide done by a team of four people.

The use of the transplanting device to re-attach *L. nigrescens* seems to affect the normal physiological processes of the kelp, as indicated by the slightly smaller size of the fronds and holdfasts as compared with control plants. It is possible that a recovery time is required by the plants to adjust to their new condition, including the time and energy invested in re-attaching to the substratum. If this is the case, fronds and holdfasts in the transplanted individuals will suffer some delay in reaching the normal size, a factor that should be taken into account in future experimental studies. An important observation is the high variability observed in the final size of transplanted kelps, which may be due to the combined effect of at least two factors. One is the mechanical damage (i.e. cuttings) suffered by fronds and stipes trapped between the plastic frames and the rocks when the corners of those frames are not fully fastened against the substratum. The other is the potential intrinsic individual differences in growth, a feature that also should be taken into account in future studies. Careful manipulation of the frame during plant anchoring has resulted in significant improvements, with survival rates after nine months of 80% or higher (Correa, pers. obs.).

Regardless of their slightly diminished growth performance, transplanted individuals reached maturity. This result justifies use of the transplanting device as a tool for establishing new recruits in areas where *L. nigrescens* is absent as a result of human or natural causes. As a restoration technique it could help the recovery of stands devastated by natural disruptive events such as the ENSO phenomenon, or by overharvesting resulting from the needs of raw material for alginate extraction (Santelices, 1996) and food for abalone farms (Castilla et al., 2005). Furthermore, in coastal areas where *L. nigrescens* has been absent since the disposal of copper mine wastes began (see Medina et al., 2005), we can now i) assess whether its absence is due to physiological or ecological constraints, ii) characterize the kinetics of copper accumulation in the plants and to unravel the in situ physiological responses triggered by the excess of metal, and iii) assess the feasibility of artificial restoration of the most impacted areas.

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