INTERTIDAL AMPHIPODS AS POTENTIAL DISPERSAL AGENTS OF CARPOSPORES OF *IRIDAEA LAMINARIOIDES* (GIGARTINALES, RHODOPHYTA)[1]

Alejandro H. Buschmann[2] *and Andrés Bravo*

Departamento de Acuicultura y Recursos Acuáticos, Instituto Profesional de Osorno
Casilla 933, Osorno, Chile

ABSTRACT

Mid and late successional benthic algae have poor dispersal capacities. Mobile herbivores may increase dispersal of some algae because spores can survive digestion by grazers and stick to external body appendages. We show that carpospores are the only type of reproductive unit of the rhodophyte Iridaea laminarioides *Bory that survive passage through the digestive tract of the amphipod* Hyale *sp. The spores also stick to the amphipods' legs and are thus carried by the amphipods in the field. Amphipods significantly increased ($P < 0.01$) the number of spores settling on artificial substrata in places where barriers prevented the normal ingress of algal propagules. The presence of artificial substrata that act as a refuge for the grazers also caused an increase ($P < 0.01$) in the number of settled spores. Amphipods also significantly increased ($P < 0.01$) the number of spores under an* Ulva *sp. canopy in laboratory experiments.*

Key index words: amphipod grazing; Hyale hirtipalma; Iridaea laminarioides; *Rhodophyta; spore dispersal; spore resistance to digestion*

Seaweeds have different dispersal capacities (Hruby and Norton 1979, Amsler and Searles 1980, Hoffmann and Ugarte 1985, Zechman and Mathieson 1985). Mid and late successional benthic algae appear to have poor dispersal capacities (Anderson and North 1966, Guzmán del Proo and de la Campa de Guzmán 1969, Dayton 1973, Paine 1979, Deysher and Norton 1981, de Ruyter van Steveninck and Breeman 1987, Hoffmann 1987). *Iridaea laminarioides* Bory is a dominant mid-intertidal alga of the wave exposed coasts of Chile (Santelices 1989). Its spores also have low dispersal characteristics (Hoffmann and Camus 1989).

Mobile amphipods that preferentially consume the cystocarpic algal tissue of *I. laminarioides* may have an ecological role as spore releasers and dispersal agents (Buschmann and Santelices 1987). Amphipods can release spores by disrupting the cystocarp of *I. laminarioides* and may also transport the propagules as they move among algal patches (Buschmann 1990). Two mechanisms exist by which the propagules could be transported: 1) by surviving digestion or 2) by becoming attached to the appendages of the amphipods.

We tested the hypothesis that amphipods present on the exposed rocky intertidal region of southern Chile are potentially important dispersal agents of the red alga *Iridaea laminarioides* by the two mechanisms described above. This study also experimentally assessed the mechanisms by which amphipods can mediate dispersal enhancement of *I. laminarioides*.

MATERIALS AND METHODS

Iridaea laminarioides plants and the amphipod *Hyale hirtipalma* (Dana) were collected in rocky intertidal habitats at Pucatrihue Beach in southern Chile (40°33′ S, 73°43′ W) and transported to the laboratory in temperature-controlled containers. Algae were sorted for reproductive structures as follows: mature cystocarpic fronds with large, brown-red cystocarps; mature tetrasporangial fronds with mature, red-purple tetrasporangial sori; and sterile thalli, lacking all macroscopic reproductive structures. Amphipods were selected by size. Algae and amphipods were washed in sterile seawater and maintained under running sea water and constant temperature (12–14° C), photon fluence rate (35–45 $\mu E \cdot m^{-2} \cdot s^{-1}$), photoperiod (12:12 h LD) and salinity conditions. Prior to each experiment, amphipods were starved for 72 h.

The capacity of spores to survive passage through the digestive tract of the amphipods was evaluated by offering reproductive carposporic, tetrasporic and sterile (control) *I. laminarioides* tissues to starved amphipods maintained in separate groups. After 24 h, 35 fecal pellets were collected from each algal type offered to the amphipods and incubated in sterile seawater in separate Petri dishes under the conditions described above. The number of spores per fecal pellet was counted under a Nikon microscope. Spores were quantified after 1 and 7 days of incubation. The total number of spores found in the fecal pellets was compared between treatments using a chi-square test including the Yate's correction (Sokal and Rohlf 1979).

Fecal pellets from amphipods were also collected in the field and washed in sterile seawater under the controlled conditions described above. After 5 days of incubation, the number of spores in the fecal pellets was counted under a microscope.

The capacity for carrying carpospores stuck to amphipod legs was measured in two ways. First, the number of spores on 15 locomotory appendages of amphipods found over mature cystocarpic fronds of *Iridaea laminarioides* in the laboratory was counted under a Nikon microscope and compared against controls, i.e. amphipods maintained on sterile algal fronds. Secondly, amphipod legs obtained directly from field collected individuals were observed under a stereomicroscope. Twenty legs obtained from amphipods collected on *I. laminarioides* and 20 legs from individuals found on *Ulva* sp. were compared. In all observations just described, only one leg was obtained randomly from each amphipod. The total number of spores on the legs for the different treatments was compared using a chi-square test including the Yate's correction (Sokal and Rohlf 1979).

The hypothesis that *Hyale hirtipalma* might facilitate spore release and dispersal while grazing and moving between algal patches was tested by placing 30 amphipods each inside replicate plastic

[1] Received 6 November 1989. Accepted 10 April 1990.

[2] Address for reprint requests.

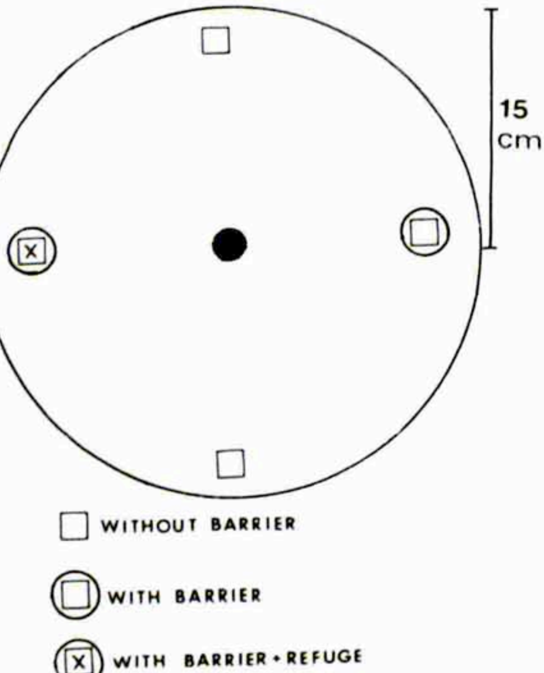

FIG. 1. Arrangement of treatments within the experimental aquaria. The black dot represents the cystocarpic *Iridaea laminarioides* frond.

containers (30 cm diam). A mature cystocarpic frond of *Iridaea laminarioides* was placed in the middle of the container, and 20 × 20 mm cover slips were placed at the bottom of each container equidistant from the center. The cover slips were arranged in three different treatments: 1) with an artificial barrier to prevent the arrival of spores without amphipods; 2) with an artificial barrier plus plastic filaments acting as refuges for amphipods and 3) controls without barriers and refuges. The artificial barriers were created by placing the cover slips inside disposable plastic cups. The spontaneous spore release to the medium without amphipods (controls) and the effectiveness of the artificial barriers in preventing the arrival of carpospores was tested in replicated containers. Figure 1 shows the arrangement of the treatments within the containers with and without amphipods. These experiments were carried out in sterile seawater. After 5 days the cover slips were removed and fixed in a Karo Syrup-formalin solution (3%) to count the number of spores settled on each slip.

A second experiment was initiated by replacing the artificial barriers and refuges with the green alga *Ulva* sp. Cover slips were fixed under the *Ulva* sp. fronds. This experiment tested the effects of the presence of an *Ulva* canopy on the arrival of spores beneath the plant. Cover slips without *Ulva* served as controls. The effectiveness of the *Ulva* fronds in preventing the access of spores in the absence of the amphipods was also assessed. Results of both experiments described were statistically analyzed using a *t*-test (Sokal and Rohlf 1979).

RESULTS

Carpospores were the only reproductive unit of *Iridaea laminarioides* able to survive passage through the digestive tract of *Hyale* (Table 1). However, their number decreased significantly ($\chi^2 = 7.62$; $P < 0.01$) after 7 days of incubation, presumably due to mortality (Table 1). *I. laminarioides* propagules also germinated in fecal pellets collected in the field; 17 germinating spores were found in 30 fecal pellets from amphipods collected on *Iridaea* patches.

TABLE 1. *Total number of spores of* Iridaea laminarioides *in fecal pellets of* Hyale *sp. (A) after 24 h of incubation; (B) after 7 days of incubation.* ** P << 0.01 ($\chi^2 = 148.3$; 2 df). * P << 0.01 ($\chi^2 = 114.5$; 2 df).

Karyological phase	Number of fecal pellets	Number of spores	Spores per fecal pellet
(A) Cystocarpic	35	63	1.8
Tetrasporic	35	7	0.2**
Sterile	35	0	0.0
(B) Cystocarpic	35	42	1.2
Tetrasporic	35	1	0.0*
Sterile	35	0	0.0

After the amphipods crawled over fertile carposporic fronds of *Iridaea*, 25 spores were found stuck to the 15 amphipod appendages. No spores were found stuck to the appendages when the amphipod crawled over infertile *Iridaea* fronds ($\chi^2 = 46.08$; $P \ll 0.001$). Also, 28 spores were found stuck to 20 appendages from amphipods caught on *Iridaea* fronds in the field. This number of spores is significantly higher ($\chi^2 = 22.86$; $P \ll 0.01$) than the seven spores found stuck to 20 amphipod legs collected on *Ulva*, suggesting that the spores could be lost as the amphipod travels between different algal patches.

When *Hyale* grazes on mature cystocarpic tissues of *Iridaea laminarioides* it scratches the cystocarp with its maxillipeds and consumes the pericarp tissue. This feeding activity can eventually perforate the pericarp, releasing a large number of carpospores into the water column. This grazing activity significantly increased ($t = 5.53$; $P < 0.001$) the number of spores released and fixed to the cover slips (Fig. 2). As previously demonstrated (Buschmann and Santelices 1987), the liberated spores are as viable and mature as those spontaneously released by the plant.

The presence of the artificial barriers placed inside the containers significantly reduced ($t = 2.72$; $P < 0.05$) the number of attached spores per cover slip in the absence of grazing (Fig. 2). However, the presence of amphipods significantly increased the number of spores ($t = 4.08$; $P < 0.01$) to as much as 50 spores per cover slip (Fig. 2). The presence of filaments acting as amphipod refuges and attaching surfaces further increased ($t = 6.17$; $P < 0.01$) the number of spores attached to the cover slips present below (Fig. 2), to an average density of 146 spores per slip. Finally, the experiments using *Ulva* as barriers demonstrated that amphipods significantly increased ($t = 4.61$; $P < 0.001$) the number of spores in the presence of *Ulva* since this alga attracts the amphipods (Fig. 3). Also, the amphipods significantly increased ($t = 3.79$; $P < 0.01$) the number of spores attached under *Ulva* (Fig. 3).

DISCUSSION

Previous results showed that the amphipod *Hyale media* (Dana) has a marked food preference for ma-

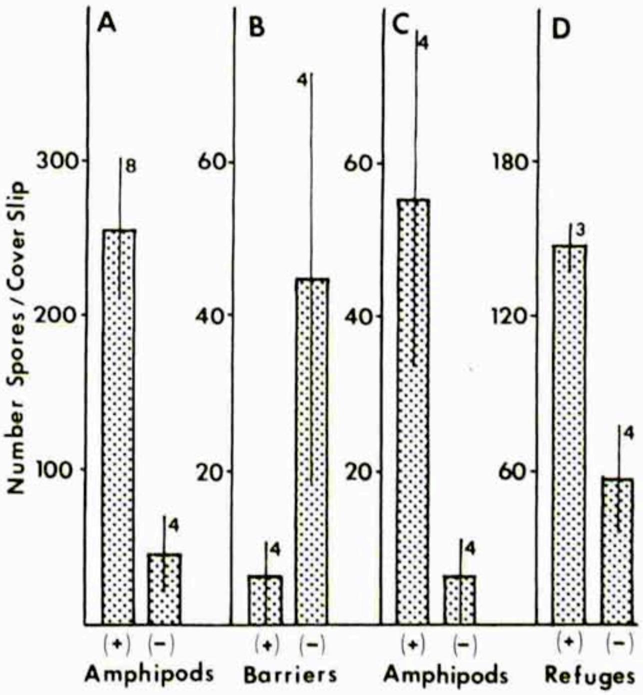

FIG. 2. Mean number of carpospores per cover slip of *I. laminarioides* released A) in the presence (+) and absence (−) of amphipods, B) in the absence of amphipods with (+) and without (−) barriers, C) on slides protected by barriers in the presence (+) and absence (−) of amphipods and D) in places with barriers and the presence (+) or absence (−) of refuges. Bars represent ±1 SD; number with each bar is the number of replicates.

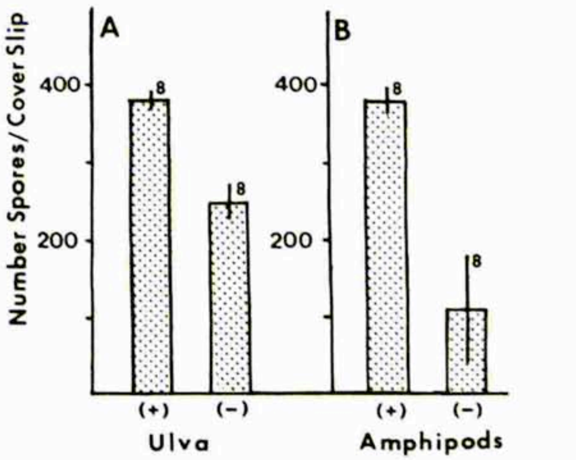

FIG. 3. Number of carpospores per cover slip of *I. laminarioides* released (A) with amphipods in the presence (+) and absence (−) of *Ulva* sp. and (B) with *Ulva* sp. in the present (+) and absence (−) of amphipods. Bars represent ±1 SD; number with each bar is the number of replicates.

ture cystocarpic *Iridaea laminarioides* (Buschmann and Santelices 1987, Luxoro and Santelices 1989). While grazing, however, the amphipods cause the release of large numbers of spores (Fig. 2) without affecting their development. Our results confirm those of Buschmann and Santelices (1987) that carpospores are the only type of reproductive unit of *Iridaea laminarioides* which survives passage through the digestive tract of amphipods. Breeman and Hoeksema (1987) also showed that algal tissues can survive passage through the gut of amphipods; however, the *Hyale-Iridaea* interaction shows a higher degree of specificity than previous reports for other algal-grazer interactions (Santelices et al. 1983, Santelices and Correa 1985, Jernakoff 1985, Santelices and Ugarte 1987, Paya and Santelices 1988, Santelices and Martinez 1988) because the consumption by the amphipods was highly specific and restricted to cystocarps of *I. laminarioides* rather than gametophytic tissues (Buschmann and Santelices 1987, Luxoro and Santelices 1989). The evidence presented in this paper suggests that this consumption might not be detrimental to *I. laminarioides*. Amphipods could facilitate the number of carpospores released, and they could also act as an active spore dispersal agent.

Our results also indicate that carpospores can stick to amphipod legs. The mucilage covering algal spores is a very common feature with several possible ecological roles: it may affect settlement efficiency, sinking rates of spores in the water column, or provide protection against desiccation during low tides (Lobban et al. 1985, Hoffmann 1987). Mucilage may also help spores stick to the legs of amphipods. This means that, in general, a close relationship between the alga and the amphipods is not necessarily required to explain the evolution of this adaptation. Indeed, in seed dispersal by adhesion to mobile animals, little structural modification appears to be required to change from wind to adhesive dispersal (Sorensen 1986).

Both methods described in this study, propagation of live carpospores from fecal pellets and the sticking of propagules to amphipod legs, may help to transport the spores to places otherwise made inaccessible by physical barriers. Turf-forming algae can inhibit the settlement of algal spores by acting as a barrier that reduces the number of spores reaching the substratum beneath (Hruby and Norton 1979, Deysher and Norton 1981). Conversely, algal turfs also enable spores already established beneath the turf to withstand longer exposure to air than those that are unprotected (Hruby and Norton 1979, Chapman 1984) since moisture retained by turf-algae can decrease desiccation stress (Hay 1981). Amphipods can potentially act as carpospore transporting agents as they pass through turf algae, such as *Ulva*, and because they can move between algal patches.

Mid and late successional forms of terrestrial plants have more species with adhesive adaptations than early successional ones (Sorensen 1986). *Iridaea laminarioides* is a typically mid successional alga in intertidal communities (Santelices 1990). Since these algae may show a limited propagule dispersal range, this study indicates that mobile amphipods could enhance dispersal of their spores that are incorporated into fecal pellets and attached to appendages.

We greatly appreciate the discussions and criticism by A. J. Hoffmann, D. A. López, P. Bernal. Our appreciation also to the reviewers for improving this manuscript and to S. Angus for reviewing the English of this manuscript. Field assistance by F. A. Kuschel, P. Vergara and many students are acknowledged with gratitude. The first author is grateful for financial support from FONDECYT Project Nr. 103/88.

Amsler, C. D. & Searles, R. B. 1980. Vertical distribution of seaweed spores in a water column offshore of North Carolina. *J. Phycol.* 16:617–9.

Anderson, E. K. & North, W. J. 1966. *In situ* studies of spore production and dispersal in the giant kelp *Macrocystis. Proc. Int. Seaweed Symp.* 5:73–86.

Breeman, A. M. & Hoeksema, B. W. 1987. Vegetative propagation of the red alga *Rhodochorton purpureum* by means of fragments that escape digestion by herbivores. *Mar. Ecol. Prog. Ser.* 35:197–201.

Buschmann, A. H. 1990. Intertidal macroalgae as refuge and food for amphipods in central Chile. *Aquat. Bot.* 36:237–45.

Buschmann, A. H. & Santelices, B. 1987. Micrograzers and spore release in *Iridaea laminarioides* Bory (Rhodophyta: Gigartinales). *J. Exp. Mar. Biol. Ecol.* 108:171–9.

Chapman, A. R. O. 1984. Reproduction, recruitment and mortality in two species of *Laminaria* in south-west Nova Scotia. *J. Exp. Mar. Biol. Ecol.* 78:99–109.

Dayton, P. K. 1973. Dispersion, dispersal and persistence of the annual intertidal alga *Postelsia palmaeformis* Ruprecht. *Ecology* 54:433–8.

de Ruyter van Steveninck, E. D. & Breeman, A. M. 1987. Deep water populations of *Lobophora variegata* (Phaeophyceae) on the coral reef of Curacao: influence of grazing and dispersal on distribution patterns. *Mar. Ecol. Prog. Ser.* 38:241–50.

Deysher, L. R. & Norton, T. A. 1981. Dispersal and colonization of *Sargassum muticum* (Yendo) Fensholt. *J. Exp. Mar. Biol. Ecol.* 56:179–95.

Guzmán del Proo, S. A. & de la Campa de Guzmán, S. 1969. Investigaciones sobre *Gelidium cartilagineum* en la costa occidental de Baja California, Mexico. *Proc. Int. Seaweed Symp.* 6:179–86.

Hay, M. 1981. The functional morphology of turf-forming seaweeds: persistence in stressful marine habitats. *Ecology* 62: 739–50.

Hoffmann, A. J. 1987. The arrival of seaweeds propagules at the shore: a review. *Bot. Mar.* 30:151–65.

Hoffmann, A. J. & Camus, P. 1989. Sinking rates and viability of spores from benthic algae in central Chile. *J. Exp. Mar. Biol. Ecol.* 126:281–91.

Hoffmann, A. J. & Ugarte, R. 1985. The arrival of propagules of marine macroalgae in the intertidal zone. *J. Exp. Mar. Biol. Ecol.* 92:83–95.

Hruby, T. & Norton, T. A. 1979. Algal colonization on rocky shores in the Firth of Clyde. *J. Ecol.* 67:65–77.

Jernakoff, P. 1985. Interactions between the limpet *Patelloidea latistrigata* and algae on an intertidal rocky platform. *Mar. Ecol. Prog. Ser.* 23:71–8.

Lobban, C. S., Harrison, P. J. & Duncan, M. J. 1985. *The Physiological Ecology of Seaweeds.* Cambridge University Press, Cambridge, 242 pp.

Luxoro, C. & Santelices, B. 1989. Additional evidence for ecological differences among isomorphic reproductive phases of *Iridaea laminarioides* (Rhodophyta: Gigartinales). *J. Phycol.* 25: 206–12.

Paine, R. T. 1979. Disaster, catastrophe, and local persistence of the sea palm *Postelsia palmaeformis. Science (Wash. D.C.)* 205: 685–7.

Paya, I. & Santelices, B. 1988. Macroalgae surviving digestion by fishes. *J. Phycol.* 25:186–8.

Santelices, B. 1989. *Algas Marinas de Chile. Distribución, Ecología, Utilización y Diversidad.* Ediciones Universidad Católica de Chile, 399 pp.

———. 1990. Patterns of organizations of intertidal and shallow subtidal vegetation in wave exposed habitats of central Chile. *Hydrobiologia.* In press.

Santelices, B. & Correa, J. 1985. Differential survival of macroalgae to digestion by intertidal molluscs. *J. Exp. Mar. Biol. Ecol.* 88:183–91.

Santelices, B., Correa, J. & Avila, M. 1983. Benthic algal spores surviving digestion by sea urchins. *J. Exp. Mar. Biol. Ecol.* 70: 263–9.

Santelices, B. & Martinez, R. 1988. Effects of filter-feeders and grazers on algal settlement and growth in mussell beds. *J. Exp. Mar. Biol. Ecol.* 118:282–306.

Santelices, B. & Ugarte, R. 1987. Algal life history strategies and resistance to digestion. *Mar. Ecol. Prog. Ser.* 35:267–75.

Sokal, R. R. & Rohlf, F. J. 1979. *Biometría.* Ediciones H. Blume, Madrid, 832 pp.

Sorensen, A. E. 1986. Seed dispersal by adhesion. *Annu. Rev. Ecol. Syst.* 17:443–63.

Zechman, F. W. & Mathieson, A. C. 1985. The distribution of seaweed propagules in estuarine, coastal and offshore waters of New Hampshire, USA. *Bot. Mar.* 28:283–94.