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Occurrence of the eelgrass pathogen *Labyrinthula zosterae* in Japan

F. T. Short[1], D. Porter[2,*], H. Iizumi[3], K. Aioi[4]

[1]Jackson Estuarine Laboratory, University of New Hampshire, 85 Adams Point Rd, Durham, New Hamphire 03824, USA
[2]Department of Botany, University of Georgia, Athens, Georgia 30602, USA
[3]Hokkaido National Fisheries Research Institute, 116 Katsurakoi, Kushiro, Hokkaido 085, Japan
[4]Ocean Research Institute, University of Tokyo, Minamidai, Nakano, Tokyo 164, Japan

ABSTRACT: The marine slime mold *Labyrinthula zosterae*, the causative agent of wasting disease of eelgrass *Zostera marina*, has been isolated from both *Z. marina* and *Z. caulescens* on the south coast of Japan. In addition, wasting-disease symptoms were produced in disease tests on leaves of *Z. japonica* and *Z. marina* using axenic cultures of *L. zosterae* isolated from *Z. caulescens* and *Z. marina*. Thus, the known host species of *L. zosterae* are expanded to include 3 species of *Zostera*. Although symptomatic necrotic lesions were observed in field-collected seagrass leaves, widespread die-off from wasting disease was not evident on the south coast of Japan.

INTRODUCTION

Eelgrass *Zostera marina* L. is a pantemperate Northern Hemisphere seagrass. *Z. marina* populations growing on the east coast of the United States and the west coast of Europe have recently experienced an epidemic disease that has, in the extreme case, led to the total destruction of localized eelgrass populations (Short et al. 1986, 1988, Muehlstein 1989). The microorganism responsible for the eelgrass disease and die-off of eelgrass in a number of locations has recently been identified as *Labyrinthula zosterae* Porter et Muehlstein (Muehlstein et al. 1988, 1991). The symptoms of this eelgrass wasting disease have been found all along the east (Short et al. 1989) and west (Muehlstein et al. 1991) coasts of the U.S. and in Europe (Short et al. 1988), but occurrence of symptoms does not always lead to widespread decline.

The die-off of eelgrass in the 1930s as a result of eelgrass wasting disease (Milne & Milne 1951, Rasmussen 1977) was a broad scale marine pandemic the cause of which was unknown at the time. The recent recurrence of eelgrass wasting disease in specific locations along the east coast of the U.S. (Short et al. 1986, 1987, 1988) has not, as yet, led to a large-scale epidemic comparable to that which occurred in the 1930s. However, since the observed disease-related decline in Great Bay (New Hampshire, USA) in 1984, symptoms of the disease have been found extensively all along the east coast of the United States. Within the last 2 years, 4 other sites on the east coast have had documented eelgrass die-offs that are the direct result of eelgrass wasting disease. These occurred in Casco Bay, Maine (Short unpubl.), in the Anasquam River in Massachusetts (Dexter 1985), in Stage Harbor on Cape Cod, Massachusetts (Short unpubl.) and in the Niantic River in Connecticut (Short et al. 1989). The eelgrass plants succumbed to massive infection resulting in the total or partial die-off of eelgrass within these estuaries. In many other estuarine areas widespread disease symptoms have been documented, but little or no actual eelgrass die-off has been observed. The exact nature of conditions that lead to plant mortality from the disease is still under investigation (Short et al. 1991).

In addition to these east coast locations where die-off or disease symptoms have been found, eelgrass on the

* Addressee for correspondence

west coast of the U.S. from San Diego to Alaska has been shown to have various levels of disease symptoms with the pathogen *Labyrinthula zosterae* present in samples showing characteristic necrotic lesions.

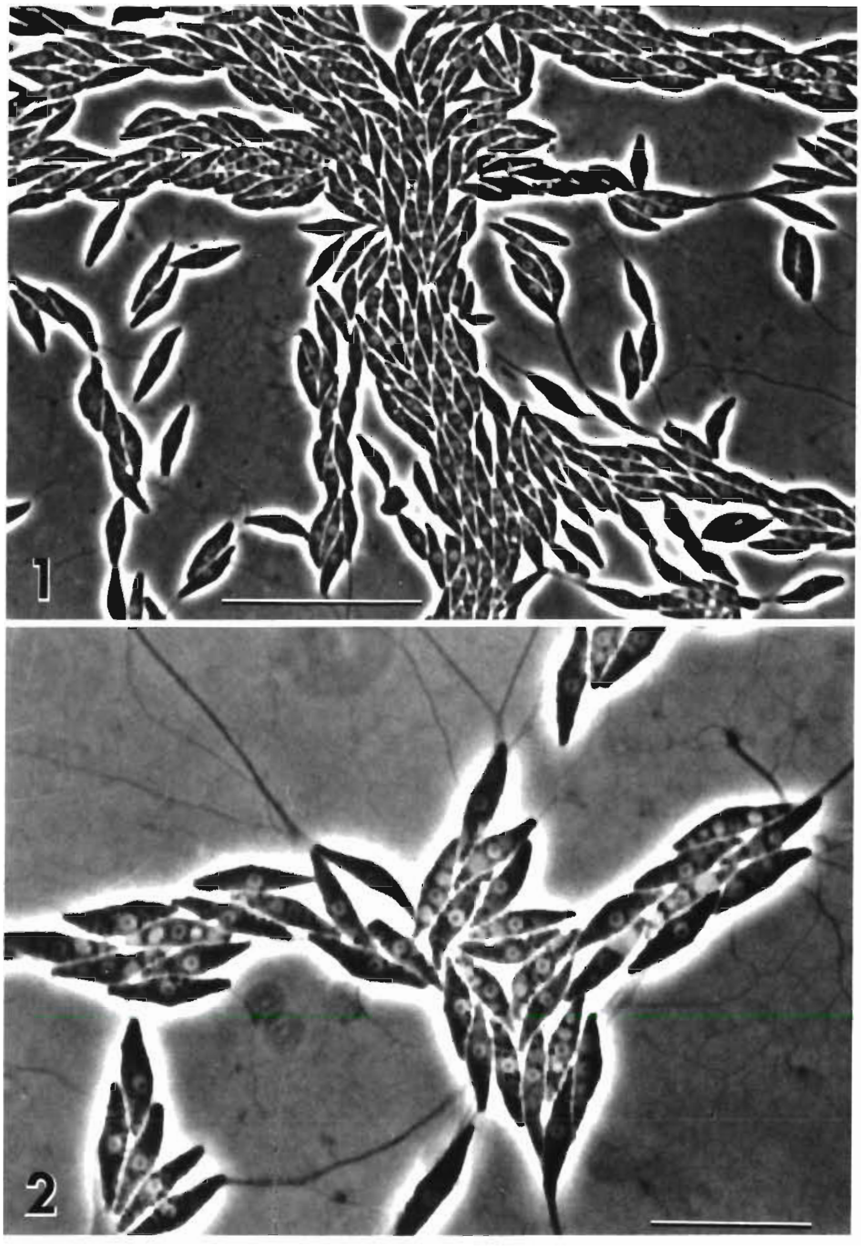
The International Ecological Congress in Japan in July of 1990 provided the opportunity to collect seagrass from a number of sites on the southeastern shore of Japan and to look for the pathogen and evidence of seagrass decline in the western Pacific.

METHODS

Seagrasses were collected from subtidal locations on the Misaki Peninsula on the southeast coast of Japan south of Tokyo. Field trips and sampling were based out of the Misaki Marine Biological Station of the University of Tokyo. Seagrasses were collected randomly by skin diving in water from 1 to 7 m in depth. Specific locations were Odawa Bay, Koajiro Bay and Moroiso Bay. Overall, 3 species of *Zostera* were collected. The first species, *Z. marina*, collected from all 3 locations, is the predominant seagrass in Japan and throughout the northern hemisphere. The second, *Z. japonica* Ascherson et Graebner, collected from Odawa Bay, is found in sheltered tidal flats on the coast of the Western Pacific from Vietnam to Sakhalin including Japan and was also recently introduced to the west coast of the United States (Phillips & Meñez 1988). The third species, *Z. caulescens* Miki, collected from Koajiro and Moroiso Bays, is found in scattered locations on the coasts of Japan and Korea where typically it grows in deeper water than *Z. marina* (den Hartog 1970).

All 3 species of *Zostera* were collected and examined for the characteristic necrotic lesions typical of eelgrass wasting disease (Short et al. 1987, Burdick et al. 1993). The plants were collected by hand while skin diving, bagged and brought back to the laboratory for analysis. All suspect black spots on the seagrass leaves were excised from the plant, sectioned into segments 5 cm long and plated on horse serum-seawater agar (SSA) emended with antibiotics (Porter 1990) for selective isolation of *Labyrinthula* species. Isolates were maintained on SSA as previously described (Muehlstein et al. 1988) for later infection experiments, microscopic examination and characterization.

Direct microscopic examination of *Labyrinthula* cultures was by phase contrast microscopy using a Zeiss



Figs. 1 & 2. *Labyrinthula zosterae* from Koajiro Bay, Japan. Two-day-old culture on agar surface. The *L. zosterae* colony consists of uninucleate fusiform cells that produce a common ectoplasmic network of branching and anastomosing filaments. Phase contrast microscopy. Scale bar = 50 µm in Fig. 1, 25 µm in Fig. 2

Table 1. Disease tests of Japanese isolates and type culture of *Labyrinthula* on *Zostera* spp.

Disease test			*Labyrinthula* isolate used as inoculum	
Test seagrass	Number attempted	Number with symptoms	Original location	Original host
Z. marina	4	4	Moroiso Bay	*Z. caulescens*
Z. marina	6	1	Odawa Bay	*Z. marina*
Z. marina	8	8	Koajiro Bay	*Z. marina*
Z. marina	2	2	Type culture of *L. zosterae*	*Z. marina*
Z. marina	4	0	Control, no inoculum	
Z. japonica	6	6	Koajiro Bay	*Z. marina*
Z. japonica	4	4	Type culture of *L. zosterae*	*Z. marina*
Z. japonica	4	0	Control, no inoculum	

RA microscope fitted with a long working distance phase contrast condenser. Micrographs were recorded on Kodak Technical Pan film.

Infection tests in the laboratory were carried out as described by Muehlstein et al. (1988). Test *Zostera* plants were inoculated by attaching a short segment of sterilized leaf, invaded by *Labyrinthula* cells from axenic culture, to a leaf of the test plant using a split section of polyethylene tubing. Plants were maintained in 2 l flasks of filtered seawater at 20 ppt salinity and were mixed by slow-bubbling air. They were located in a constant-temperature room at 20 °C illuminated for 14 h d^{-1} at 200 $\mu E\ m^{-2}\ s^{-1}$.

RESULTS

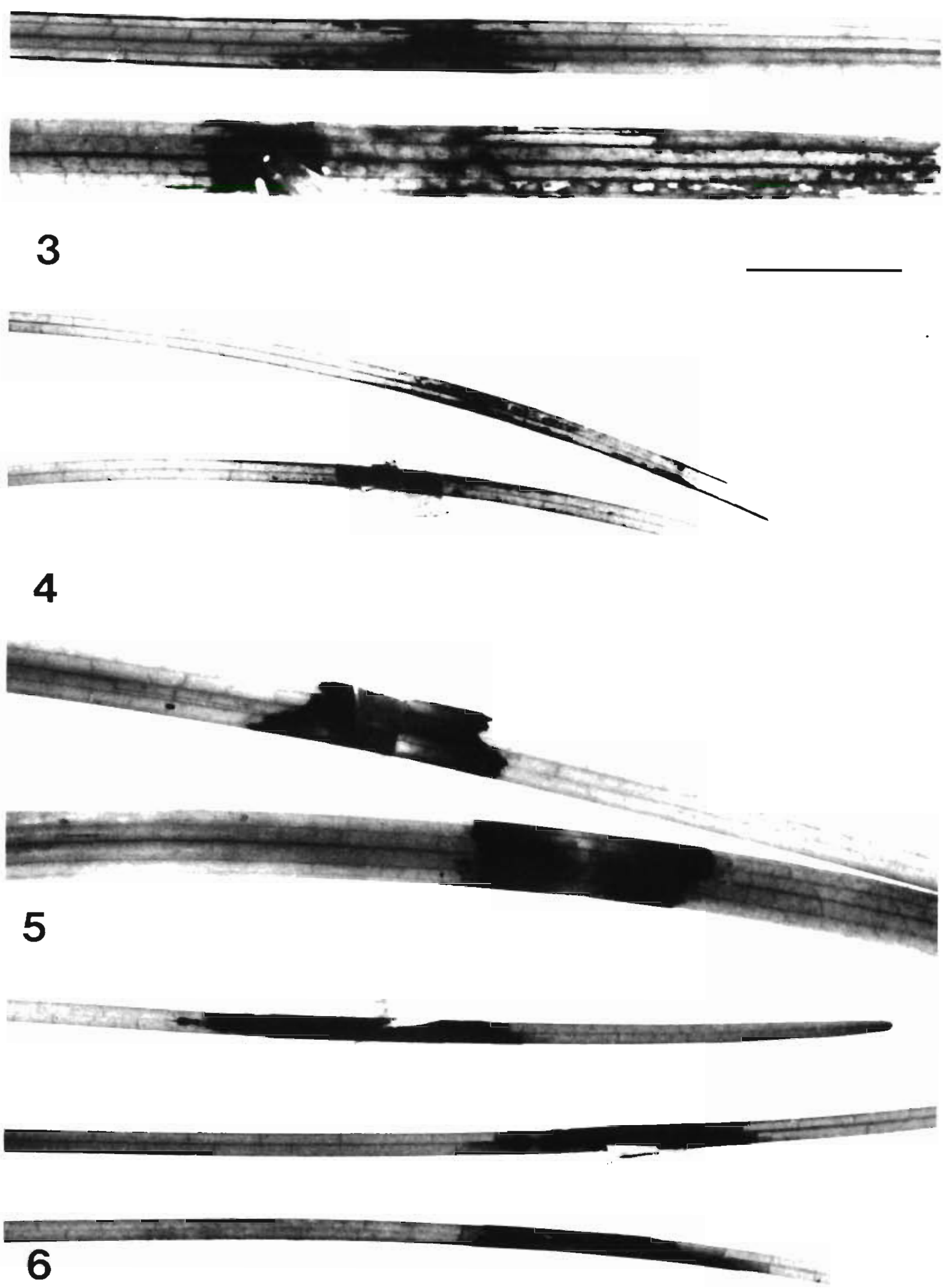
Our field survey found blackened necrotic lesions on mature and aging leaves of all 3 species of *Zostera* sampled. In all 3 species, the mature leaves had small and sparsely distributed black necrotic lesions. Many of the older leaves had larger black necrotic areas. The black spots occurring on the healthier mature leaves were often isolated with only 1 or 2 spots found per plant. Black spots or larger necrotic lesions were most abundant on *Z. marina*. Fewer necrotic spots were found on *Z. caulescens* and only a very few spots were found on *Z. japonica*. Overall, the occurrence of black lesions on any of the sampled seagrass species in Japan was low compared to symptoms of the wasting disease found along the east coast of the United States.

Isolation of *Labyrinthula* from the black lesions on the seagrass leaves was performed at the Misaki Marine Biological Station. Of the 33 leaf segments plated out, 5 labyrinthulids appeared on the isolation plates. Four of these were from *Zostera marina* and 1 from *Z. caulescens*. Of the 4 isolates from *Z. marina*, 1 was identified as the saprobic *Labyrinthuloides yorkensis* Perkins and not studied further; the other 3 produced a growth pattern on agar which was similar to the eelgrass pathogen *Labyrinthula zosterae*. The 1 isolate from *Z. caulescens* appeared also to be similar to *L. zosterae*. The *Labyrinthula* isolate from a necrotic lesion on a leaf of *Z. marina* collected from Koajiro Bay is shown in Figs. 1 & 2. The cells' measurements (value ± SD) are 16.5 ± 1.9 × 4.1 ± 0.9 µm (n = 25). They contain 1.3 ± 1.6 vacuoles $cell^{-1}$ and 27.2 ± 9.2 granules $cell^{-1}$. The granules (which are probably lipid food reserve) average 0.4 ± 0.1 µm in diameter. The cells of the *Labyrinthula* isolate from a necrotic spot on a leaf of *Z. caulescens* from Moroiso Bay were 15.3 ± 3.0 × 3.7 ± 0.5 µm (n = 30) with 3.8 ± 4.8 vacuoles $cell^{-1}$ and 29.9 ± 11.5 granules $cell^{-1}$. These values are comparable to those reported for *L. zosterae* (Muehlstein et al. 1991).

To test pathogenicity and host specificity of the Japanese isolates, healthy *Zostera marina* and *Z. japonica* plants (grown in mesocosms at Jackson Estuarine Laboratory, New Hampshire, USA) were inoculated in 2 l flasks with 3 Japanese isolates of *Labyrinthula* and the type culture of *L. zosterae*. The results of these disease tests are shown in Table 1 and Figs. 3 to 6. All tests were successful except those performed with the Odawa Bay isolate (1 of 6 produced symptoms). Unfortunately this isolate was lost before we made a quantitative micrographic examination to see how it compared with the type culture of *L. zosterae*. Inoculated leaves of both *Z. marina* and *Z. japonica* from disease tests with the Koajiro Bay isolate of *L. zosterae* showed a more diffuse pattern of the infection symptoms (Figs. 3 & 4) when compared with the dense black infection symptoms produced by the type culture of *L. zosterae* (Figs. 5 & 6).

DISCUSSION

The isolates of *Labyrinthula* obtained from *Zostera marina* and *Z. caulescens* in Japan were classified as *L. zosterae*, the eelgrass wasting disease pathogen. Thus, *L. zosterae* has both a broader range of host species and a broader geographical range than originally reported (Muehlstein et al. 1991). The significance of our results is that we have demonstrated *L. zosterae* in the Western Pacific, supporting the hypothesis that the pathogen occurs throughout the extensive north temperate range of its host *Z. marina*. In addition to the many locations in North America cited in Short et al. (1988) and Muehlstein et al. (1991), *L. zosterae* has



Figs. 3 to 6. *Zostera* spp. leaves from disease tests 3 d after inoculation with isolates or type culture of *Labyrinthula zosterae*. The disease symptoms are the darkened necrotic streaks and patches. These symptoms spread out from the inoculation location. All at same magnification. Scale bar = 10 mm. Fig. 3. Koajiro Bay isolate on *Z. marina*. Fig. 4. Koajiro Bay isolate on *Z. japonica*. Fig. 5. Type culture on *Z. marina*. Fig. 6. Type culture on *Z. japonica*

been isolated from the coast of Europe (Vergeer & den Hartog 1991). Prior to our observations of the eelgrass pathogen in Japan, there had been no reports of eelgrass wasting disease or evidence of eelgrass die-off from wasting-disease infection on the coast of Japan or elsewhere in the western North Pacific. In New Zealand, Armiger (1964) did report a *Zostera* sp. dieoff which she described as caused by a species of *Labyrinthula*. However, the New Zealand *Labyrinthula* differs from *L. zosterae* in having smaller cells (average 12 × 3 µm) and forming sorus-like bodies (Armiger 1964).

The host range of *Labyrinthula zosterae* is now shown to be broader than originally described as only *Zostera marina* by Muehlstein et al. (1991), but is still restricted to certain species of Northern Hemisphere *Zostera*. We report isolation of *L. zosterae* from *Z. caulescens*. Recently Vergeer & den Hartog (1991) reported isolation of *L. zosterae* from *Z. noltii* Hornem. in the Netherlands and France. In 1987 Muehlstein (pers. comm.) isolated *L. zosterae* from a necrotic lesion on a leaf of *Z. japonica* growing in Padilla Bay, Washington, USA.

The significance of disease symptom variation (Figs. 3 to 6) is not known. Variation such as this is noticed in diseased eelgrass plants in the field, in mesocosm tanks and in laboratory disease tests (Burdick et al. 1993, Porter & Short unpubl.). These observations suggest that there may be a genetic component of the pathogen in the manifestation of the disease symptoms.

Our observations were too limited on which to judge the extent of the wasting disease in Japan and the threat to the survival of Japanese seagrass. Quantitative assessment of the extent of *Labyrinthula zosterae* infection remains to be made. However, it appears from our findings that the pathogen occurs fairly frequently within a population of *Zostera*, but plays a minor role as a disease agent. This low level infection is in contrast to the active disease symptoms that are currently observed on both coasts of the Atlantic.

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