Infectivity of *Mikrocytos mackini*, the causative agent of Denman Island disease in Pacific oysters *Crassostrea gigas*, to various species of oysters

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ABSTRACT: In addition to *Crassostrea gigas*, *Mikrocytos mackini* Farley 1988, a pathogenic intracellular protistan of unknown taxonomic affiliations, produces disease and mortalities in other species of economically important oysters (*Crassostrea virginica*, *Ostrea edulis* and *Ostrea conchaphila*). Preliminary evidence suggests that these alternate species may be more susceptible to infection and the resulting disease than the usual host *C. gigas*. *M. mackini* isolated from *C. virginica* and *O. edulis* were infective for oysters. Warm temperatures (above 15°C) prevented the development of *M. mackini* in *C. gigas*, *C. virginica* and *O. edulis*.

KEY WORDS: *Mikrocytos mackini* · *Crassostrea gigas* · *Crassostrea virginica* · *Ostrea edulis* · *Ostrea conchaphila* · Protista · Denman Island disease · Infectivity

INTRODUCTION

In British Columbia, Canada, Denman Island disease, caused by *Mikrocytos mackini* (Farley et al. 1988), can account for about 30% mortality in market-sized *Crassostrea gigas* (Pacific oysters) on certain beaches in the spring of some years (Bower 1988). *M. mackini* is a tiny protistan (about 2 µm in diameter) of unknown taxonomic affiliations that inhabits the vesicular connective tissue cells of its host. Infection with this parasite often results in the development of greenish coloured lesions (pustules, up to 1 cm in diameter) in the oyster tissues, and the parasite can be transmitted directly between oysters. To date, all infected oysters obtained from the field were either collected off a beach or, if from hanging culture, had been held on the beach during the spring months. Thus, natural transmission is known to occur on certain beaches in British Columbia but transmission between oysters being cultured only via suspension techniques has not yet been verified.

Prior to this study, *Mikrocytos mackini* was only known in *Crassostrea gigas* from British Columbia. Because *C. gigas* is an important species to shellfish aquaculture in many countries around the world, concern was raised about the potential risks involved with the inadvertent introduction of this parasite to other countries in conjunction with global transfers of *C. gigas*. Thus, it was important to ascertain the susceptibility of other species of oysters to this parasite and the disease it causes.

Although *Mikrocytos mackini* could be transmitted between *Crassostrea gigas* by cohabitation in the laboratory and by incubation in a slurry of homogenized diseased oysters (Quayle 1961), these methods of exposure are unreliable for testing the host specificity of *M. mackini*. It is impossible to determine if each oyster used in the experiment had been exposed (or remained closed for the exposure period) and if the extent of exposure was similar for all exposed oysters. Also, these methods of exposure usually had a long prepatent period (3 to 6 mo) and resulted in prevalences of infection less than 50% among exposed oysters. Recently, techniques were developed to isolate viable and infective *M. mackini* from heavily

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infected oysters dying from Denman Island disease and the parasite could reliably be transmitted between *C. gigas* by inoculation of the isolate into the adductor muscle of uninfected oysters (Hervio et al. 1996).

In the present study, inoculation of *Mikrocytos mackini* isolates was used to assess the infectivity of *M. mackini* to other economically important species of oysters currently available in British Columbia. This procedure was also used to determine if *M. mackini* isolated from alternate species of oysters were infective and if warm temperatures impeded the development of *M. mackini* in alternate species as in the usual host, *Crassostrea gigas*. In addition to this artificial mechanism of assessing susceptibility, results were confirmed by exposing the various species of oysters to natural transmission in the environment.

MATERIALS AND METHODS

Oysters. Large *Crassostrea gigas* [122.2 ± 25.0 mm (mean $\pm$ standard deviation) in shell length, $n = 213$, and over 2 yr of age], small *C. gigas* (59.3 ± 8.9 mm in shell length, $n = 59$ at 8.5 mo and 64.3 ± 8.9 mm, $n = 43$ at 12 mo of age), *Crassostrea virginica* (eastern oysters, 75.1 ± 11.5 mm in shell length, $n = 186$), *Ostrea edulis* (flat oysters, 79.1 ± 8.7 mm in shell length, $n = 160$), and *Ostrea conchaphila* (the only species of oyster indigenous to British Columbia, 41.8 ± 5.2 mm in shell length, $n = 67$) were obtained from various locations in southern British Columbia. Oysters that were not used in experiments within 2 wk of collection were held in lantern nets suspended in Departure Bay, Nanaimo, British Columbia. The holding period varied between 14 and 24 mo for the large *C. gigas* and was less than 5 mo for all other species including the small *C. gigas*. In the laboratory, groups of 60 or fewer oysters were held in 50 l tanks supplied with flowing seawater (2 to 4 l min⁻¹) of ambient salinity (28 to 30 parts per thousand) and temperatures of about 10°C unless otherwise indicated. Experimentally exposed and control oysters were always held in separate tanks. In the laboratory, oysters were examined for mortalities and fed 5 times per week with a variety of cultured algae [i.e. each tank received about 2 l containing about 6×10^6 cells ml⁻¹ of *Chaetoceros gracilis* (clone NRC 108), *Isochrysis* sp. from Tahiti (clone Bigalow CCMP 1324), or 3H *Thalassiosira pseudonana* (clone Bigalow CCMP 1335)].

***Mikrocytos mackini* isolation and transmission by inoculation.** For all laboratory transmission experiments, oysters were exposed to *Mikrocytos mackini* by inoculation. Although *M. mackini* originated from *Crassostrea gigas* stocks cultured in British Columbia, the isolates used in these studies had been maintained in the laboratory for up to 2 yr by passage (inoculation)

between *C. gigas* every 6 to 8 wk with passage to other species of oysters for experimental purposes. The parasite was isolated from experimentally infected oysters as described previously (Hervio et al. 1996). Briefly, labial palps and adductor muscle from heavily infected oysters were excised, rinsed in filtered seawater (FSW at 0.22 μ m) supplemented with 1% Tween 80 (FSWT), homogenized, sieved successively through 200, 100, 60, and 20 μ m nylon meshes and centrifuged for 30 min at $2000 \times g$. The resulting pellet was either: (1) washed twice in FSW (resuspension followed by centrifugation) to remove the Tween; or (2) resuspended in FSW, layered onto a 15 to 30% (w/w) sucrose/FSWT gradient, centrifuged as above, the sucrose solution decanted and the pellet washed once in FSW. For both methods, the washed pellet was resuspended in a desired volume of FSW. The relative intensity of *M. mackini* in the inoculum was determined from a smear prepared with about 20 μ l of the inoculum. The smear was air dried, and fixed and stained with a commercial modification of a Wright-Giemsa staining procedure (Diff Quick Stain Set, Baxter Healthcare Corporation, McGraw Park, IL, USA) following the manufacturers protocol. The average number of *M. mackini* per field (200 μ m in diameter at 1000 $\times$ magnification) was determined and was used as the relative measure of intensity. For details on the species of oyster from which *M. mackini* was obtained and the relative intensity of *M. mackini* in the inoculum see Tables 1 & 2.

Oysters exposed to *Mikrocytos mackini* by inoculation and control oysters, inoculated with an equivalent volume of FSW, were prepared for inoculation by mechanically grinding the edge of both valves at the level of the adductor muscle to make a small aperture into the mantle cavity. To relax the adductor muscle to facilitate inoculation, these oysters were immersed in an anesthetic solution of 7% magnesium chloride hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) in seawater for 3 to 5 h prior to inoculation. All oysters were inoculated into the adductor muscle using a 25 gauge 1½ 'inch' needle. Each oyster received 0.1 ml of the parasite suspension or FSW, except for the small *Crassostrea gigas* and the *Ostrea conchaphila* that received only 0.05 ml due to their small size. Oysters receiving a similar inoculum were grouped into 1 tank as described above. Oysters were monitored daily and weak or dead individuals were removed and examined for *M. mackini* (method described below). Surviving oysters were examined between 49 and 91 d after inoculation.

In order to determine if warm temperature impedes the development of Denman Island disease in inoculated oysters as it does in oysters exposed by incubation in a homogenate from infected oysters (bath exposure) (Hervio et al. 1996), a preliminary experiment

was conducted with large *Crassostrea gigas*. Oysters receiving a similar inoculum were divided into 2 groups. One group ($n = 20$) was incubated at $10.0 \pm 1.0^\circ\text{C}$ and the other group ($n = 38$) was incubated at $17.9 \pm 0.1^\circ\text{C}$. During the third month of incubation, all oysters held at the cold temperature and 19 oysters from the group at the warm temperature were examined for *Mikrocytos mackini*. All remaining oysters ($n = 19$) held at the warm temperature were then transferred to $10.2 \pm 0.7^\circ\text{C}$ for an additional 84 d of incubation prior to examination for *M. mackini*. Controls ($n = 13$) were maintained as for the oysters that were initially incubated at the warm temperature and then transferred to the cold temperature.

The effect of incubation at a warm temperature on the development of Denman Island disease in other species of oysters was also tested. Replicate groups of inoculated and control *Ostrea edulis*, *Crassostrea virginica* and large *C. gigas* were incubated in cold ($9.2 \pm 2.0^\circ\text{C}$) or warm seawater ($17.9 \pm 0.5^\circ\text{C}$). Oysters were maintained and monitored as in other laboratory experiments and surviving oysters were examined between 51 and 59 d after inoculation.

For all laboratory experiments, all oysters were examined for *Mikrocytos mackini* using stained tissue imprints of the adductor muscle, palps and/or mantle as described by Hervio et al. (1996). The intensity of infection (as defined by Margolis et al. 1982) was qualitatively evaluated from the number of *M. mackini* observed in oyster tissue imprints. A heavy intensity was assigned to oysters with more than 5 *M. mackini* per field (200 μm in diameter at 1000 $\times$ magnification). All other oysters with *M. mackini* were considered to be lightly infected.

Transmission by field exposure. A more natural field challenge was accomplished by holding oysters on a beach, where *Mikrocytos mackini* is enzootic, during the spring when transmission occurs. On March 11, 1993, 15 small *Crassostrea gigas*, 15 large *C. gigas*, 15 *C. virginica*, 15 *Ostrea edulis*, and 15 *O. concaphila* were placed in each of 5 plastic perforated trays (Nestier trays, 56 cm square and 5 cm high) and covered with plastic predator mesh. A sixth covered tray contained oysters as for the other trays but lacked the 15 *O. concaphila*. The 6 trays were distributed at 30 m intervals along the beach in Henry Bay (Denman Island, British Columbia) at the 0.75 m tide level where the disease was first encountered in 1960 (Quayle 1961, Bower 1988). The oysters were left on the beach from March 11 to June 3, 1993. At monthly intervals, each tray was checked and mortalities recorded. On April 8, 1993, 78 resident *C. gigas* in Henry Bay at the 0.6 to the 0.9 m tide level were collected and examined for *M. mackini*. On June 3, 1993, the 6 trays were returned to the laboratory. All re-

maining oysters from each tray were placed in separate 50 l tanks and maintained at about 10°C as for oysters in the inoculation experiments. Control oysters were kept in the laboratory at about 10°C for the duration of the experiment (field exposure and laboratory incubation period).

On September 16, 1993, surviving oysters were examined for the presence of *Mikrocytos mackini*. Because examination of histological sections is more sensitive for detecting naturally acquired infections of *M. mackini* in oysters with lesions (Hervio et al. 1996), all oysters with lesions (focal green pustules characteristic of Denman Island disease), all small *Crassostrea gigas* and all *Ostrea concaphila* that were too small for the detection of lesions were examined histologically as described by Hervio et al. (1996). The other oysters were examined by the tissue imprint technique as described for the inoculation experiments because this technique was shown to be more sensitive when no lesions are observed (Hervio et al. 1996).

RESULTS

Transmission by inoculation

All 4 species of oysters were susceptible to *Mikrocytos mackini* that was isolated from *Crassostrea gigas*, and *M. mackini* from both *C. virginica* and *Ostrea edulis* were infective to exposed oysters (Table 1). In all 4 laboratory experiments reported in Table 1, the prevalence of infection in *C. virginica* and *O. edulis* (from 89 to 100%) was greater than in the usual host, *C. gigas* (from 47 to 66% regardless of size/age), regardless of the oyster species from which the inoculum originated and the relative number of *M. mackini* inoculated. The same trend occurred for the percentage of oysters that was found moribund during the incubation period and for the percentage of heavily infected oysters. In Expt 2 (Table 1), *O. concaphila* also had a greater prevalence of infection (96%) than *C. gigas* of similar size (48%) despite the extra 9 d that the infection had to develop in the *C. gigas*. The same trend occurred for the percentage of moribund oysters found (89 versus 17%) and for the percentage of oysters with a heavy intensity of infection (82 versus 28%). Among all control groups, 1 large *C. gigas*, 1 *C. virginica* and 3 *O. concaphila* were infected with *M. mackini*, indicating that the oysters had been exposed to this parasite in the field prior to setting up the experiment. However, in all cases, the prevalence of infection and the percentage of heavily infected oysters was considerably greater for the groups exposed by inoculation.

Table 1. Infectivity of *Mikrocytos mackini* to various species of *Crassostrea* and *Ostrea* following inoculation with a suspension of *M. mackini* isolated from infected oysters. Inoc. = inoculated

	Large <i>C. gigas</i>		Small <i>C. gigas</i>		<i>C. virginica</i>		<i>O. edulis</i>		<i>O. conchaphila</i>	
	Controls	Inoc.	Controls	Inoc.	Controls	Inoc.	Controls	Inoc.	Controls	Inoc.
Expt 1 (from <i>C. gigas</i> with 50 per field) ^a										
Number of oysters	30	32	–	–	30	35	28	32	–	–
Incubation period (d)	61	80	–	–	66	77	62	61	–	–
% moribund	0.0	6.3	–	–	0.0	20.0	0.0	90.6	–	–
% infected	0.0	65.6	–	–	0.0	100.0	0.0	100.0	–	–
% heavily infected ^b	0.0	12.5	–	–	0.0	80.0	0.0	62.5	–	–
Expt 2 (from <i>C. gigas</i> with 50 per field) ^a										
Number of oysters	20	30	30	29	30	30	28	16	27	29
Incubation period (d)	88	90	88	91	85	85	85	74	82	82
% moribund	0.0	6.7	0.0	17.2	0.0	23.3	0.0	100.0	14.8	89.7
% infected	5.0	46.7	0.0	51.7	3.3	96.7	0.0	100.0	11.1	96.5
% heavily infected ^b	0.0	26.7	0.0	34.5	3.3	63.3	0.0	93.7	7.4	75.9
Expt 3 (from <i>C. virginica</i> with 5 per field) ^a										
Number of oysters	20	10	–	–	20	10	20	10	–	–
Incubation period (d)	59	50	–	–	59	50	58	51	–	–
% moribund	0.0	0.0	–	–	0.0	0.0	0.0	0.0	–	–
% infected	0.0	60.0	–	–	0.0	100.0	0.0	90.0	–	–
% heavily infected ^b	0.0	20.0	–	–	0.0	30.0	0.0	40.0	–	–
Expt 4 (from <i>O. edulis</i> with 12 per field) ^a										
Number of oysters	20	10	–	–	20	10	20	9	–	–
Incubation period (d)	59	49	–	–	59	49	58	49	–	–
% moribund	0.0	0.0	–	–	0.0	0.0	0.0	66.7	–	–
% infected	0.0	60.0	–	–	0.0	90.0	0.0	88.9	–	–
% heavily infected ^b	0.0	0.0	–	–	0.0	0.0	0.0	66.7	–	–

^aOyster species from which the inoculum was isolated and the relative intensity of *M. mackini* in the inoculum presented as the average number per 200 µm diameter field at 1000× magnification

^bOysters with greater than 6 *M. mackini* per field (200 µm in diameter at 1000× magnification) in stained tissue imprints

The preliminary temperature experiment indicated that infections of *Mikrocytos mackini* did not develop in large inoculated *Crassostrea gigas* incubated at warm temperatures (about 18°C). Only 2 of 19 inoculated oysters incubated at this warm temperature for 74 d had infections which were barely detectable. However, all 20 inoculated oysters incubated at about 10°C for 71 d were infected and the intensity of infection in 15 of these oysters was heavy. Two of the 19 inoculated oysters incubated at about 18°C for 74 d and transferred to about 10°C for a further 84 d of incubation had light infections, whereas none of the 13 controls treated in a similar fashion were infected.

Mikrocytos mackini was not detected in *Crassostrea gigas*, *C. virginica* or *Ostrea edulis* incubated at warm temperatures for about 55 d (Table 2). Unlike the 4 laboratory experiments reported in Table 1, the prevalence of infection in oysters incubated at the cold temperature was similar for all 3 species of oysters (Table 2). Nevertheless, the percentage of oysters that was found moribund during the incubation period and the percentage of heavily infected oysters was greatest for *O. edulis*.

Transmission by field exposure

During the field and laboratory incubation periods of the field exposure experiment, 25 exposed oysters were either lost (n = 6), consumed by crabs (n = 8) or decomposed prior to examination (n = 11) and were excluded from the analysis. The distribution of excluded oysters among the species and trays can be easily determined from Table 3 by comparing the number of oysters examined (value in parentheses) with 15 (the original number used for each sample). Four of the exposed oysters (1 *Ostrea conchaphila* and 3 *O. edulis*) became moribund during the laboratory incubation period and all 4 were found to be infected with *Mikrocytos mackini*. Of the 410 exposed oysters, only 30 became infected with *M. mackini* and over half (19) came from 2 of the 6 trays (Table 3). However, infections were observed in at least 6% of all 4 species of oysters with *O. edulis* and *Crassostrea virginica* having the highest prevalences (12.4 and 10.0% respectively). Although all 30 infected oysters had lesions characteristic of Denman Island disease, only 9 (1 *O. conchaphila* and 8 *O. edulis*) had heavy infections. Thirty-one other oysters representing all species had

Table 2. Effect of temperature on infectivity of *Mikrocytos mackini* to *Crassostrea* spp. and *Ostrea edulis* following inoculation with a relative intensity of 20 *M. mackini* per 200 μ m diameter field at 1000 $\times$ magnification isolated from infected *C. gigas*. Inoc. = inoculated

	Large <i>C. gigas</i>		<i>C. virginica</i>		<i>O. edulis</i>	
	Controls	Inoc.	Controls	Inoc.	Controls	Inoc.
Incubated at 9.2 $\pm$ 2.0°C						
Number of oysters	20	19	20	19	20	19
Incubation period (d)	59	54	59	52	58	51
% moribund	0.0	10.5	0.0	5.3	0.0	47.4
% infected	0.0	89.5	0.0	89.5	0.0	100.0
% heavily infected ^a	0.0	57.9	0.0	42.1	0.0	73.7
Incubated at 17.9 $\pm$ 0.5°C						
Number of oysters	—	20	—	17	—	20
Incubation period (d)	—	55	—	55	—	52
% moribund	—	0.0	—	0.0	—	0.0
% infected	—	0.0	—	0.0	—	0.0
% heavily infected ^a	—	0.0	—	0.0	—	0.0

^aOysters with greater than 6 *M. mackini* per field (200 μ m in diameter at 1000 $\times$ magnification) in stained tissue imprints

tissue lesions (focal greenish discoloration in living tissues), but *M. mackini* was observed in neither tissue imprints nor histological sections of these oysters.

Six of the 26 *Ostrea conchaphila* held in the laboratory for the duration of the field exposure (controls) were infected with *Mikrocytos mackini*. *M. mackini* was not observed in any of the other 109 control oysters (Table 3). Seventeen percent of the 78 *Crassostrea gigas* resident in Henry Bay on April 8, 1993 were infected with *M. mackini*.

DISCUSSION

All species of oysters tested for susceptibility to *Mikrocytos mackini* became infected regardless of the method of challenge (i.e. by inoculation in the laboratory or by exposure to natural transmission in the field). In most experiments, the severity of infection was greater in species other than the usual host (*Crassostrea gigas*), suggesting that alternate species of oysters may be more susceptible to infection and the resulting disease. Also, because *M. mackini* from at least 2 of the alternate host species were infective, the threat posed to the culture of oysters in other regions of the world by accidental introduction of this pathogen warrants that precautions be taken when transplanting oysters from British Columbia.

One factor that provides oyster culturists potential control of *Mikrocytos mackini* is the sensitivity of this parasite to warm temperatures. The inability of *M. mackini* to develop in oysters (*Crassostrea gigas*, *C. virginica* and *Ostrea edulis*) held at about 17°C may explain why this parasite and Denman Island disease have not been reported south of British Columbia, despite the historic exchange of live oysters between British Columbia and Pacific states of the United States to the south (Quayle 1988). Apparently, *M. mackini* can survive in oysters exposed to these warm temperatures for prolonged periods. In the preliminary temperature experiment reported above and in Hervio et al. (1996), some *C. gigas* incubated at about 17°C for

about 3 mo following exposure developed patent infections on return to cold (about 10°C) water. Thus, it is not likely that exposure to warm temperatures can be used as a method of removing *M. mackini* from an infected oyster population.

The apparent greater susceptibility of alternate species of oysters to *Mikrocytos mackini* suggests that *Crassostrea gigas* may be the indigenous host of this parasite. However, *M. mackini* has not been reported from locations outside of British Columbia even though

Table 3. Infectivity of *Mikrocytos mackini* to various species of *Crassostrea* and *Ostrea* via natural exposure in the field for 12 wk during the spring followed by 15 wk of incubation in the laboratory at 10°C. Data recorded as percentage of oysters infected with *M. mackini* followed by the number of oysters examined in parentheses. Control oysters were held in the laboratory for the entire 27 wk period and not intentionally exposed to *M. mackini*

Oyster species	Tray 1	Tray 2	Tray 3	Tray 4	Tray 5	Tray 6	Totals
Large <i>C. gigas</i>							
Controls	—	—	—	—	—	—	0 (19)
Exposed	6.7 (15)	13.3 (15)	0 (15)	20.0 (15)	0 (14)	6.7 (15)	7.9 (89)
Small <i>C. gigas</i>							
Controls	—	—	—	—	—	—	0 (30)
Exposed	0 (14)	0 (13)	0 (15)	0 (11)	0 (12)	0 (14)	0 (79)
<i>C. virginica</i>							
Controls	—	—	—	—	—	—	0 (30)
Exposed	0 (15)	6.7 (15)	6.7 (15)	13.3 (15)	26.7 (15)	6.7 (15)	10.0 (90)
<i>O. edulis</i>							
Controls	—	—	—	—	—	—	0 (30)
Exposed	0 (14)	20.0 (15)	0 (15)	20.0 (15)	33.3 (15)	0 (15)	12.4 (89)
<i>O. conchaphila</i>							
Controls	—	—	—	—	—	—	23.1 (26)
Exposed	— ^a	0 (14)	7.1 (14)	7.7 (13)	10.0 (10)	8.3 (12)	6.4 (63)
Totals	1.7 (58)	8.3 (72)	2.7 (74)	13.0 (69)	15.3 (66)	4.2 (71)	

^aNo *O. conchaphila* were placed in this tray

C. gigas is not native to the Pacific coast of North America and was introduced, mainly from Japan (Quayle 1988). The small size of *M. mackini* makes it very difficult to detect, unless the observer is familiar with its morphological characteristics. A micrograph published in an early report on oyster mortalities in Matsushima Bay, Japan (Numachi et al. 1965, Fig. 15 therein) contains structures that appear to be *M. mackini*. Perhaps *M. mackini* was introduced into British Columbia along with its host *C. gigas*. To verify this speculation, surveys for *M. mackini* will have to be conducted on beach-cultured oysters in Japan during the spring.

In British Columbia, the prevalence of *Mikrocytos mackini* varies yearly in oysters on Henry Bay, Denman Island (between 11 and 39% from 1960 to 1987, Bower 1988). During the field exposure component of the study reported here, 17% of the resident *Crassostrea gigas* on the beach in Henry Bay in April 1993 were infected. Consequently, less than 13% of each of the oyster species exposed to natural transmission in Henry Bay in 1993 became infected. Variation in the prevalence of infection in oysters between trays (from 1.7 to 15.3%, Table 3) suggests that the distribution of the infection in Henry Bay is not uniform. This result supports casual observations noted during surveys for Denman Island disease in oysters (Bower unpubl.).

None of the small *Crassostrea gigas* developed detectable infections. This result confirms earlier investigations by Quayle (1988), who found oysters less than 2 yr old to be less affected. This apparent lack of infection in young *C. gigas* may be attributable to their small size. Possibly insufficient water was filtered by these small oysters for them to be exposed to infectious *Mikrocytos mackini*, or the number of infectious forms that were acquired were too few for the infection to be detectable when the experiment was terminated in September 1993. In the field, exposed resident oysters do not develop detectable infections until the following spring (after about a 1 yr prepatent period). To accelerate the development of the disease and to reduce inadvertent losses among experimental oysters left in the field, the oysters were returned to the laboratory after the spring exposure period and incubated at cold temperatures (about 10°C). (In the field, warm summer temperatures delay the development of the infection; Hervio et al. 1996) The 15 wk incubation period in the laboratory may have been too short for the development of patent infections in oysters that were exposed to very few infective forms. Nevertheless, 15 wk was sufficient for the development of infections in some individuals of all species tested, thus confirming the susceptibility of all species under natural conditions.

The occurrence of *Mikrocytos mackini* in control groups of oysters was not unexpected because all oysters used in this study originated from beaches in

British Columbia. However, this was the first time that this parasite has been observed in *Crassostrea virginica* and *Ostrea concaphila* from the field. From 1903 to 1940, extensive attempts were made to introduce *C. virginica* into at least 5 locations in southern British Columbia (Quayle 1988). However, a remnant population survives in only 1 location: the upper river estuaries of Boundary Bay. In areas of higher salinity at this location, *C. gigas* cohabits with *C. virginica* and could have been the source of infection for the *C. virginica* in one of the control groups (Table 1). Denman Island disease may be one of the factors that currently limits the expansion of *C. virginica* populations in British Columbia.

Prior to 1940, a fishery for *Ostrea concaphila* existed and attempts to culture this species were made in British Columbia. However in the early 1940s, a drastic drop occurred in *O. concaphila* populations in Boundary Bay and Ladysmith Harbor, British Columbia, for 'no perceptible reason' (Quayle 1988). In the decade prior to the 1940s, millions of *Crassostrea gigas* seed had been imported into British Columbia from Japan. Perhaps *Mikrocytos mackini* had inadvertently arrived in British Columbia with this seed and had precipitated the drastic reduction in *O. concaphila* populations. Although *O. concaphila* still occurs in regions where *M. mackini* is considered enzootic, *O. concaphila* is no longer abundant and is rarely found where *C. gigas* is cultured. A similar scenario could occur with other endemic species of oysters in other parts of the world if *M. mackini* is inadvertently introduced during transplantations of the usual host *C. gigas*.

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