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Life cycle studies of Perkinsus marinus : host specificity : final report, grant # NA16FL0399-01

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FINAL REPORT

Project title: Life cycle studies of *Perkinsus marinus*- Host Specificity; Grant # NA26FL0380-01

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Introduction

This project represents a renewal of Grant # NA16FL0399-01 designed to further investigate the host specificity of *Perkinsus marinus* by surveying for its presence in molluscs obtained from Virginia's coastal and estuarine waters. As a result of that survey, determinations were made as to the host species most appropriate for use in transmission-of-infection experiments.

Methods

Host Ranges and Incidences of Infection- As stated in the proposal, this part of the study was designed to find, out of 10 specified species of molluscs, which ones are infected at least part of the year. From that list *Mytilopsis leucophaeata, Petricola pholadiformis, Littorina irrorata, Melampus bidentatus, Buscyon carica* and *Eupleura caudata* were examined and *Cratena kaoruae* was substituted for *Cratena pilata* because the latter could not be found. *Diastoma varium, Lyonsia hyalina* and *Dipolthyra smithii* could not found either. To compensate I substituted *Argopecten irradians* for one of them and significantly expanded the effort to reevaluate 11 species of both bivalves and gastropods which had been surveyed the first year of this study (Table I). The species which were not found are often episodic in their occurrence and it is not surprising that such a problem would arise.

The selection of *A. irradians* was based on the belief that it will be a species of significance in future aquaculture efforts therefore it would be useful to know if it were susceptible to infection by *Perkinsus* sp. The population from which the scallops

came was set at the Wachapreague, VA laboratory of VIMS, grown to maturity over 12 months at Cherrystone Creek and then held for 2 months at Wachapreague before being evaluated. Thus, the scallops had an opportunity to become infected at two localities where *P. marinus* is endemic.

The sampling areas for all of the molluscs were from subtidal and intertidal sediments within a 10 mile radius of Gloucester Point, VA and a 10 mile radius of Wachapreague, VA. The former had a salinity range of 10 to 22 ‰ and the latter a range of 27 to 32‰.

Evaluations of whether the molluscs were infected with *Perkinsus* sp. or spp. were accomplished using a slightly modified Ray technique (Ray, 1954). This consisted of placing tissues of each animal in 10ml of fluid thioglycollate medium (FTM) with 6.1mg/10ml (4,680 units/10ml) of streptomycin sulphate and 3.0mg/10ml (4710 units/10ml) of penicillin-G (potassium salt), and 0.04mg/10ml of Nystatin. In the cases where the molluscs were small enough ($\leq$0.5 cm^3 of tissue volume), the whole animal was placed in a 15X125 mm culture tube with 10 ml of medium. From the larger individuals samples of four different organs were obtained from each mollusc. The size of each organ sample was such that at least 40%, up to about 75%, of the areas under a 22X22 mm square coverslip was occupied when the tissues were squashed under the coverslip for microscopic examination. The organs sampled from *C. virginica* were gill, rectum, mantle and hepatopancreas. If the mollusc had a foot and/or siphon one of the two were sampled instead of the rectum. As with the oyster the gill, mantle and hepatopancreas were also sampled.

The tubes with FTM and tissues were held for about 5 days at room temperature before assessments were made using the squashing method. All of each tissue sample was examined for the presence of prezoosporangia and infections were quantitated using the scale proposed by Mackin (1962). In recording the numbers of prezoosporangia, whether or not clusters of cells were found and the number of cells per cluster were noted. This provided an insight into whether multiplication of parasite cells had occurred in the host's (or carrier's?) tissues after filtration from the ambient water.

It should be noted that I use the term "infected" to denote the fact that *Perkinsus* spp. cells were observed in or on mollusc tissue. This is a term of convenience and does not mean that I determined whether the presence of the cells denoted that 1) the cells had established themselves as parasites or 2) they were simply entangled in the mucus and cilia or folds and/or microvilli of epithelial surfaces and were transient residents with limited or no proliferative capacity.

Transmission-of-Infection Experiments- This phase of the study

was conducted in the Virginia Institute of Marine Science's wet laboratory facilities at Gloucester Point and Wachapreague. Estuarine water was pumped into the laboratory and filtered through three, 1 μm pore-size cartridge filters arranged in tandem followed by one carbon cartridge filter. The water was then directed into two 35 gal circular, fiberglass tanks connected to each other so that water could flow from one to the other. The water was then pumped continuously at a rate of ca. two liters/min past a 25 watt UV germicidal lamp (Aquanetics), generally at least 24 hours before use. The bottom of the inflow tank was covered by a layer of crushed oyster shell (chicken grit), raised off the bottom one inch to serve as a subbottom filter. Water from the tanks was gravity fed into the containers holding the experimental molluscs.

Food for the molluscs was provided by adding suspensions of either *Thalassiosira pseudonana* or *T. weissflogii* so that 1 to $2X10^9$ cells per liter of the former and 810^7 to $2X10^8$ cells per liter of the latter were present in the holding containers shortly after adding the algae. The molluscs were fed at intervals of every one to three days (averaging every 1.3 days). Before each feeding there was an 70 to 80% change of the water in the holding container.

The molluscs in each TIE were held in 10 gal aquaria with 30-32 l of estuarine water from the above-mentioned system. For each TIE, either 25 control molluscs and 25 challenged molluscs or 10 control and 10 challenged molluscs were placed in each of two aquaria. The aquaria were aerated and had lids to prevent splashing and aerosols from carrying infective cells from one aquarium to the other. Uninfected oysters for the TIE's were obtained from Mook Sea Farm of Damariscotta, Maine. In recent years *P. marinus* has been found as far north as Massachusetts and possibly as far north as Maine. Even though there are unpublished reports of *P. marinus* being found in Maine, I have found no evidence that any of the Maine oysters I used were infected before being treated at VIMS. Thus far, over 1,500 unchallenged oysters from Mook Sea Farm have been examined at VIMS by myself and other workers over the period of January, 1992 to present.

Two shipments of *Macoma balthica* were obtained from Eastport, Maine on June 30 and August 20, 1994 to serve as uninfected controls. However, cells of *Perkinsus* sp. were found in 8 of 34 unchallenged clams from the first shipment (6 light and 2 light-moderate infections) and 2 of 73 clams from the second shipment (one light and one light-moderate); therefore, unlike the *C. virginica* from Maine, the Maine clams could not be regarded as ideal controls. They were used nevertheless because another source from further north of Maine was not identified. No population of *M. balthica* from Virginia was found to be free of the parasite. There is the possibility that the holding system used in this study permitted secondary infections to occur but this appears to be unlikely because none of the unchallenged oysters obtained from

Maine were shown to have infections despite, in some cases, being held for 4 months in the system. Unfortunately I neglected to examine clams from the first shipment before they were placed in the holding system and the 8 infections were discovered after 44 days in the system. The second shipment was sampled before being placed in the system and out of 25 clams, none were infected. The two infected ones were found after 25 and 34 days in the system. In the experiments where challenges were conducted, the controls were uninfected.

Obtaining other species of uninfected molluscs from Virginia's waters was a problem in this study because the parasite is widespread; however, I was fortunate to select populations which did not demonstrate *Perkinsus* sp. or spp. when used as controls in the transmission-of-infection experiments (exceptions: TE-26 where one *Mya arenaria* contained one cluster of 8 cells and TE-27 where 3 *T. plebeius* were lightly infected and one was very lightly infected). Possibly this was in part due to the very wet spring and accompanying low salinities which one would expect would suppress the multiplication of the parasite.

When holding the sediment-dwelling bivalves, *Macoma balthica, Tagelus plebeius* and *Mya arenaria* in aquaria, they were placed in a bed of sand obtained from above the mean high water mark to lessen the probability of unintentionally introducing *Perkinsus* spp. cells and to lessen the strain on the adductor muscle. Underlying the bed of sand was a standard "under-gravel" filter purchased from a pet store and operated by an air lift. The filter was used to lessen the stresses caused by anaerobiosis.

When meronts, merozoites and schizonts of *P. marinus* from *C. virginica* were used as infective cells to challenge molluscs, suspensions of the parasite were prepared and cell numbers were estimated as follows: Oysters were determined to be infected by looking at fresh squashes of adductor muscle under oil immersion, phase contrast optics. Cells of *P. marinus* were easily located by looking for the vacuoplast. Since the cells are surrounded by host muscle fibers and very few hemocytes, they are not camouflaged by the presence of other cells. The oyster was then finely chopped with a razor blade, repeatedly pulled into and expelled from a 10 ml hypodermic syringe (without a needle) until a fine suspension of cells was obtained. The suspension was then sequentially filtered through 90, 35, and 20 μm Nitex screens. An aliquot of the 20 μm filtrate was placed in a known volume of FTM and the number of prezoosporangia was estimated after 24 hr using a Petroff-Hauser counting chamber and a 40X, phase contrast objective. Ten replicate counts were used. The rest of the cell suspension was immediately added, after obtaining the aliquot, to an aquarium with 25 molluscs at 22-24°C (no 24 hr delay). Vigorous aeration was used to keep the cells in suspension as much as possible to give the molluscs a chance to filter the cells from the water. Four to five days were allowed to elapse before changing the water. If

there were more than one addition of parasite cells to the aquarium the time between additions varied from one to 12 days. The numbers of cells listed in Table II represent the numbers added to each aquarium, each of which contained 30 l of estuarine water. Thus, for example in TE-35 the oysters were exposed to a total of 70 cells of *Perkinsus* sp. per ml in the aquarium followed by 163/ml then 247/ml. Obviously the number of *Perkinsus* sp. cells which entered the mantle cavity of each oyster is not known because 1) the pumping rates of the individual oysters is unknown and 2) the exact settling rates of the suspended cells is not known. However, an estimate of settling rates and length of time the cells were viable was obtained. In two experiments it was found that 46 to 64% of the cells were still in suspension in the top 5 mm of water in the aquarium after 24 hr and 0.4 to 1.4% of the cells could be detected after 5 and 4 days. Thus the molluscs had a reasonable amount of time to filter cells of the parasite from the water.

When cells of *P. marinus* and *P. atlanticus* from axenic cultures were used to challenge molluscs, exponential phase cultures were used and they were simply added while in the culture medium to the aquaria (TE-19 & -23 to -25) or injected into the mantle cavity of the mollusc (TE-30 to -33). In the latter case the number of cells injected was estimated by counting in a Petroff-Hauser counting chamber and the number listed in Table II is the number injected into each mollusc. After injection the molluscs were held out of water for 3 hours before adding them to the aquaria. For the experiments where the cells were added as a suspension into the aquarium water, the number listed in Table II is the number of cells added to each aquarium, each of which contained 30 l of estuarine water.

The culture medium for the parasites was that of Kleinschuster and Swink (1993). I isolated *P. marinus* from a York River *C. virginica* and *P. atlanticus* was isolated from a York River *Macoma balthica* by Dr. Steve Kleinschuster. I am using the name *P. atlanticus* because all morphological characteristics of the parasite and the host tissue response is exactly the same as that of *P. atlanticus* described by Azevedo (1989) from Portuguese *Ruditapes decussatus*. It should be noted that due to the lower virulence of the cultured cells as compared to *Perkinsus* spp. cells obtained directly from infected molluscs and used without being cultured, it was considered necessary to inject cultured cells into the mantle cavity to induce significant infections as opposed to feeding the cells to the molluscs by suspending them in the aquarium water. The is the only part of the study where the injection method was used.

In the case of challenges to *Crepidula plana* and *C. fornicata* it can be assumed that the molluscs both filtered cells of the parasite from suspension and grazed them from the bottom of the aquaria. Since *Urosalpinx cinerea* is not a filter feeder, they were challenged by placing heavily infected oysters, with the right

valve removed, in the aquaria with the gastropods. One oyster per 25 *U. cinerea* used, with the oysters being replaced two times at plus 25 then plus 3 days during the experiment. Although it was not confirmed whether every gastropod fed on the oyster meats, it is known that the large majority of them did feed at one time or another.

At the end of each TIE the molluscs were opened and samples of four organs were obtained from each mollusc for culture in FTM as in the field sampling program (see above).

Although highly unlikely, the oysters and clams from Maine could serve as carriers of some infectious disease organism or other species not found locally. Therefore, the used water from the holding containers was chlorinated by a one time addition of 100 ml Clorox/20 gal of estuarine water for 1 to 3 days at room temperature. The water was then dechlorinated and dumped onto a beach outside the wet laboratory.

Results

Host Ranges and Incidences of Infection- During the nine months of this project the following molluscs were examined for the presence of *Perkinsus* spp. (Table I):

As was observed in the first year's study, unless the gastropods were in close association with infected bivalve molluscs they contained few if any cells of *Perkinsus* spp. The *Urosalpinx cinerea* used in this year's study were not obtained from bivalves nor were the *Crepidula* spp. Last year the same was the case except for the sample of *C. fornicata* from Wachapreague which was obtained from the shells of infected oysters and showed 31% infections.

The bivalves had markedly higher levels of infections than the gastropods with 5 of 9 species containing cells of *Perkinsus* spp. The two species of shipworms (*Bankia gouldi* and *Teredo navalis*) had the highest levels.

Transmission-of-Infection Experiments- Results are contained in Table II with the numbered series representing findings obtained at Gloucester Point and the lettered series, at Wachapreague. "Meronts/schizonts" refers to the use of suspensions of homogenized, filtered suspensions of infected mollusc tissues. The intensities of infections are provided in Table III with 0.5= very light, 1= light, 2= light-moderate, 3= moderate, 4= moderate-heavy and 5= heavy.

The use of axenic cultures of *P. marinus* to provide suspensions of cells for filtration by the challenged oysters (TE-19 & -25) resulted in no infections despite the repeated additions of higher concentrations of cells than those which yielded 68, 56 and 76% infections in the first year's study (TE-4, -6 & -7). In the latter phase of the study similar experimental conditions were used except that fewer cells were added to the aquaria (1.1 X 10^9, 7.0 X 10^7 and 6.1 X 10^7 cells/30 l, respectively). When axenic cultures of *P. atlanticus* were used to challenge *C. virginica* (TE-24) by the feeding method, two oysters were found to contain very light levels of infection in the digestive gland and mantle with no indication of proliferation. Most probably the cells observed were filtered from the water and entrapped on the epithelial surfaces. Using cultured *P. atlanticus* to feed *M. balthica* (TE-23) resulted in three light infections in 10 clams.

When axenically cultured cells were injected into the mantle cavity, more and higher level infections were established. *P. marinus* (TE-30) established one light-moderate, 2 light and one very light infections in nine oysters examined with proliferation of the parasite in a number of loci. When *P. marinus* was used to challenge *M. balthica* (TE-33), one light-moderate and one moderate-heavy infection were observed in 5 oysters. Thus it was obvious that proliferation had occurred. When cultured cells of *P. atlanticus* (TE-32) were used to challenge *M. balthica* 2 light-moderate and one light infections in 5 clams were observed. The same type of cells injected into the mantle cavity of *C. virginica* (TE-31) resulted in 3 very light and one light infections in 10 oysters examined.

Despite the high numbers of cells inserted into the mantle cavities of the molluscs, the incidences and intensities of infections were not striking thus it was reassuring to realize that the best choice of cells to use in challenges is meronts, merozoites and schizonts from infected molluscs, used without culturing. The reason that axenically cultured cells were evaluated was that challenges could be conducted using a clean preparation generated under controlled and reproducible conditions which would have been highly desirable. However, use of the cells does not appear to be recommended. Although injections of uncultured cells of *Perkinsus* spp. were not used as a method of challenging molluscs in this 2 year study, the evidence from TE-4, -6 & -7 vs. TE-19 & -25 as well as from the literature (Mackin, 1962) where injection of suspensions of homogenized, infected oyster tissues was used, led me to the conclusion that uncultured cells should be used. In other words considering the concentrations of cells used, the challenges accomplished in TE-19, -25 and particularly TE-30 should have yielded 100% or nearly 100% infections with intensities ranging from light to heavy in 28 to 56 days after being challenged.

Of greatest interest is the observation that axenically

cultured cells of *P. marinus* can establish themselves and multiply to significant levels in *M. balthica* (TE-33). This occurred when the cells were injected.

When uncultured cells of *Perkinsus* sp. in homogenates of infected *Tagelus plebeius* and *Bankia gouldi* were used to challenge *C. virginica*, no infections were observed (TE-34 & -35). The *T. plebeius* used in TE-34 were obtained from TE-ES-J where 100% of the clams were infected using *P. marinus* from homogenized *C. virginica*. Unfortunately, the number of cells used from *T. plebeius* (TE-34) had to be estimated from a knowledge of the intensities of infection in each of the clams, because when the attempt to count the number of *P. marinus* was made, not enough antibiotics were used to suppress bacterial growth in the FTM. The resulting lack of infections could have been due to the relatively small number of *P. marinus* cells used. Only 10, instead of the standard 25, oysters were used because of the small number of *P. marinus* available.

The lack of more challenge experiments involving use of *Perkinsus* sp. cells from non-oyster molluscs to challenge *C. virginica*, is due to the fact that I was not able to find heavy enough infections in such molluscs to serve as a source of sufficient numbers of cells to warrant conducting an experiment.

A variety of results were obtained in the experiments involving use of *P. marinus* in homogenates of infected *C. virginica* to challenge six other species of molluscs. The *T. plebeius* challenges yielded 0 to 100% infections. This range of results may have been due to the difficulty with keeping the clams alive in the experimental system over 1.5 to 2 months. If they were not pumping water at normal rates this would have influenced the number of infective cells taken into the mantle cavity and thus biased the results. Of course other mechanisms involving the physiological and immunological condition of the clams would also be altered. In TE-ES-J the high level of infections could be related to the higher number of cells used in the challenge. The parasite did multiply in the tissues of the clam, infections rising to moderate and moderate-heavy levels in some individuals.

Although several small clusters of the parasite were observed in challenged *Mya arenaria*, all infections were very light and it is concluded that the cells observed were only transients and had not established viable infections. In *Crepidula plana* and *C. fornicata* the infections were all very light or light except for one individual which was light-moderate. In *Urosalpinx cinerea* where exposures to the parasite presumably occurred directly by feeding on infected tissues of the oysters provided in the experiment, the infections were all very light or light except for two individuals which were marginally light-moderate. Very few small clusters were observed indicating that cells of the parasite were transient inhabitants of the host and had not established viable infections.

Discussion

The primary goal of this study was to determine which molluscs serve as hosts of *Perkinsus marinus* and which are simply carriers. Unfortunately a clear picture did not materialize. Unless one engages in an more intensive, multiyear field sampling program coupled with a more intensive transmission-of-infection effort for each species, then proof of which are carriers and which are significantly parasitized will not be provided. However, I believe that reasonable insights relevant to the species examined have been provided by this effort. I have noted which mollusc species permit multiplication within their tissues as evidenced by formation of clusters of parasite cells and have noted the intensity of the infections. From the field studies conducted in this year's part of the study it appears that *Bankia gouldi and Teredo navalis* serve as hosts for a species of *Perkinsus*, because a significant percentage of two populations (49 and 43%) were infected and the intensities were as high as moderate. The unknown species of *Perkinsus* which infects the ship worms does not appear to be as virulent as *P. marinus* in oysters because heavy infections were not observed; however, the infections appeared to be widespread in the host in that the body wall, digestive gland and gills were infected. I was not able to design an experimental procedure for holding the shipworms for transmission-of-infection experiments under controlled conditions.

In examining the transmission-of-infections experiments it was found that *T. plebeius* served as a host for *P. marinus* (TE-27 & TE-ES-J) but only when challenged with larger numbers of parasite cells and more frequently than was necessary when *C. virginica* was challenged. When fewer numbers of cells were used no infections materialized (TE-ES-D & E). The numbers of cells required to initiate infections appear to be in a narrow range (see TE-ES-D & E vs. TE-27). Experiment TE-20 was not allowed to progress a sufficient period of time to obtain results which could be used to compare with the other *T. plebeius* challenges. It was designed to see if the clams would accumulate large numbers of *P. marinus* cells and before they had a chance to depurate them the plan was to harvest them and use them as a source of cells for challenging *C. virginica*. Obviously the plan did not work. It appears that *T. plebeius* must be exposed to large numbers of infective cells to become infected with *P. marinus*. Whether this occurs under natural conditions is not known. The field samples of *T. plebeius* from both years of this study contained 10% infected individuals however they were never more than lightly infected.

Table I. Summary data concerning incidences of naturally occurring *Perkinsus* sp. or spp. in molluscs from tidal waters of Virginia.

GASTROPODS

Location*	Species	No. examined	Total no.(%) w/*Perkinsus*	Sample Times and Sizes ( ) = No. with *Perkinsus* Spring	Summer	Fall
Wach.	*Busycon canaliculatum*	20	0	–	–	20
"	*Busycon carica*	26	0	26	–	–
York R.	" "	30	0	–	5	25
"	*Cratena kaotuae*	72	0	–	–	72
"	*Crepidula fornicata*	61	2 (3)	–	49 (2)	12
"	*Crepidula plana*	56	0	–	35	21
"	*Eupleura caudata*	45	0	–	45	–
"	*Littorina irrorata*	49	0	–	49	–
Wach.	" "	92	0	38	54	–
"	*Melampus bidentatus*	50	0	–	50	–
York R.	*Mitrella lunata*	28	0	–	–	28
"	*Urosalpinx cinerea*	42	0	–	42	–
Wach.	" "	75	0	–	75	–

BIVALVES

York R.	*Anomia simplex*	13	0	–	13	–
C. K.	*Argopecten irradians*	51	0	–	51	–
York R.	*Bankia gouldi*	110	54 (49)	–	110 (54)	–
"	*Geukensia demissus*	75	0	–	50	25
"	*Mya arenaria*	62	2 (3)	–	62	–
James R.	*Mytilopsis leucophaeata*	56	3 (5)	–	26	24
Wach.	*Petricola pholadiformis*	50	0	–	50	–
"	*Tagelus plebeius*	87	0	–	87 (0)	–
York R.	" "	83	6 (7)	–	83 (6)	–
"	*Teredo navalis*	13	0	–	13 (0)	–
Wach.	" "	53	23 (43)	–	53 (23)	–

*Location: C. K. = Cherrystone Creek
James R. = James River
Wach. = Wachapreague
York R. = York River

Table II. Results from attempts at transmission of infections using cells of *Perkinsus marinus* and *P. atlanticus*.

Code #	Infective Cells	Cell Counts	Origin of Infective Cells	Challenged Mollusc (# challenged, # read, % infected)	Time of exposure (d)
TE-19	Axenic culture of *P. marinus*	8.9×10^6 8.4×10^8 3.4×10^8	*Crassostrea virginica*	*C. virginica* (25,25,0)	56
TE-25	"	1.4×10^8 7.6×10^6 7.8×10^7	"	" (10,10,0)	43
TE-30	"	1.1×10^5 3.3×10^5	"	" (10,9,44)	28
TE-24	Axenic culture of *P. atlanticus*	5.6×10^7 4.0×10^7 1.1×10^8 1.3×10^8	*Macoma balthica*	" (10,7,29)	36
TE-31	"	2.7×10^4 1.2×10^5	"	" (10,10,40)	28
TE-34	meronts/schizonts	ca. 10^5	*Tagelus plebeius*	" (10,8,0)	68
TE-35	"	2.1×10^6 4.9×10^6 7.4×10^5	*Bankia gouldi*	" (25,25,0)	47
TE-20	"	1.1×10^8 2.0×10^8	*C. virginica*	*T. plebeius* (25,21,0)	10
TE-27	"	3.0×10^8 4.0×10^9	"	" (25,9,56)	65
TE-ES-D	"	1.0×10^8	"	" (25,13,0)	46
TE-ES-E	"	2.4×10^8	"	" (25,19,0)	46
TE-ES-J	"	1.7×10^9 1.6×10^9 4.0×10^8	"	" (25,22,100)	46
TE-21	"	1.6×10^8 1.4×10^8	"	*Mya arenaria* (25,24,8)	16
TE-26	"	3.8×10^8 4.0×10^9	"	" (25,10,20)	65
TE-28	"	2.2×10^9 1.7×10^9 1.6×10^9	"	*Crepidula plana* (25,23,13)	49
TE-29	"	2.2×10^9 1.7×10^9 1.6×10^9	"	*C. fornicata* (25,18,11)	49
TE-ES-I	"	5.0×10^8	"	" (25,25,0)	15
TE-ES-F	"	Heavily infected oysters	"	*Urosalpinx cinerea* (25,19,40)	49
TE-ES-G	"	"	"	" (25,15,47)	33
TE-ES-H	"	"	"	" (25,25,24)	48
TE-22	"	1.8×10^9	"	*M. balthica* (25,18,50)	49

Table continued on next page.

Table II - continued.

Code #	Infective Cells	Cell Counts	Origin of Infective Cells	Challenged Mollusc (# challenged, # read, % infected)	Time of exposure (d)
TE-23	Axenic culture of *P. atlanticus*	5.6×10^7 4.0×10^7 1.1×10^8 1.3×10^8	*M. balthica*	*M. balthica* (10,10,30)	36
TE-32	"	5.0×10^4	"	" (10,5,60)	28
TE-33	Axenic culture of *P. marinus*	1.3×10^5	*C. virginica*	" (10,5,40)	28

Table III. Weighted intensities of infections.

Code #	Challenged Mollusc	Donor Mollusc	Weighted Intensities (range; mean) Digestive Gland	Gill	Other	Mantle	All Four Organs
TE-19	*C. virginica*	*C. virginica**	0	0	0	0	0
TE-25	"	" *	0	0	0	0	0
TE-30	"	" *	0-2; 0.44	0-1; 0.11	0-1; 0.11	0-2; 0.44	0-2; 0.28
TE-24	"	*Macoma balthica***	0-0.5; 0.14	0	0	0-0.5; 0.07	0-0.5; 0.05
TE-31	"	" **	0-1; 0.10	0-0.5; 0.05	0	0-0.5; 0.15	0-1; 0.08
TE-34	"	*Tagelus plebeius*	0	0	0	0	0
TE-35	"	*Bankia gouldi*	0	0	0	0	0
TE-20	*T. plebeius*	*C. virginica*	0	0	0	0	0
TE-27	"	"	0-2; 0.61	0-2; 0.40	0-0.5; 0.06	0-1; 0.11	0-2; 0.30
TE-ES-D	"	"	0	0	0	0	0
TE-ES-E	"	"	0	0	0	0	0
TE-ES-J	"	"	0-4; 1.7	0-4; 1.9	0-1; 0.5	0-2; 1.3	0-4; 1.4
TE-21	*Mya arenaria*	"	0	0-0.5; 0.25	0	0	0-0.5; 0.07
TE-26	"	"	0-0.5; 0.05	0-0.5; 0.09	0	0	0-0.5; 0.04
TE-28	*Crepidula plana*	"	0-2; 0.09	0-1; 0.08	0	0	0-2; 0.04
TE-29	*C. fornicata*	"	0-1; 0.11	0	0-2; 0.11	0	0-2; 0.06
TE-ES-I	"	"	0	0	0	0	0
TE-ES-F	*Urosalpinx cinerea*	"	0-2; 0.68	--	0-1; 0.05	0-1; 0.11	0-1; 0.28
TE-ES-G	"	"	0-2; 0.37	--	0-0.5; 0.03	0-1; 0.17	0-2; 0.19
TE-ES-H	"	"	0-2; 0.26	--	0	0-1; 0.02	0-2; 0.09
TE-22	*M. balthica*	"	0-0.5; 0.17	0-0.5; 0.11	0-1; 0.5	0	0-1; 0.20
TE-23	"	*M. balthica***	0-0.5; 0.05	0	0-0.5; 0.15	0-0.5; 0.05	0-0.5; 0.06
TE-32	"	" **	0-2; 1.0	0-0.5; 0.1	0	0-1; 0.2	0-2; 0.32
TE-33	"	*C. virginica**	0-5; 1.4	0-3; 1.0	0-2; 0.4	0-4; 1.0	0-5; 0.95

*Cells from axenic culture of *P. marinus*.

**Cells from axenic culture of *P. atlanticus*.