REPORT ON STUDIES OF OYSTER PARASITES IN THE ARANSAS BAY AND GALVESTON BAY AREAS OF TEXAS

(An annual progress report on an inter-agency contract study for the period September 1962 through August 1963 between Texas A&M College and the Texas Game and Fish Commission)

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The first collection of oysters for this study was taken off Fulton Beach, Aransas Bay on January 1, 1962. Subsequently, 34 collections were made, most of them in Aransas Bay, but several collections were made in Mesquite, Tres Palacios, Corpus Christi, and Galveston Bays. These oysters were sectioned and slides made, a total of more than 1300. All collections were of live oysters, and the reading of the slides was a slow process. Live oysters were used because the platforms which were to hold trays of oysters were delayed in construction. Hence, no data on mortality are available. The period from January 1, 1962, to June 26, 1962, was used to determine roughly from the slides what parasites and diseases were present and where they were located. The data on collections are listed in Table 1.

Some of these collections were ruined by holding out of water at the Rockport station before being fixed for shipment to College Station. The time of holding out of water varied from one to twelve days, and certain collections (see Table 1) were dead before fixation and were useless for study. Others which were held out of water for one to several days but which did not actually die were very difficult to interpret because such treatment causes changes in the histology which closely resembles disease caused by some types of parasites, and also causes invasion of tissues by saprophytic bacteria, which normally do not invade oyster tissues. Because of these factors, some of the data appeared to be questionable and are not reported until such time as normal checks may be made.

One complication arose when oysters were shipped to College Station for studies in aquaria. Oysters thus placed in aquaria died rapidly within a few days of being placed in aquaria. Oysters brought all the way from Louisiana as controls failed to die in this rapid manner. This phenomenon is discussed in the section on bacterial diseases, as is the appearance of the flagellate parasite Hexamita. Mr. Frank Schlicht wrote his master's thesis on the Hexamita studies, and a copy of his thesis will be provided as soon as possible. Hexamita is discussed only briefly in this report.

Bacterial Diseases

1. Occurrence of bacteria in aquarium oysters.

Several lots of oysters were brought to College Station alive where they were studied in closed aerated aquaria. In every case it was found that the oysters died very rapidly, beginning in three days to a week. These oysters were studied carefully. A large number of slides were made, and fresh smears of stomach content of dead oysters were made in most cases in an effort to determine what was the nature of the organism responsible for these deaths.

A sample of Louisiana oysters was brought to College Station and kept in the closed aquaria to determine whether or not the deaths were due to equipment or handling, or to something peculiar to Texas oysters. The Louisiana oysters failed to die in the rapid manner shown by Texas oysters. They lasted for a considerable period, finally dying of Dermocystidium disease after an epidemic of this fungus got started in the aquaria. This is what is expected of Louisiana oysters and parallels the sequence of events which we had learned to expect in past experimentation at Grand Isle.

Both Galveston Bay oysters and Aransas Bay oysters showed the tendency to early deaths in aquaria. Time out of water had no effect on the quick mortalities; the Louisiana oysters were out of water for about four days, more than twice the period of the Texas oysters, and showed no ill effect. Rough handling also was ruled out as a factor.

It was quickly established that Hexamita, a flagellate protozoan parasitic in oysters, appeared in the oysters during the mortalities. However, a careful study by Mr. Frank Schlicht showed that the mortalities began prior to appearance of the flagellates and that many subsequent deaths failed to show Hexamita. Thus there was a general correlation of the appearance of Hexamita and deaths of oysters, but on close examination of individual oyster deaths the correlation appeared to be incomplete. It was tentatively decided that Hexamita could have been a contributing cause of death but probably was not a primary cause. However, study of the Hexamita problem in Puget Sound, Washington, in the summer of 1963 indicates that Hexamita may invade live oysters, and further studies on this protozoan in Texas oysters is needed.

Sections of oysters stained to show bacteria indicate that the deaths were probably due to either one of two bacteria. One of these was a short bacillus resembling a Pseudomonas. Sections showed large numbers of these in leucocytes and other tissue cells and concentrated just under the epithelium of the mantle and body wall. The fact that they were intracellular indicates that they were phagocytized by amoebocytes while the oysters were still alive. In many cases oysters which had gaped were still alive, as shown by the strong ciliary action, sensitive mantle, or heartbeat. These sometimes had heavy bacterial loads. It is tentatively

believed that this bacterium thus associated with the deaths may have accounted for some of the deaths, if not all. This hypothesis will have to be tested further. Several investigators have reported killing oysters with a Pseudomonas or similar bacillus, but these reports (to meetings) were not followed up with publications. A. K. Sparks of the University of Washington made such a report as did Dr. Leslie Stauber of Rutgers University. Another bacterium probably tied in with the aquarium mortalities seems to be one of the slime bacteria, close to Chondrococcus which parasitizes fishes. This is only a tentative identification. These organisms, if correctly identified, may be widespread in oysters. It should be noted that these do not seem to be the same as mycelial disease. It was originally believed that they were the same organism, but these studies indicate that this is not true (see section on mycelial disease).

No attempt to culture the slime bacteria was made, though this should be relatively easy if they are indeed Chondrococcus. Most slime bacteria culture readily on cellulose fibres (cotton, cigarette paper) laid on the surface of neutral agar plates.

This bacterium showed up not only in the aquarium gapers, but were apparently present in tissues of occasional live oysters from the Port Aransas area. It is very difficult to recognize, since it is usually in the coccus form and stains and looks like a small solid nucleus. However, in some dead oysters, undoubted Cytophaga or Chondrococcus were frequently observed which were in filamentous form. However, oysters with this form were usually in advanced stages of disintegration, and the coccus form and filament form may not be the same organism.

It is obvious that thisstudy should be followed up. Attempts to isolate the bacteria and controlled experimentation to check on their effect when applied to oysters in aquaria should be made. It is hoped to set up some studies of this nature next year.

TABLE 1.

Live oysters collected and sent to College Station for studies of disease in order of dates processed

Collection No. and Date	No. Oysters	Reef	Bay	Remarks
1-29-62 + 2-60	30	off Fulton Beach	Aransas	no D. marinum data
2-21-62 + 2-20	10	Pintail	Aransas	*
2-21-62 +22-40	10	Halfmoon	Aransas	*
2-21-62 +42-60	10	Long Reef	Aransas	*
3- 5-62 + 2-20	10	Lap Reef	Copano	*
3- 5-62 +22-40	10	Bray's Cove	Mesquite	*
3- 6-62 + 2-12	6	Hanna's	Galveston	no thio record
3- 6-62 +14-24	6	Todd's Dump	Galveston	no thio record
3- 6-62 +26-36	6	Humble Well	Galveston	taken from boat; no thio record
3-19-62 + 2-20	10	Pintail	Aransas	*
3-23-62 + 2-20	10	Bray's Cove	Mesquite	*
3-23-62 +22-40	10	Halfmoon	Aransas	*
3-23-62 +42-60	10	Long Reef	Aransas	*
3-23-62 +42-78	9	Lap Reef	Copano	*
4-11-62 + 2-20	10	Middle Ground	Tres Palacios	
4-11-62 +22-40	10	Oliver's	Tres Palacios	
4-20-62 + 2-20	10	Bray's Cove	Mesquite	*
4-20-62 +22-40	10	Pintail	Aransas	*
4-20-62 +42-50	5	Long Reef	Aransas	*
4-20-62 + 1-19	10	Lap Reef	Copano	* Died while holding;
4-20-62 +21-39	10	Halfmoon	Aransas	* these 2 collections were rotten; not sectioned
5-11-62 + 1-19	10	Bray's Cove	Mesquite	* Died while holding
5-11-62 +21-39	10	Long Reef	Aransas	* Died while holding
5-11-62 +41-59	10	Pintail Reef	Aransas	* Died while holding
5-11-62 + 2-20	10	Halfmoon	Aransas	*
5-11-62 +22-40	10	Lap Reef	Copano	*
6-20-62 + 2-20	10	Alta Vista	Corpus Christi	
6-25-62 + 2-20	10	Pintail	Aransas	*
6-25-62 +22-40	10	Bray's Cove	Aransas	*
6-25-62 +42-60	10	Halfmoon	Aransas	*
6-25-62 +62-80	10	Lap Reef	Copano	*
6-25-62 +82-100	10	Long Reef	Aransas	*
6-26-62 + 2-20	10	Hanna's Reef	Galveston	*
6-26-62 +22-40	10	Todd's Dump	Galveston	*
	Total 342			

*with D. marinum data

Mycelial Disease

Mycelial disease appeared in Aransas Bay at Fulton Beach and Halfmoon Reef, in Copano Bay at Lap Reef, in Corpus Christi Bay at Alta Vista Reef, and in Tres Palacios Bay at Middle Ground Reef. It is obvious that it is widespread, and probably occurs nearly everywhere. But these studies showed that infection incidence was not high except at Fulton Beach and Lap Reef. Where it was in high incidence most infections were light in individual oysters. It is possible that this is due to the fact that we were sectioning live oysters presumably healthy, and that, if gapers had been available, the concentration might have been heavier. As it stands the infections observed did not appear to have been comparable to those studied in Louisiana and elsewhere.

Placement of these peculiar bacteria has been a puzzling problem. It was thought early in these studies that the problem had been solved when a paper was found which identified similar organisms as slime bacteria. A careful study of the paper, however, indicates that the author was guessing at the placement. He presented no data to actually connect the organisms with any of the known slime bacteria.

Certain structural peculiarities now indicate that the "mycelia" of oysters may not be slime bacteria. Specifically, the lack of a gelatinous sheath and the presence of a thin hard cuticle indicate that they may not be slime bacteria. Doubt has been cast also on part of the branching of the filaments. It appears that separate individuals attach to each other with a flattened end, giving an impression of branching, when in fact these probably are masses of interlocked individuals. However, true branching does occur.

Only one group of the bacteria has characters similar to this. This is the group of the Caulobacteria, a little known aberrant order, found in lakes, ponds, and streams. The group was set up by Henrici and Johnson in 1939. Most species attach to some kind of substrate by means of waxy stalks. These may often be dissolved with treatment with alcohols which may account for their apparent absence in some preparations of oysters.

At least two species of this peculiar group are parasitic. Pasteuria ramosa parasitizes Daphnia pulex and D. magna, living in the body cavity (haemocoele). Vibriothrix tonsillaris is found in crypts of the human tonsils where they form greyish or yellowish granules. This one is often mistaken for an Actinomycete. Judging from figures of species of the genus Caulobacter, the oyster parasites probably will be found to belong here.

Some species of the Caulobacteriales can be cultured, but some others have not been cultured. A common collecting method (for free-living species) is to set out blank glass slides in racks in the water. Species of various genera of the order attach to these plates and may be stained

in situ on the slides. An attempt to determine whether or not the oyster species is saprophytic as well as parasitic will be made using this method.

TABLE 2

MYCELIAL DISEASE

Positive Samplings:

1-29-62 (1). Fulton Beach - Aransas Bay - 81.5% infected

4-11-62 (2). Middle Ground Reef - Tres Palacios Bay - 20.0% infected

5-11-62 (3). Halfmoon Reef - Aransas Bay - 50% infected

5-11-62 (4). Lap Reef - Copano Bay - 80% infected

6-20-62 (5). Alta Vista Reef - Corpus Christi Bay - 20% infected

Dermocystidium marinum

The records show that Dermocystidium marinum is in heavy incidence in every area in which checks were made. The data are condensed in Table 3. In this table the data for each station are in chronological order. Collections were made at most of the stations beginning in the winter or early spring and continuing up to the latter part of June. With the exception of Bray's Cove in Mesquite Bay each of the stations shows a comparatively low incidence up to the beginning of the summer when the data ended. This is the usual picture. It seems certain that D. marinum is a factor in mortality in summer since the weighted incidence would be expected to continue to increase up to August or September.

Data are too meager for the Galveston Bay area to perceive any trend in D. marinum development. Those June records available appear to indicate a little less D. marinum in Galveston Bay than is in Aransas Bay, but again the data are too skimpy to be certain about this.

Bray's Cove station oysters had a very heavy infection as far back as March and continued high to June. It seems evident from the data that all oysters from this station must be almost continually infected. It must be kept in mind, however, when assessing the data on D. marinum in this study, that the oysters collected in the Rockport area were often held out of water for considerable periods before fixation and culturing for D. marinum. The author has shown (Publications of Institute of Marine Science, Vol. 7) that holding infected oysters out of water greatly accelerates the intensity of infection. This factor may have introduced considerable error in the data and must be taken into account.

TABLE 3

Dermocystidium marinum

Incidence and Weighted Incidence

Date	Reef	Bay	No. Oysters Checked	Incidence (%)	Weighted Incidence
1-29-62	Off Fulton Beach	Aransas	not checked	---	---
2-21-62	Pintail	"	5	20	0.20
3-19-62	"	"	10	0	---
4-20-62	"	"	10	60	0.70
5-11-62	"	"	10	40	0.40
6-25-62	"	"	10	70	1.10
2-21-62	Halfmoon	"	5	40	0.60
3-23-62	"	"	10	50	0.50
4-20-62	"	"	10	40	0.70
5-11-62	"	"	10	100	1.70
6-25-62	"	"	10	100	2.10
2-21-62	Long Reef	"	5	60	1.00
3-23-62	"	"	10	100	1.50
4-20-62	"	"	10	100	2.10
5-11-62	"	"	10	100	2.00
6-25-62	"	"	10	100	2.40
3- 5-62	Lap Reef	Copano	10	90	1.50
3-23-62	"	"	9	55	0.55
4-20-62	"	"	10	50	0.80
5-11-62	"	"	10	90	1.60
6-25-62	"	"	10	70	1.20
3- 5-62	Bray's Cove	Mesquite	10	100	2.20
3-23-62	"	"	10	100	1.30
4-20-62	"	"	10	90	1.60
5-11-62	"	"	10	100	1.80
6-25-62	"	"	10	100	2.10
3- 6-62	Hanna's	Galveston	6	no record	
6-26-62	"	"	10	60	0.70
3- 6-62	Todd's Dump	"	6	no record	
6-26-62	"	"	10	80	1.30
3- 6-62	Humble Well	"	6	no record	
4-11-63	Middle Ground	Tres Palacios	10	no record	
4-11-62	Oliver Reef	"	10	no record	
6-20-62	Alta Vista	Corpus Christi	10	no record	

Larval Cestodes

Larval cestodes have only recently been reported for oysters. They probably occur sparingly in most producing areas. Several slides from the present series showed the presence of these interesting parasites, but the number was insufficient to make any kind of critical study. No more than one cyst has been found in any one oyster. In every case the worms are encased in a thick fibrous capsule laid down by the host. This isolation process appears to be unique: Oysters normally react to foreign bodies by heavy concentrations of leucocytic cells which do not produce a cyst. In some cases (as for bacteria) the leucocytes isolate by forming an epithelial wall (as in pearl formation).

The group to which the cestodes belong could not be determined from the few slides showing the parasite. However, Burton (1963) reported on a cestode larva in oysters, which is probably the same species, which he called Tylocephalum.

Bucephalus

Bucephalus was observed to occur in light concentration at 3 stations. The following Table 4 presents the data:

TABLE 4

Records of Bucephalus in Oysters

Date	Reef	Bay	Incidence %
1-29-62	Fulton Beach	Aransas	3.7
3- 6-62	Todd's Dump	Galveston	10.0
4-11-62	Middle Ground	Tres Palacios	10.0

When incidence is as low as in these oysters, it probably means that most reefs have Bucephalus, but that the samples were too small to determine whether or not the trematodes were generally present, or, as indicated, occur in limited areas.

One infection observed here showed cercariae leaving the host. They were present in large concentrations throughout the host tissue which was a shambles of mechanical destruction. It is believed that when the worms leave the host the latter dies. However, the period of leaving is usually late, and most oysters are harvested prior to that time.

All hosts carrying Bucephalus are castrated by the worms. This is not a matter of concern in the Gulf States where spatfall is too heavy generally.

Nematopsis sp.

Approximately all oysters were infected with at least a few spores of Nematopsis. Some few were heavily infected, the infections being considerably heavier than those observed under natural conditions in Louisiana or elsewhere. From the location of the infections (mostly gill and around the gut) it is believed that the common species is Nematopsis prytherchi Sprague, but it is possible that N. ostrearum Prytherch also occurs.

Since all studies of the effect of Nematopsis on oysters have used N. ostrearum and no one has tested N. prytherchi, it may be that it would be profitable to test N. prytherchi experimentally. It is not anticipated that it will be found that this species is lethal to oysters, but there may be other effects of a physiological nature.

Sex Ratios of the Oysters Studied

It was noted early in the study that males were very scarce in the oyster collections. The very first group studied was 70.3 per cent female and 3.7 per cent male with 26 per cent undetermined. Something near this ratio was continued at other stations except the Alta Vista station in Corpus Christi Bay where the one collection of 10 oysters was nearly evenly divided.

It is possible that the wide variance in number of males and females could be due to conscious selection of large oysters. On the assumption that oysters are first male then in their second season reverse to become females, one would expect a discrepancy in size of males and females. If this is the explanation, one would expect the males of the collections to be smaller than the females. The data on sizes in Table 5 does not bear out this assumption, but this is not necessarily a valid test. It would be interesting to sample a population and to check the sex of all sizes of oysters and determine relative sizes.

This point of interest is mentioned because mycelial disease is selective of males, i.e., the data show that vastly more males than females die in an epidemic of the disease. This suggests that mycelial disease may attack young oysters more than older oysters, a theory which has never been checked. In fact reflection indicates that little has been done in the way of studying early spat for disease. Loosanoff and Davis described a fungus disease of spat in aquaria but failed to make an effort to project their studies to the field. (*)

(*) Since this was written, a new bacterial disease of spat has been described.

TABLE 5
SEXES OF OYSTERS SECTIONED

Date	Reef	Bay	♂	♀	Undetermined or Asexual	Mean Size, mm. ♀	♂
1-29-62	Fulton Beach	Aransas	1	19	7	-	-
2-21-62	Pintail	"	2	8	0	62	
3-16-62	"	"	1	9	0	72	68
4-20-62	"	"	0	10	0	73	-
6-25-62	"	"	1	9	0		
2-21-62	Halfmoon	"	2	7	1	69	59
3-21-62	"	"	1	9	0	73	82
5-11-62	"	"	3	6	1	83	76
6-26-62	"	"	1	9		-	-
2-21-62	Long	"	1	9	0	67	-
3-21-62	"	"	0	10	0	83	-
4-20-62	"	"	1	6	3	-	-
3- 5-62	Lap	Copano	1	9	0	90	89
3-21-62	"	"	1	7	2	73	73
5-11-62	"	"	2	8	0	65	73
6-25-62	"	"	0	10	0		
3- 5-62	Bray's Cove	Mesquite	1	9	0	81	84
3-21-62	"	"	3	7	0	78	73
4-20-62	"	"	1	9	0	64	62
6-25-62	"	"	2	8	0		
3- 6-62	Hanna's	Galveston	1	4	1	-	-
6-26-62	"	"	3	6	1	-	-
3- 6-62	Todd's Dump	"	0	5	1	-	-
6-26-62	"	"	0	10	0	-	-
3- 6-62	Humble Well	"	0	5	1	-	-
4-11-62	Middleground	Tres Palacios	1	8	1	-	-
4-11-62	Oliver's Reef	"	3	7	0	-	-

Summary

The first six month's study indicated that D. marinum is the primary parasite in Texas oysters and that mycelial disease is present generally but not in great concentration. Two possible new bacterial diseases were observed but need much more study before they can be assessed since so far they have been observed in aquarium oysters only. Nematopsis often produces heavy infections in Texas oysters, and miscellaneous other interesting but unimportant parasites were recorded.