Second Annual

OYSTER MORTALITY CONFERENCE

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In cooperation with:
Bureau of Commercial Fisheries
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Summary of Conference
by

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1. Participants
2. Outline of Discussion Topics
3. Summary of Discussions

1. Studies by the Virginia Fisheries Laboratory:

A. Field and Laboratory report by J. D. Andrews.

Early last year Virginia Fisheries Laboratory instituted a general monitoring program on mortalities on the Virginia seaside and in the Chesapeake, employing tray techniques in use at Gloucester Point since about 1950.

Seaside: Trays here showed appreciable mortality only between mid-May and mid-June when the kills ranged from 10 to 20%. This may fit in with Sieling's report of May, 1958 mortalities in the Chincoteague. There has been no significant mortality since mid-June. It was again emphasized that Dermocystidium has not been important on the Eastern Shore. Various oystermen on the Eastern Shore report that the 1959 set was good, market oysters are scarce, and the prices are high. There were no complaints of mortalities in contrast to the sporadic kills in 1958.

Eastern Bayside: Trays here were all of James River stock, 2-3 years old. No appreciable mortalities occurred until mid-August, and this mortality fits with the incidence of Dermocystidium along the shore and in the creeks.

Western Bayside: Trays were set up here in May and June to cover the salinity ranges in the various areas. Locations included Wreck Shoal, Hampton Bar, York River, Rappahanock and Gloucester Point. The results are complicated by the fact that 1959 was a very bad Dermocystidium year. The death rate at Gloucester Point was the highest since observations started here in 1951. In 1954 Dermocystidium killed 50%, and the 1959 kill is higher. Approximately 90% of the summer gapers showed heavy to moderate Dermocystidium infection.

In this Western Bayside area, older oysters on the grounds apparently suffered an end-of-winter and possibly a May-June mortality. For example, 15-20 of Ballard's grounds surveyed in late February, 1959 showed no signs of mortality. These same grounds checked on mid-August had 20-45% losses estimated to be 3-4 months old. Miles brought samples from Mobjack Bay also showing a high mortality, but claimed there had been no spring mortality on his grounds. The Dermocystidium season, based on positive gapers, in lower Chesapeake is from 1 July to 1 November. Apparently Chesapeake deepwater beds suffered a 60-65% loss in 1959 of which about 1/3 to 1/2 occurred in spring and is unexplained. There is also some uneasiness about the summer losses which are greater than may be accounted for by Dermocystidium.

Dr. Andrews also reported briefly on a technique he has been using for shell growth measurements. This is based on underwater weights of oysters which is essentially a shell weight. At Gloucester Point healthy oysters grow from spring until fall. Those infected with Dermocystidium show no increase in weight. In 1959 half of the oysters under observation showed no shell production, and when checked for Dermocystidium had very little. This implies some other cause of "sickness" for these oysters.

B. Laboratory Studies reported by John L. Wood.

These studies got underway in the second half of 1959. Over 1800 oysters, some gapers and some alive, have been processed; and approximately 800 are on slides. Lantern slides were shown of a variety of the slides. An organism called "HBO" (just found in material from Hampton Bar in mid-September) has subsequently been found in several locations. Its distribution in gaper and live oyster samples was shown by the following table:

	LIVE OYSTERS	HBO	% HBO	GAPERS	HBO	& HBO
Moljack Bay	132	23	17.4			
York River	116	8	6.9	143	11	7.7
James River	82	3	3.7	37	1	2.7
Rappahanock	25	13				
Seaside Va.	48	4	8.3	42	7	16.6
Seaside Del.	—	—	—	12	4	33.3

Following a study of slides of both "HBO" and "MSX", Dr. Wood agreed that these are probably the same organism.

2. Additional Studies on Virginia Seaside:

A. Bureau of Commercial Fisheries reported by J. B. Engle.

The Franklin City Laboratory, under direction of Tom Carver, established 5 tray locations in the Lower Chincoteague area early in 1959. These are at Purnell Point, Guys Point, Cockle Point, Watts Bay and Toms Cove. At each location, oysters taken locally from plant grounds were compared, in trays, with "control" oysters brought in from the James River. By early January, 1960 the natives had total mortalities ranging from 16 to 44% while the "control" ranged from 3 to 10%. Most of the mortality in the natives occurred in May and June.

Also reported was a 1957 oyster kill in the Chincoteague of 57-75% based on one oysterman's records of his box counts. This is an addition to the record of Chincoteague mortalities reported last year.

3. Studies by the Chesapeake Biological Laboratory:

A. The Maryland Chesapeake reported by Francis Beaven.

The Maryland-Virginia boundary approximates the 15% salinity line in Chesapeake Bay which means that, in contrast to Virginia, Maryland lacks many oyster predators and parasites. Over the last year, the CBL has made an extensive box survey on 200 bars in tributaries and in bays, and this was repeated in October. The only unusual mortality was in Holland Straits. It was recalled that this area received a "shot" of 02-poor water in 1957; and this may have occurred again in 1959, causing increased oyster mortality. To provide a "base line" for future work, oysters from each bar were fixed for histological study.

Trays have also been set up and stocked with seed oysters from Fishing Bay:

Solomons - March 13, 1959-January 14, 1960	3.4% mortality
Holland Straits - September 3, 1959-January 11, 1960	2.5% mortality

Local oysters in Holland Straits showed an 8% kill in the September-January period. This group also had a Dermocystidium weighted incidence of 1.04.

Some of the changes in operation of the Maryland industry over the years were noted. The planters are now getting seed by shelling grounds in southern Virginia waters. Years ago they were getting seed from the Virginia Seaside and some from Delaware Bay. When the Chesapeake corporation was planting in trays, Long Island seed stock was used for several years.

B. Chincoteague Bay Studies reported by Frad Sieling.

Four tray stations were established March, 1959 in the Maryland Chincoteague at Rattlesnake Landing, Whales Gizzard, Sinepuxent Bay and Grays Creek. These parallel the trays set up to the South by the Franklin City Laboratory. At each location oysters from local grounds planted at least nine months, "natives," were placed in trays to compare with "control" stocks brought in from Fishing Bay. Samples were fixed every 2 weeks at all locations. Total mortalities from March to January, 1960 range from 16-37% for the "natives," and from 3-6% for the "controls." The major losses again occurred in May and June, and there have been virtually no losses since mid-October. It was noted that losses, in general, over the 1959 year were light.

It is planned to continue these trays through another season.

4. Laboratory Advances at Rutgers:

A. Preparation for eveing demonstrations - L. A. Stauber

In the absence of John Myrhe of the Bivalve Laboratory staff, Dr. Stauber reviewed Myrhe's work on preparation of pericadial smears with the Schaudinn technique. By this method, a permanent stained slide of leukocytes and other cellular materials in the pericardial fluid is obtained in approximately three hours. Demonstrations of clearly differentiated "msx," and various types of leukocytes were presented. Dr. Stauber suggested that the Schaudinn technique might be combined with a tissue biopsy technique for the detection of "msx" infection in tissues rather than just in pericardial fluid.

B. Evaluation of the Fresh Smear Technique - W. J. Canzonier

In the fall of 1958, we began the examination of pericardial fluids of oysters to diagnose infection with "msx." Pericardial fluid is stained on a slide with methylene blue (50 mg. per 100 ml. oz. dist.). The results are variable and, with decomposing gapers, the staining is frequently not precise; leading to doubtful diagnoses classified as "possible" and "probable". With tray gapers subsequently fixed, sectioned, and studied from the August-September, 1959 mortalities, an evaluation of the reliability of the fresh smear examination has been attempted. Infections are classified in permanent slide sections as follows:

Heavy	- average more than 5 organisms	per oil immersion field.
Moderate	- 1 - 5 organism	per oil immersion field.
Light	- less than 1 organisms	per oil immersion field.
Rare	- 1 or more per section, but less than 1	per oil immersion field.
Absent	-	

Comparison of fresh-smear examination with permanent slide:

		FRESH-PERICARDIAL SMEAR			
		NEGATIVE	POSSIBLE	PROBABLE	DEFINITE
SLIDE COUNT	Absent	20	0	0	0
	Light	14	0	1	0
	Moderate	1	4	0	6
	Heavy	(1)	3	3	10

This comparison shows quite clearly that the fresh, pericardial smear examination misses the light infections, but is quite reliable for the moderate and heavy infections. In no case has a positive fresh diagnosis been proved wrong by fixed material.

C. Leukocyte Activity to low temperatures - John Dupuy.

The work of Dr. Stein and Dr. Mackin reported at the NSA meeting in Washington, July, 1959 clearly shows the development of heavy Hexamita infections in oysters held experimentally at low temperature. Mackin has rearlier reported the presence of intracellular stages of Hexamita in oyster leukocytes. At the suggestion of Dr. L. A. Stauber, these experiments were designed to test the activity of oyster leukocytes at 5°C. Tripp, (1958) working in this laboratory, has shown that avian corpuscles injected intracardially at room temperature are engulfed by leukocytes, and are digested and eliminated from the oyster within 12-16 days. In the experiments reported here, 2 groups of 12 oysters were injected intracardially with avian corpuscles and held at 5°C in sea water of salinity 12. Four oysters from each were examined at 14, 21, and 28 days. Fresh smears were made from various parts of the oyster. Blood smears throughout the experiment showed almost all the leukocytes engorged with avian corpuscles. This was verified in Giemsa preparations with no appreciable change in size and shape of the avial cells. Hexamita were also observed in some of the blood smears. Other fresh smears revealed ciliates and Hexamita in stomach contents; and Hexamita, ciliates and occasionally leukocytes engorged with avian corpuscles in the gill minces. These observations are interpreted to mean that although the leukocytes can engolf the inert avian corpuscles at 5°C, they cannot digest them normally nor effectively transport them from the circulation. This suggests as an alternate interpretation of Mackin's observations of intracellular stages of Hexamita, that at low temperatures the leukocytes have engulfed Hexamita, but have been unable to digest them completely.

Another interesting observation by Dupuy is that oysters kept in air storage at 5°C did not show Hexamita infection within three months, in contrast to those held in sea water.

D. Inoculation studies reported by Sung Yen Feng.

A Pseudomonas-like bacterium, A-3, originally isolated from "gapers" on heavy-kill grounds in Delaware Bay in September, 1958, was injected intracardially into apparently healthy oysters to study the course of infection. Times required to destroy 50% of A-3 in homogenate samples during the early phase of the experiment was 2½, 20 and 204 hours at 23°, 15.7° and 9.4°C respectively. At 23°C, the initial rapid loss of A-3 was followed by a rise both in blood and homogenate samples. Three such cycles occurred in a 20-day period. At 15.7°, A-3 reached its lowest point on the 4th day, and then rose rapidly to nearly the original level by the 8th day. This level was then maintained for the duration of the experiment. At 9.4° the numbers of A-3 declined steadily, though slowly, throughout the experiment. Mycobacterium smegmatis also injected intracardially was removed rapidly from the blood and did not subsequently rise in level. In tissues the level remained high for 6 days and then dropped gradually to extinction in the next 6 days.

Response of oysters to injection of various soluble substances, especially protein solutions, is also under investigation. The fates of injected diphtheria toxoid, bovine hemoglobin, human albumin and rhodamine-conjugated human gamma globulin were followed colorimetrically, by serological techniques, and fluorescence microscopy. These substances have been detected within oyster leukocytes minutes after intracardial injection. It is assumed that the method of ingestion (pinocytosis) of soluble, high-molecular substances by oyster leukocytes is similar to that observed by Lewis (1931) and Mast and Doyle (1934) for macrophages and Amoeba.

E. Transmission Studies - Rudolf Scheltema

Several attempts have been made to transfer "msx" to healthy oysters. In the most recent attempt, not yet completed, but reported here, uninfected Wreck Shoal oysters (James River) were placed in aquaria with oysters taken from stocks dying rapidly in Cape Shore Trays. The tanks were set up in triplicate sets with adequate uninfected controls and all were held at laboratory temperature approximately 25°C. All inflowing water was passed through sand filters that removed about 90% of the bacteria, and presumably any infective spores. As expected, the infected stocks died most rapidly in the early stages of the experiment followed by mortalities in the oysters mixed with them. After 60 days or so, however, the control mortalities increased and overtook those of the experimental stocks. Histological work on the gapers is yet to be completed. Thioglycollate cultures of gapers in the latter half of the experiment show that practically all tanks were heavily infected with Dermocystidium, and little optimism is held for a definitive result on "msx" transmissions.

5. Field and Tray Studies in Delaware Bay - H. H. Haskin:

A. The planted grounds.

There was no industry planting in 1959, but the 1958 plants which had suffered a total mortality of 52% by the end of 1958 were kept under surveillance. Six to eight grounds were sampled monthly for recent boxes, old boxes and living oysters. By the end of March, total mortality reached 67%, and by late fall averaged 80%. These are minimal figures because of errors caused by break-up of boxes. Over half of the total mortality from June to October, 1959 was drill kill. The surviving oysters grew well and have excellent meats. The yield, however, on grounds dredged for market since last October has been 1 bushel of market oysters per 10 bushels of seed planted.

One ground planted experimentally with a single deck-load of seed oysters from Arnold's Bed in October, 1958 is of particular interest as a unique planting. The seed were moved from an area lightly touched by the epidemic kill of 1958 to planted grounds while oysters were still dying just after the peak kill. By December, 1959 approximately 65-70% of this planting haddied with a small peak in kill in April-May, and a sustained summer peak between June 8 and September 23.

B. The Seed Beds.

The epidemic kill first hit the Seed Beds in fall of 1958, and by June, 1959 had killed 60-70% of the oysters except on Arnold's Bed which showed a total mortality of 20%--based again on box counts. There was no indication of significant fall kill here in 1959. A few new boxes in the December sampling indicated recent kill of 2 to 5%. The 1958 set (yearlings) are living well to date. We are especially interested in following in detail the events that befall this 1958 set and the set of subsequent years. In these we may find our first evidence of development of resistant stocks to the pathogen.

C. Experimental Tray Studies.

Survivors of the trays set up on the Cape Shore Tidal flats in June, 1958 were carried through this past year, together with a variety of additional stocks of oysters from Delaware Bay and other coastal areas. A total of about 15,000 oysters were under close study. Objectives were:

1) to determine if the epizootic pathogen were still present.
2) to find evidences of resistant stocks or strains of oysters.
3) to check patterns of kill by the pathogen.

The model epizootic in tray stocks followed almost exactly the 1958 pattern, except that the onset of peak kill was about 2 weeks earlier, mid-August rather then early September. The tabulation below indicates the relative susceptibility of the various major stocks under study over the period of peak kill and through the fall. The pattern of kill may be summarized for both 1958 and 1959 as follows: Stocks introduced into the lower Bay from March to June show low (less than 3%) monthly mortalities through the summer until the late summer peak kills when the monthly rate climbs to 25-50%. Stocks introduced in late summer and fall show a spring kill, followed by a lower summer rate, though consistently higher (10-20% monthly) than that for stocks introduced in spring. Stocks previously exposed in the lower Bay that have passed through one or more years of heavy mortality show comparatively a uniform death rate throughout the year, with summer monthly rates from 3-10% and spring and fall peaks grouped around 10-18%.

SUMMARY OF TRAY KILLS

# of Trays	Stock	6 Aug.-15 Nov.	27 May-15 Nov.
7	Delaware Bay Natives Survivors of 1958 kills	12-20%	28-35%
3	Long Island Stocks	31-43%	
7	Virginia Seasides	39-41%	
3	Delaware Bay-Arnold's	42-46%(64*)	44%
3	James Rivers-(23 April)	55-59%	66%
1	James Rivers-(15 June)	47%	
3	Mid-Chesapeakes	67-83%*	
3	Navesink Rivers	68-78%	77%

*The mid-Chesapeakes from Holland Straits apparently brought a concentration of Dermocystidium to our trays. The Arnold's tray with the aberrant 64% mortality was alongside the Holland Straits stock. In mid-August a sample of gapers cultured for Dermo indicated about 5% Dermo-infected. By early September this had risen to 10%, and by early November to 30%.

6. Field Studies - Mullica River and Great Bay by James B. Durand:

A. The 1959 patterns of mortality in this small estuary were strikingly similar to those reported for Delaware Bay. Native oysters were suspended in baskets at three locations from July 10 to October 8. Dredge samples at 6 stations were taken periodically from April 11 to October 8. In both types of study the oyster mortality increased oceanward. At French Point (upriver) basket mortality was 17%; at Turtle Island (river mouth) it was 37%; and at Cape Horn (down-bay) it was 46%. Comparable cumulative mortalities (April-October) in dredge samples were 2.6%, 50% and 54% respectively. The highest mortality was in a Sanctuary population near Turtle Island. This population had been introduced from the Navesink in September, 1958. Comparing the detailed mortality figures with those in Delaware Bay, the relatively high consistent mortality throughout the spring and summer in the Mullica indicates that all stocks here had been exposed previously to the pathogen.

7. Comments on Leukocytes by T. C. Nelson:

A. Observations were reported of the activity of leukocytes in possible removal of MSX from infected oysters and their passage to uninfected oysters. Great numbers of leukocytes are voided in the feces, and extended in mucus eliminated from the gills, and blown out with rejected mud and sand. Since oyster leukocytes can live at least 2 weeks outside the body in sea water, here is a possible means through which this oyster disease may be spread from infected to non-infected oysters.

8. Future Objectives - All Laboratories Represented:

A. Virginia Fisheries Laboratory.

The program underway will be continued and probably extended. With the knowledge that "msx" is in the lower Chesapeake, it will now be necessary to assess its role in mortalities and to determine whether it is spreading or maintaining its position. Histological studies will be continued in connection with the field program in a search for pathogens and their life cycle stages. Dr. Hargis spoke briefly of a general expansion of staff and programs at V. F. L. in the basic marine science.

B. Chesapeake Biological Laboratory.

The Maryland Laboratory will continue its monitoring program, and is particularly on the alert for signs of "msx" in the Maryland Chesapeake. Tray studies in the Chincoteague will be continued for at least another year to follow the mortalities in tray stocks exposed to "msx" through at least two seasons. It is anticipated that this laboratory will be able to handle its own histological preparations.

C. U. S. Fish and Wildlife Laboratory--Annapolis.

Mr. Engle spoke briefly of general plans for the new Oxford, Maryland Laboratory. It is anticipated that this laboratory will embark on a general shellfish mortality program in cooperation with the various state programs. Mr. Richard Burton at the Annapolis Laboratory is now fully employed in shellfish histology.

D. Delaware Marine Laboratory.

Here also a histological study will get underway. Miss Belz, recently added to the staff, is a trained histological technician. Dr. Shuster plans to tackle the problems of tissue culture with oyster preparations as part of a basic program of oyster disease studies.

E. New Jersey Oyster Research Laboratory.

The present emergency program of study of the Delaware Bay Oyster mortalities will continue. It was pointed out that this laboratory has been attempting to gather all information possible on an epizootic in progress. A high priority has been placed on close observation of events in oyster populations on the planted grounds and seed beds of Delaware Bay to determine the patterns of kill. These have been supplemented and extended by close observations of a variety of stocks in trays. Tray and aquarium studies will be expanded to attempt to work out the life cycle

stages of the pathogen and to obtain transmission under controlled experimental conditions. Dr. John Mackin has placed "msx" in the genus Haplosporidium because of the close resemblance of certain of the stages. We are not yet certain, however, of the definitive spore stages. The highest priority will be given to the continuing search for resistant stocks of oysters to the Delaware Bay pathogen.