IMPORTANT DISEASES OF CULTURED OYSTERS

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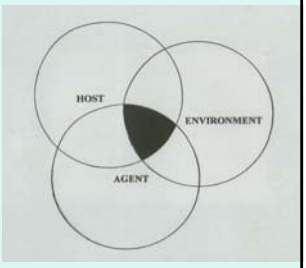
WHAT IS DISEASE?

- **WHAT IS DISEASE?**
- **All disease is the result of the interaction of 3 factors:**
  - **host susceptibility**
  - **agent (pathogen) virulence**
  - **environmental conditions.**
- **The elimination or modulation of any one of these factors can decrease or eliminate the occurrence of disease.**
- **Environment is especially important with bivalves (and all invertebrates)!**



WHAT PROMOTES DISEASE IN BIVALVES?

- **Disease Occurrence in bivalves (and the bivalves themselves!) are greatly affected**
  - **–water temperature**
  - **–salinity**.
- **And**
  - **– FOOD SOURCES**
  - **– WATER QUALITY**

Definitions

Pathogen:

any disease-producing agent, especially a virus, bacterium, or other microorganism

Pathogenesis:

the development of morbid conditions or of disease; more specifically the cellular events and reactions and other pathologic mechanisms occurring in the development of disease. adj., *adj* pathogennet´ic.

Disease:

an impairment of the normal state of the living animal or plant body, or one of its parts, that interrupts or modifies the performance of the vital functions

WHEN SHOULD WE TEST BIVALVES?

- **TIMES OF TRANSPORT OF SEED/ADULTS**
  - **– HATCHERY/NURSERIES SELL SEED TO AQUACULTURISTS**
  - **– ADULTS ARE MOVED FROM ONE AREA TO ANOTHER**
- **TIMES OF MORBIDITY AND MORTALITY**
- **MONITORING?**

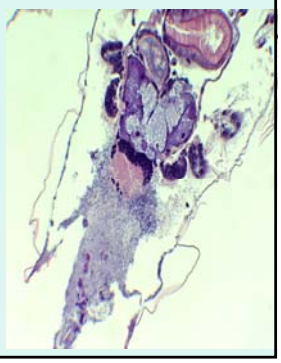
Diseases Of Oysters

DISEASES OF JUVENILE AND LARVAL STAGES

Bacillary Necrosis
OSHV
JOD (ROD)

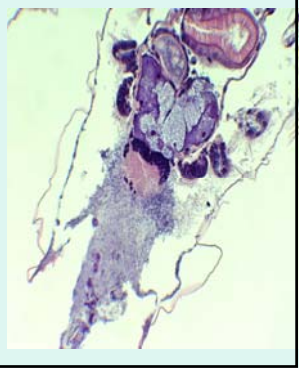
BACILLARY NECROSIS
(LARVAL AND JUVENILE VIBRIOSIS)

- **HOST: LARVAL AND JUVENILE BIVALVES**
- **PATHOGEN: *VIBRIO sp. Bacteria***
- **GROSS APPEARANCE: Sudden death of many animals in the culture**



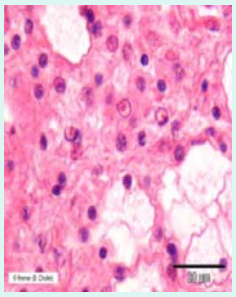
BACILLARY NECROSIS

- DIAGNOSTIC METHODS:
  - GROSS AND MICROSCOPIC EXAMINATION
  - CULTURE OF BACTERIA
- MANAGEMENT METHODS
  - REDUCE DENSITY
  - CLEAN/STERILIZE FACILITIES AND EQUIPMENT
  - PROVIDE QUALITY FOOD



OYSTER HERPES VIRUS (OsHV)

- in *C. gigas* (Pacific Oysters)
  - INFECTION OF LARVAL AND JUVENILE OYSTERS
  - BUT UP TO 30% OF ADULTS CAN BE AFFECTED TOO
  - MOST OFTEN ASSOCIATED
    - SUMMER TEMPERATURES
    - CULTURED ANIMALS
  - HATCHERY DISEASE
    - ADULTS ARE CARRIERS
  - MORTALITY OF LARVAE/JUVENILES CAN REACH 100%



http://wwz.ifremer.fr/cri/mollusc/main_activities__1/tutorials/herpes_virus_oshv_1

Herpes Viruses

- OYSTER HERPES VIRUS (OsHV) in *C. gigas* (Pacific Oysters)
- Pacific oysters are cultured on the west coast of the U.S.
  - First identified in Tomales Bay, California, 2005
  - Where from?

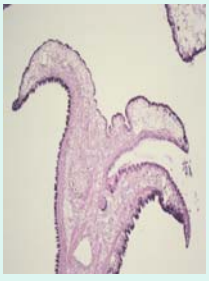
Herpes Viruses

- **OYSTER HERPES VIRUS (OsHV1)**
  - Severe epizootics of OsHV-1(more pathogenic strain!) in France in 2008
  - now in England (and Scotland)
    - *C. gigas* (not the local oyster!)
    - Juveniles
    - High mortality (20 to 80%)

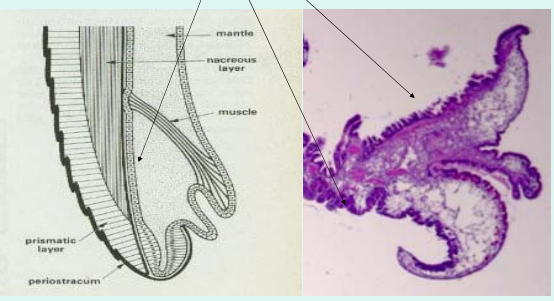
JUVENILE OYSTER DISEASE (JOD/ROD)

- **HOST**
  - ***CRASSOSTREA VIRGINICA* (EASTERN OYSTER)**
- **PATHOGEN**
  - ***Roseobacterium marinus***
  - **ALPHA-PROTEOBACTERIA**
- **GEOGRAPHIC LOCATION**
  - **MA, RI, NY, MAINE**

The mantle and its tissues

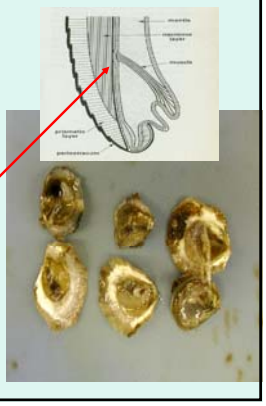


MANTLE – two major types of epithelium
mantle cavity
shell epithelium



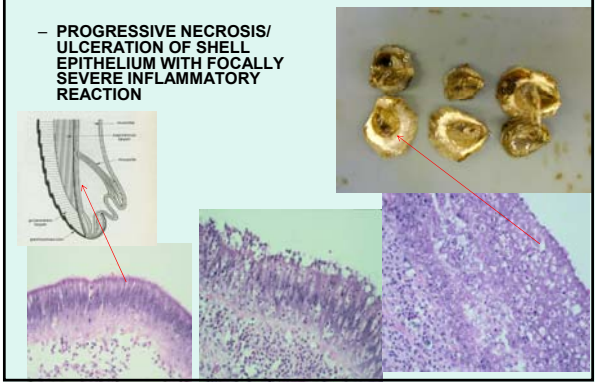
JUVENILE OYSTER DISEASE (JOD/ROD)

- GROSS APPEARANCE
  - MANTLE RETRACTION
  - DEEP CUPPING OF THE LEFT VALVE
  - CONCHIOLIN DEPOSITION ON THE INNER SURFACES OF VALVES
    - Between shell epithelium and the nacreous layer!
  - REDUCED CONDITION AND DEATH



JUVENILE OYSTER DISEASE (JOD or ROD)

- PATHOGENESIS
  - PROGRESSIVE NECROSIS/ ULCERATION OF SHELL EPITHELIUM WITH FOCALLY SEVERE INFLAMMATORY REACTION



JOD/ROD

- **CHARACTERISTICS OF DISEASE**
  - JUVENILES < 25 MM IN SHELL HEIGHT
  - TEMP 21-22° C (70 to 72°F)
  - SALINITIES 25 TO 32 PPT (INHIBITED AT LOWER SALINITIES)
  - LARGER ANIMALS (25-40 MM) SHOW SIGNS OF INFECTION, BUT DO NOT DIE
  - HIGH MORTALITY FOUR TO SIX WEEKS AFTER FIRST SIGNS

JUVENILE OYSTER DISEASE

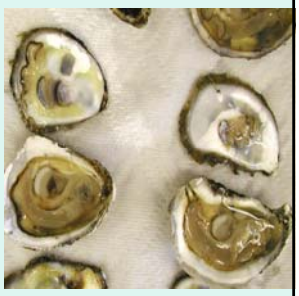
- DIAGNOSTIC METHODS
  - GROSS AND HISTOLOGICALLY EXAMINATION
  - PCR
- MANAGEMENT METHODS
  - SPAWN BROODSTOCK TO ALLOW FOR SEED >25 MM IN EARLY SUMMER
  - DECREASE DENSITY
  - INCREASE WATER FLOW
- DEVELOP RESISTANT ANIMALS
  - F1 RESISTANCE!

DISEASES OF ADULT OYSTERS

- Perkinsus marinus (DERMO)
- MSX
- SSO
- QPX

DERMO DISEASE

- **HOST**
  - ***CRASSOSTREA VIRGINICA* (EASTERN OYSTER)**
- **PATHOGEN**
  - ***PERKINSUS marinus***
    - **DINOFLAGELLATE (KINDA)**
- **GROSS APPEARANCE**
  - **WATERY, POOR CONDITION**

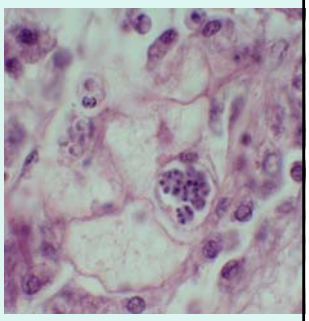


DERMO DISEASE GEOGRAPHICAL LOCATION

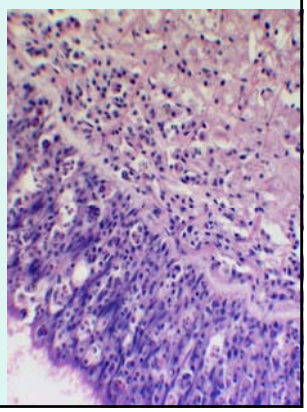
- **1940 – CAUSED MORTALITY IN GULF OF MEXICO**
- **Now found from Gulf of Mexico to Maine!**

Dermo

- **FORMS IN TISSUE AND IN CULTURE MEDIA**
  - **SIGNET RING WITH VACUOLE (MATURE MERONTS)**
  - **ROSETTE (SPORANGIUM)**
- **FORM IN SEAWATER**
  - **ZOOSPORANGIA**



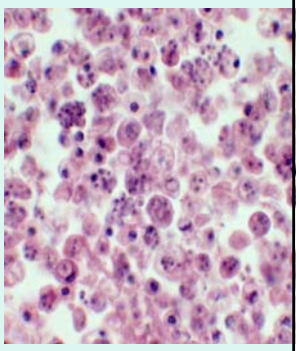
DERMO DISEASE



- INFECTS IN DIGESTIVE TRACT
- GILL
- MANTLE

DERMO DISEASE

- PATHOGENESIS
  - HEMOCYTES ENGULF BUT CANNOT DESTROY (AT LEAST NOT ALL)
  - PROLIFERATION IN HEMOCYTES
  - SPREAD THROUGH THE BODY DEATH CAN TAKE 1-2 YEARS
  - HIGHEST MORTALITY IN THE FALL (SEPT/OCT IN MA)



DERMO DISEASE

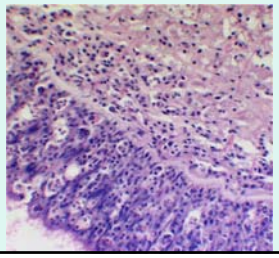
- EPIDEMOLOGY
  - DIRECTLY INFECTIVE

CHARACTERISTICS OF INFECTION

- >18° C (>64 °F)
- SALINITIES OF 15 TO 30 PPT PREFERED
- CAN TOLERATE LOW SALINITIES (3PPT)

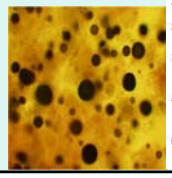
Detection

- Histology
  - Fix tissue, embed in paraffin, stain with H&E



Detection

- Ray/Mackin Fluid Thioglycollate media (RFTM) assay
  - Culture tissue in media for 4-7 days
  - Stain with Lugol's Iodine
  - Examined under microscope for black spheres
  - Rank Mackin Index 0-5



RANK	INTENSITY	DESCRIPTION
0.5	VERY LIGHT	1-20 CELLS IN THE ENTIRE TISSUE
1.0	LIGHT	20 TO 100 CELLS IN THE ENTIRE PREP
2.0	LIGHT TO MODERATE	LOCALIZED INFECTIONS OF 25-50 CELLS OR UNIFORMLY DISTRIBUTED CELLS
3.0	MODERATE	ALL FIELDS AT 100X TOTAL MAGNIFICATION SHOW SEVERAL PARASITES
4.0	MODERATE TO HEAVY	LARGE #S OF PARASITES. LOCALIZED AREAS APPEAR BLUE/BLACK WHEN STAINED
5.0	HEAVY	ENORMOUS NUMBERS OF PARASITES. MAJORITY OF TISSUE STAINS BLUE/ BLACK

DERMO Detection

•Real Time PCR (quantitive)

Remove tissue,

↓

Extract DNA (Chelex)

↓

Run Real Time PCR, quantitatively using a standard curve

COMPARISON WITH RAY/MACKIN THIOGLYCOLLATE METHOD

Mackin Index	Threshold cycle	Cell Density
0	30+	<1
0.5	27-30	1-50
1	23-26	50-250
2	19-22	250-1000
3	15-18	1000-10000
4	11-14	+10000

MUTINUCLEATED SPHERE UNKNOWN (MSX)

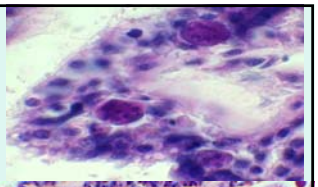
- HOST
  - *CRASSOSTREA VIRGINICA* (EASTERN OYSTER)
  - *PATHOGEN: HAPLOSPORIDIUM NELSONI*
    - PROTOZOA
- GROSS APPEARANCE
  - GAPPERS
  - POOR WATERY CONDITION OF TISSUES

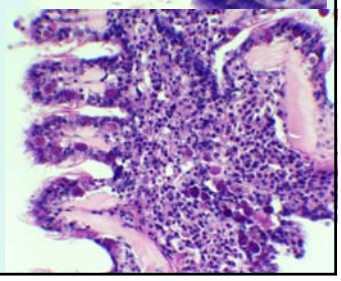
MSX

- GEOGRAPHICAL LOCATION
  - FLORIDA TO MAINE
  - CANADA!
  - Esp. FROM CHESAPEAKE BAY TO MA
  - INTRODUCED TO THE EAST COAST FROM WITH PACIFIC OYSTER (WEST COAST)

MSX

- PLASMODIA
- INITIAL LOCATION
  - GILL EPITHELIUM



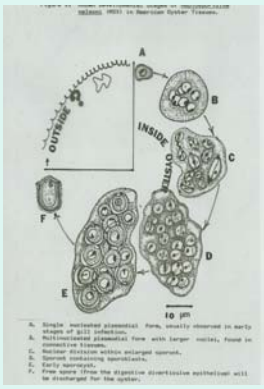


MSX

TISSUE FORMS (CAN'T GROW IN CULTURE!)

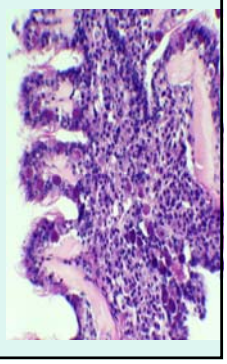
- EPIDEMOLOGY
  - INDIRECTLY INFECTIVE

  (INTERMEDIATE HOST?)



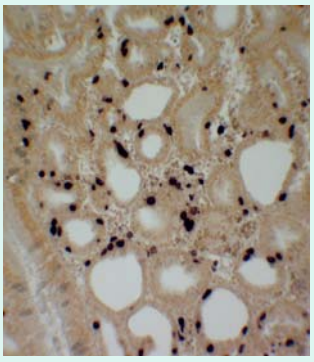
MSX

- CHARACTERISTICS OF INFECTION
  - SALINITIES > 15 PPT TO 28 PPT
  - DOES NOT SURVIVE AT LOW SALINITIES
  - >APPROX. 18° C (64°F)
  - MOST MORTALITY OCCURS IN AUG/SEPT



MSX

- DIAGNOSTIC METHODS
  - HISTOPATHOLOGY
  - Quantitative real time PCR

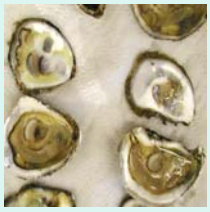


Resistance to MSX and Dermo

- Strains have been developed
- When to use resistant strains?
- Why doesn't everyone use resistant strains?

SEASIDE ORGANISM (SSO)

- **HOST**
  - ***CRASSOSTREA virginica* (EASTERN OYSTER)**
- **PATHOGEN**
  - ***HAPLOSPORIDIUM costale***
  - **PROTOZOAN**
- **A relative of MSX!**

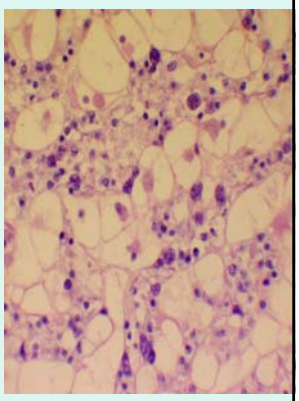


SSO

- **EPIDEMOLOGY**
  - **SIMILAR TO MSX**
  - **EXCEPT:**
    - **IN EARLY SPRING INCREASED NUMBERS OF PLASMODIA IN INFECTED ANIMALS**
    - **SYNCHRONOUS SPORULATION IN SPRING RESULTING IN MORTALITY OF MANY ANIMALS (MAY/JUNE IN NORTHEAST)**
  - **SALINITIES >25 PPT**
    - **PARASITE DOES NOT EXIST AT LOWER SALINITIES**

SSO

- PATHOGENESIS
  - ANIMALS WITH LOW NUMBERS OF PLASMODIA ARE CLEARED OF THE DISEASE DURING SPORULATION
  - ANIMALS WITH MODERATE TO SEVERE INFECTIONS DIE DURING SPORULATION

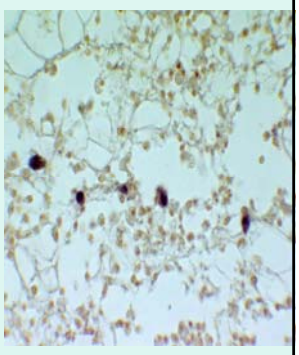


SSO

- GEOGRAPHICAL LOCATION
  - VIRGINIA TO MA

SSO

- DIAGNOSTIC METHODS
  - HISTOPATHOLOGY
    - OFTEN HARD TO DIFFERENTIATE MSX FROM SSO HISTOLGICALLY
    - PCR/QPCR



MANAGEMENT METHODS FOR ADULT OYSTER DISEASES

- SPAWN AND RAISE SEED AT LOW SALINITIES TILL LATE IN FIRST YEAR
- PLANT SEED OUT AT END OF FIRST SUMMER
- REQUIRE HEALTH EXAMINATION BEFORE SHIPPING SEED
- PROHIBIT RELAYS
- DEVELOP RESISTANT OYSTERS
- LET INFECTED LEASES/PLOTS LIE FALLOW FOR 1-2 YEARS
- MONITOR POPULATIONS IN THE FLATS

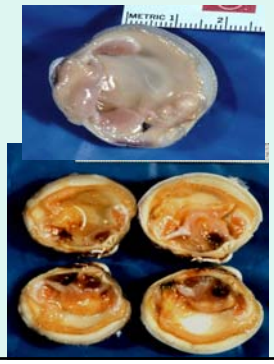
CLAM DISEASES

- Mercenaria mercenaria (hard clam, quahog)
  - Quahog parasite unknown
  - tumor
- Mya arenaria (soft shell clam)
  - tumor

Quahog Parasite Unknown
(hard clam disease)

- APPROXIMATELY TWO YEARS OF AGE
- 30PPT SALINITY
- CLAMS LYING ON THE SURFACE OF THE SEDIMENT

QPX DISEASE IN HARD CLAMS



NODULES AND IRREGULAR SWELLING IN THE MANLTLE EDGES

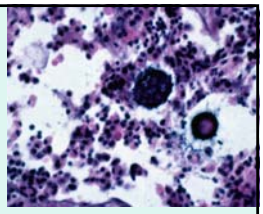
CANNOT CLOSE SHELLS COMPLETELY

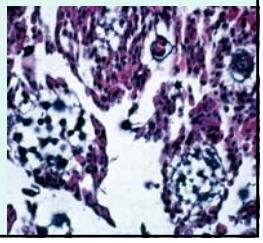
- **Inability to function**
- **Secondary infections**

Locations of clams with QPX disease

- RI
- NY
- VA
- St. Lawrence River/Canada

QPX

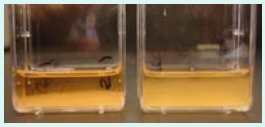


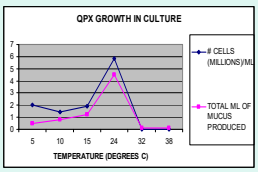


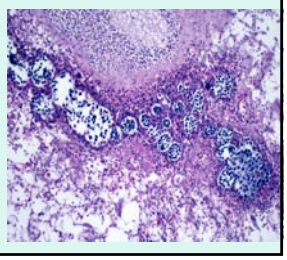
- **PATHOGEN**
  - **PROTOZOAN**
- **THALLI, SPORANGIA, ENDOSPORES**
- **IS DIRECTLY INFECTIVE**

QPX – IN CULTURE

- GROWS BEST AT 24°C DEAD AT 32°C
- SOME STRAINS PRODUCE COPIOUS AMOUNTS OF MUCUS (ATCC)

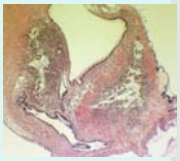


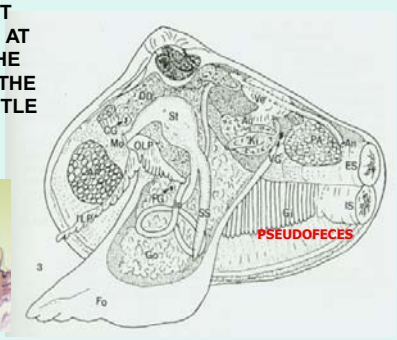




PATHOGENESIS OF INFECTION

INFECTION MOST OFTEN OCCURS AT THE BASE OF THE SIPHON AND IN THE ADJACENT MANTLE

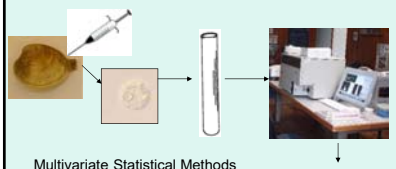




SPRING VS SUMMER

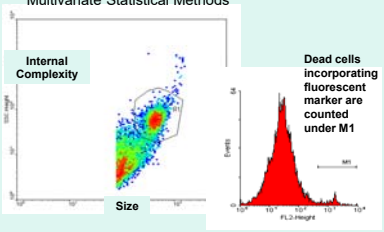
- MORTALITY IS OFTEN SEEN IN THE SPRING AND FALL
- ASSOCIATED WITH THE CLAMS INABILITY TO MOUNT AN EFFECTIVE RESPONSE UPON INITIAL INFECTION?

Hemocyte measurements



Flow cytometer:
- Size
- Complexity
- Green fluo.
- Orange fluo.
- Red fluo.

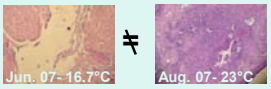
Multivariate Statistical Methods

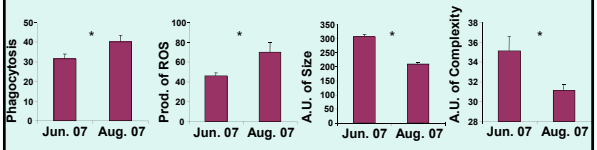


HEMOCYTE PARAMETERS:

Concentration
Morphology
(Size and Complexity)
% dead hemocytes
Phagocytosis
Production of ROS
Apoptosis

What are the differences between the Spring and the Summer?





In Scudders Lane (QPX-infected site), from the Spring to the Summer

Increase
↑ Number of circulating hemocytes,
Phagocytosis,
Production of ROS

Decrease
↓ Size,
Complexity,
Apoptotic hemocytes

Stronger immune status in the summer

EFFECTS CLAM STRAIN

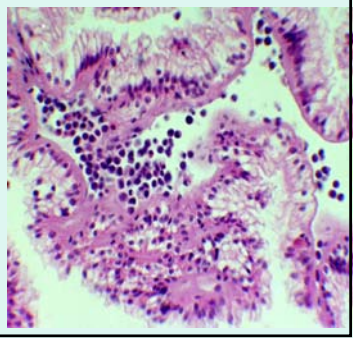
- **MORTALITY IS OFTEN SEEN IN CLAMS SPAWNED FROM BROODSTOCK CONDITIONED TO SOUTHERN WATERS**
- **ASSOCIATED WITH THE CLAMS INABILITY TO MOUNT AN EFFECTIVE RESPONSE UPON INITIAL INFECTION**

TUMORS OF CLAMS

- **HEMOCYTIC SARCOMA (LEUKEMIA)**
  - **CLAMS, OYSTERS AND MUSSELS/HOST SPECIFIC**
- **ALTERNATE NAMES**
  - **HEMATOPOIETIC SARCOMA**
  - **HEMATOPOIETIC NEOPLASIA (HN)**
  - **DISSEMINATED NEOPLASIA (DN)**

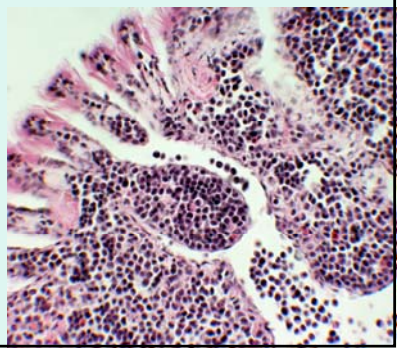
TUMORS OF BIVALVES
HEMOCYTIC LEUKEMIA

- **Mya arenaria**
  - **SOFT SHELL CLAM**
- **Mercenaria mercenaria**
  - **HARD CLAM**



CHARACTERISTICS OF HEMOCYTIC NEOPLASIA

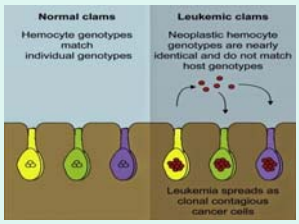
- **NEOPLASIC CELLS CIRCULATE IN THE HEMOLYMPH RESULTING IN:**
  - **OBSTRUCTION OF THE VASCULAR SYSTEM**
  - **LOSS OF NORMAL HEMOCYTES**



CAUSE OF THE NEOPLASTIC DISEASE

- POLLUTION
  - CONSISTANTLY HIGH LEVELS OF PCB AND OTHER POLLUTANTS– NBH
  - ASSOCIATION WITH CONTAMINENTS- CHLORDANE
- RETOVIRUS (TYPE B) (OPRANDY ET AL, 1981)
- Recent findings: cells?
  - Metzler et al., 2015

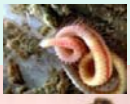
http://www.cell.com/cell/abstract/S0092-8674(15)00243-3?_returnURL=http%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0092867415002433%3Fshowall%3Dtrue

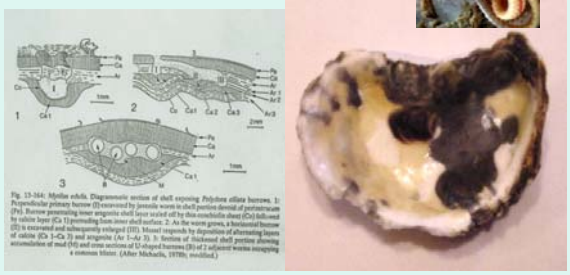


OTHER CONDITIONS

POLYDORA SP.

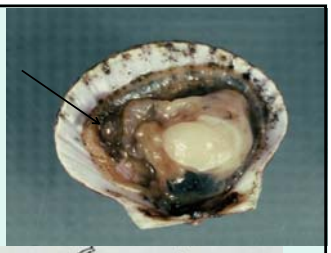
- POLYCHAETE (MUD WORMS)





PEA CRABS

- PINNOTHERES SP.



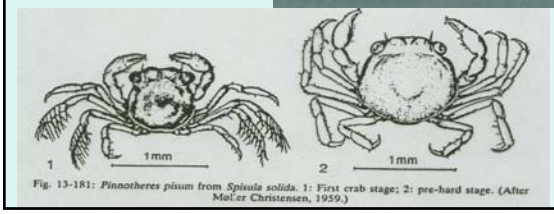
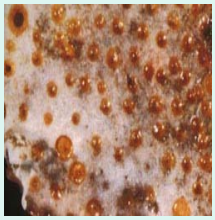


Fig. 13-181: *Pinnotheres pisum* from *Spisula solida*. 1: First crab stage; 2: pre-hard stage. (After Møller Christensen, 1959.)

CLIONA SP.

- BURROWING SPONGE (HONEYCOMB SHELL)



CLIONA SP

- BURROWING SPONGE


Gastropods and Bivalves!
J
Atlantic Oyster Drill
Urosalpinx cinerea
Gastropoda • Sorbeoconcha
C $ ♀♂
Range: Native to Atlantic Coast, introduced Pacific Coast and Europe
Habitat: Benthic epifauna, mobile
Notes: Lives in inter- and sub-tidal zones. Uses acid and radula to drill a hole into bivalve shells and uses proboscis to reach the tender meat inside. They have caused significant damage to bivalve populations.