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This document was created in response to a Freedom of Information request made to CSIRO.

FOI Number:	FOI2011/69
Date:	31 July 2012
Request:	Documents relating to the outbreak of a herpes-like virus in Victoria abalone, now known as Abalone Viral Ganglioneuritis ("AVG") which commenced during December 2005 (the "Victorian Outbreak")
Documents:	Part 5 – Documents 78-85

For more information, please refer to CSIRO's FOI disclosure log at www.csiro.au/FOILog

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From: Caroline.McGowan@dpi.vic.gov.au
Sent: Friday, 22 September 2006 2:32 PM
To: Crane, Mark (LI, Geelong)
Subject: RE: Powerpoint presentation
Attachments: FINAL Abalone Virus Scientific Forum Carolyn Friedman -Introduction to the Infectious Diseases.pdf; FINAL Abalone Virus Scientific Forum Carolyn Friedman - Lessons from California Final.pdf

Follow Up Flag: Follow up
Flag Status: Completed

Mark

Apologies - I missed Carolyn Friedman's presentations.

They are attached now.

Kind Regards

Caroline McGowan
EA to Dr Mehdi Doroudi DVM, Phd
Research Director
Marine and Freshwater Systems
PIRVic
Department of Primary Industries

Ph: +61 3 5258 0266
Fax: +61 3 5258 0270
Email: caroline.mcgowan@dpi.vic.gov.au

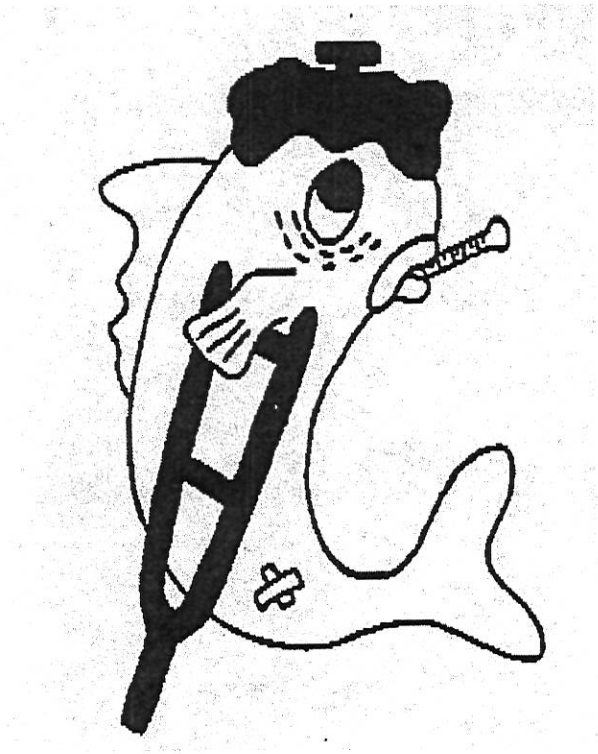
(See attached file: FINAL Abalone Virus Scientific Forum Carolyn Friedman -Introduction to the Infectious Diseases.pdf)(See attached file: FINAL Abalone Virus Scientific Forum Carolyn Friedman - Lessons from California Final.pdf)

Introduction to the Infectious Diseases

- Organisms have a natural tolerance to infection
  - Varies with life stage, molt cycle, genetic make-up and amount of stress experienced
- Immune system suppressed by
  - Adverse temperature, salinity, DO & pollutants

- ***Potential pathogens usually present in the system but do not always cause disease***

What is Disease?

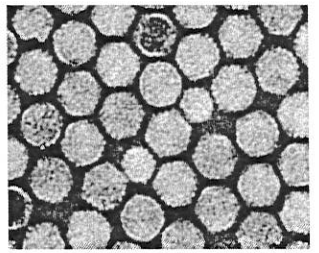


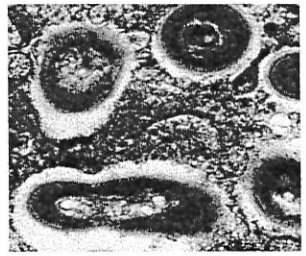
Definitions

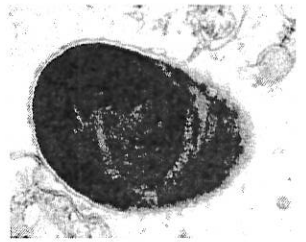
- ***Disease***: an alteration from the normal state
- ***Parasite***: an organism (often microscopic) that is metabolically dependent on its host (gets its energy or food from its host)
- ***Infectious***: transmissible (e.g. virus, bacteria, protozoa, metazoa)
- ***Pathogen***: an organism that is able to cause disease. Parasites, under certain conditions, may cause disease.
- ***Virulence***: **characteristics of the pathogen** that allow it to infect, multiply and spread in or among hosts
- ***Pathogenicity***: the ability to cause disease within a host
  - ***Presence of a parasite (such as a virus) does not equate to presence of a disease***

Causes of Infectious Disease

- Infectious - Microbial Pathogens
  - Viruses
  - Bacteria
  - Protozoa
  - Fungi
  - (Metazoan)

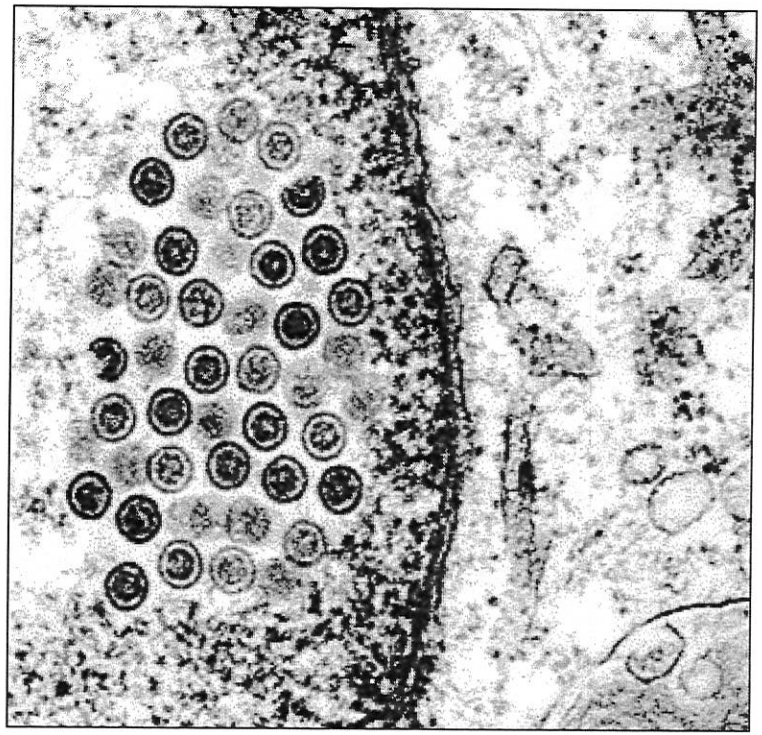




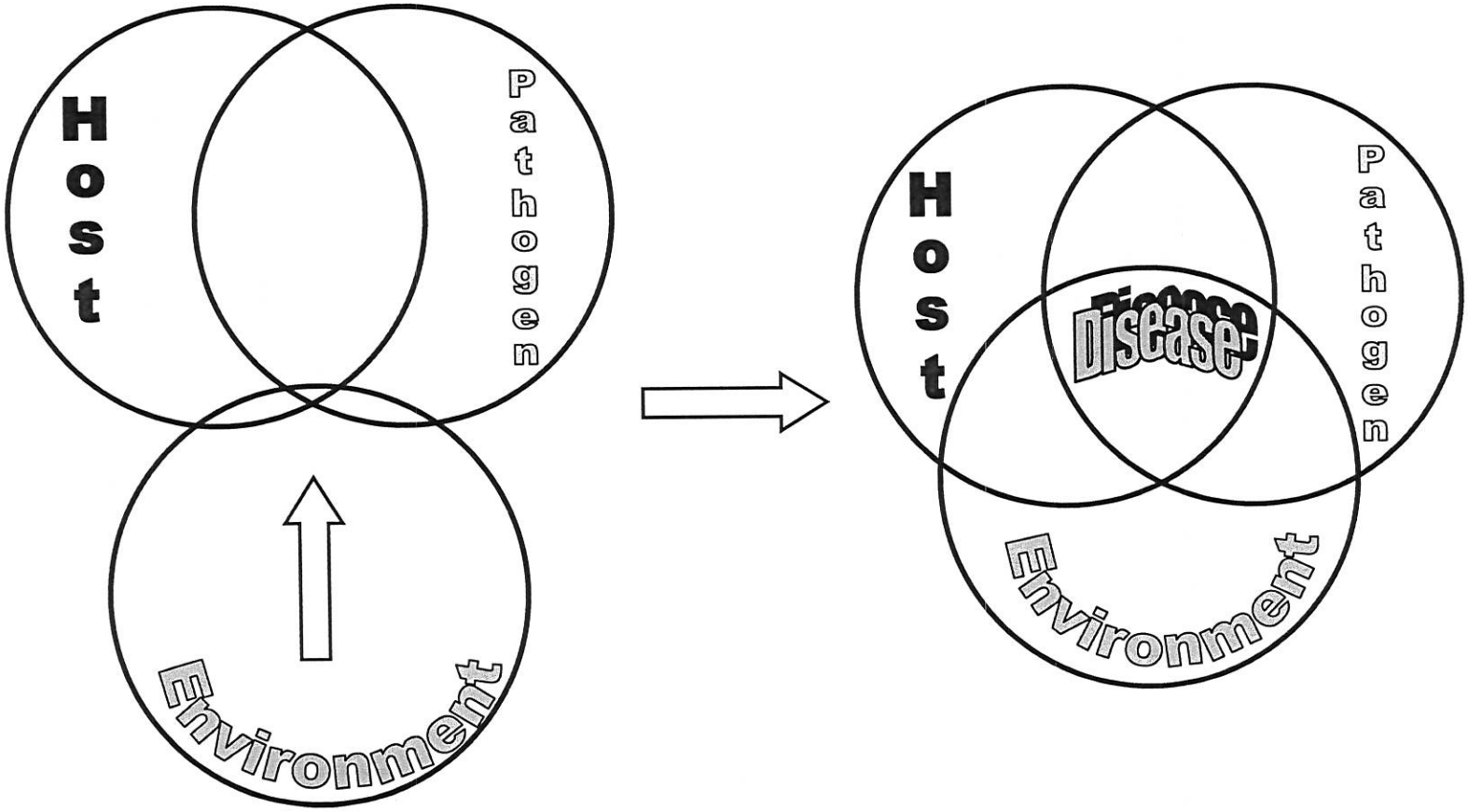


Definitions of Specific Parasites I

- ***Virus*: sub-microscopic agents made up of**
  - **Nucleic acid, in a protein or protein and lipid coat**
  - **Depend on the host cell's machinery to reproduce itself**
  - **Quiescent outside host**
  - **Very small agents usually less than 200 nm**
    - **Herpes often 90-120 nm**
    - **One nm is one thousanth of a micron which is one millionth of a meter**



Development of disease



Common diagnostic methods

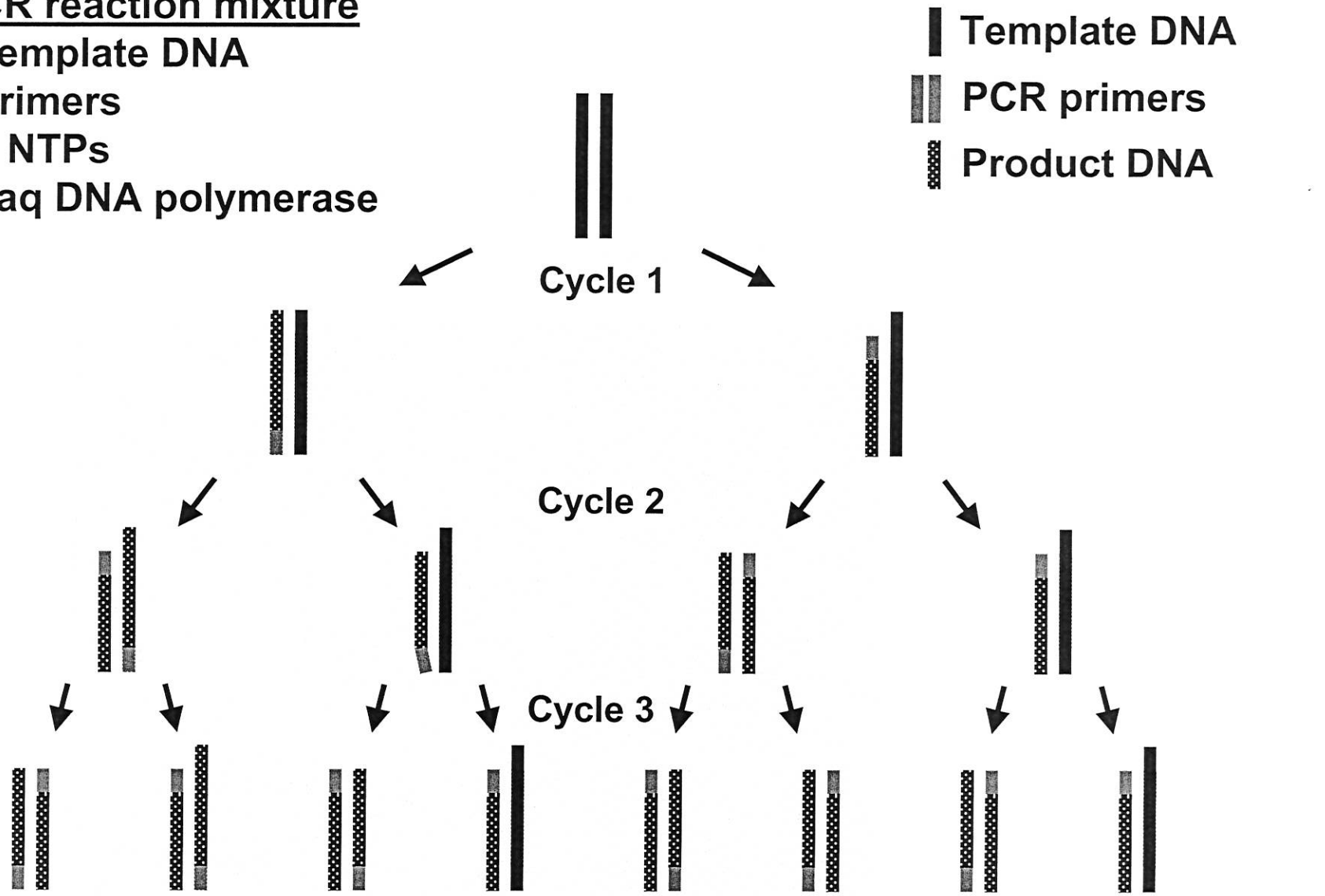
- Traditional methods
  - ***Gross examination***: visual observation of animal
  - Culture of the agent
  - ***Histology***
    - Hematoxylin & eosin stained, paraffin-embedded tissue sections to see host tissues and certain parasites
  - ***Transmission electron microscopy (TEM)***
    - Very high magnification – needed to see virus
- DNA-based tests
  - Polymerase chain reaction (PCR & Q-PCR)
    - Amplification of pathogen-specific nucleic acid
  - *In situ* hybridization (ISH)
    - Uses histology sections and probes with PCR primers +/- a fluorescent probe and view with microscope

Polymerase Chain Reaction (PCR)

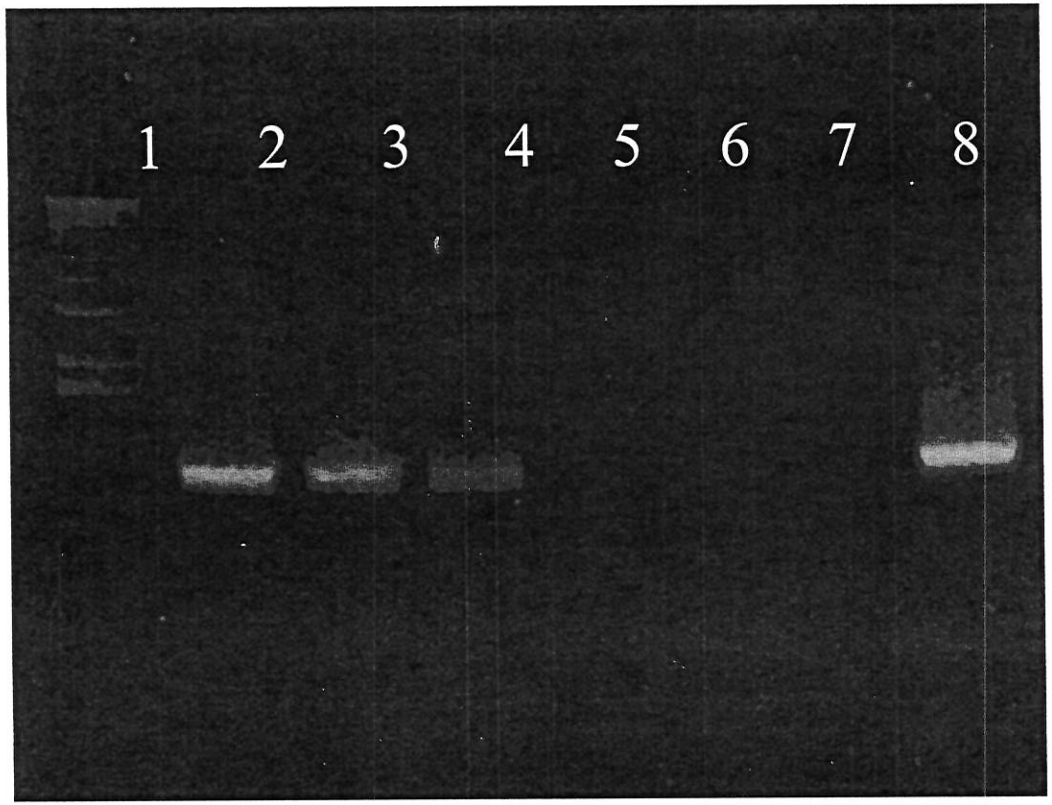
- Billion-fold amplification of a specific DNA sequence via ~30 theoretical doublings
  - (2^{30} = 1 billion fold amplification of target DNA)
- Why PCR?
  - More sensitive and specific than other tests
  - Faster and less expensive, especially for large numbers of samples
  - But it only detects the DNA of the agent and does NOT tell you if it is alive (viable) or causing an infection or disease
    - Must combine with histology or other test to show infection and disease – such as in new cases

PCR reaction mixture

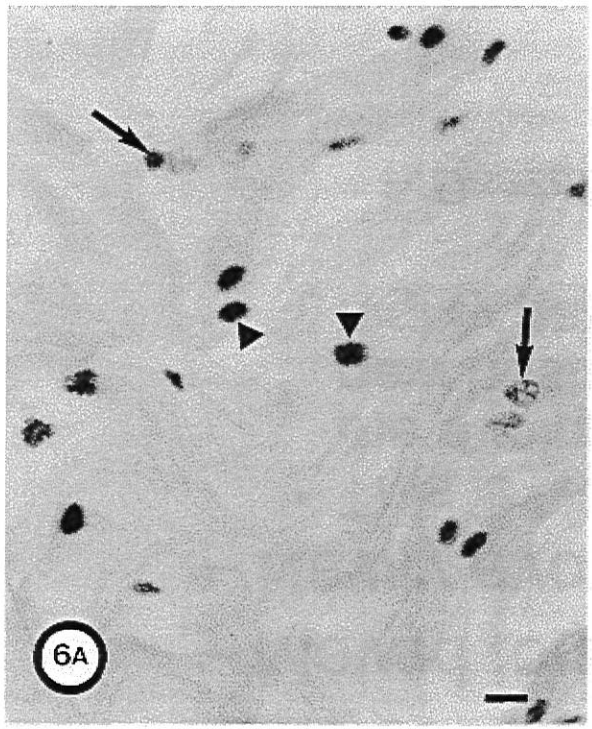
- Template DNA
- Primers
- d NTPs
- Taq DNA polymerase

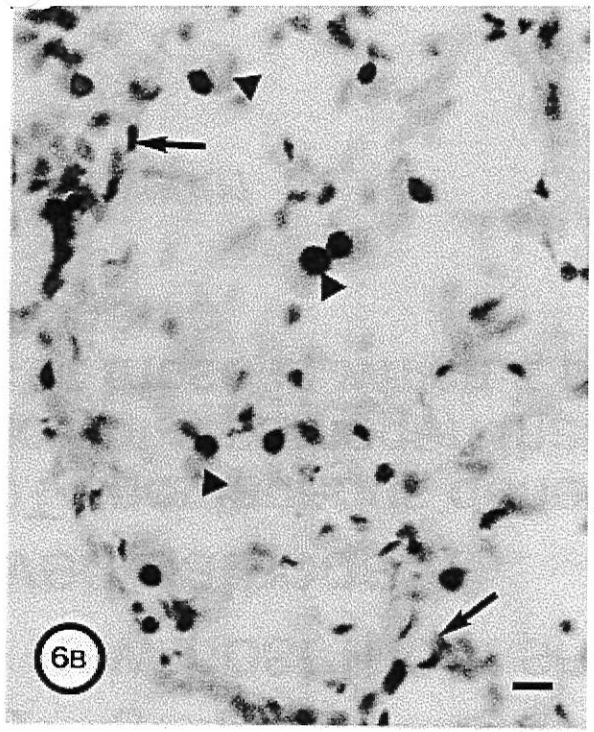


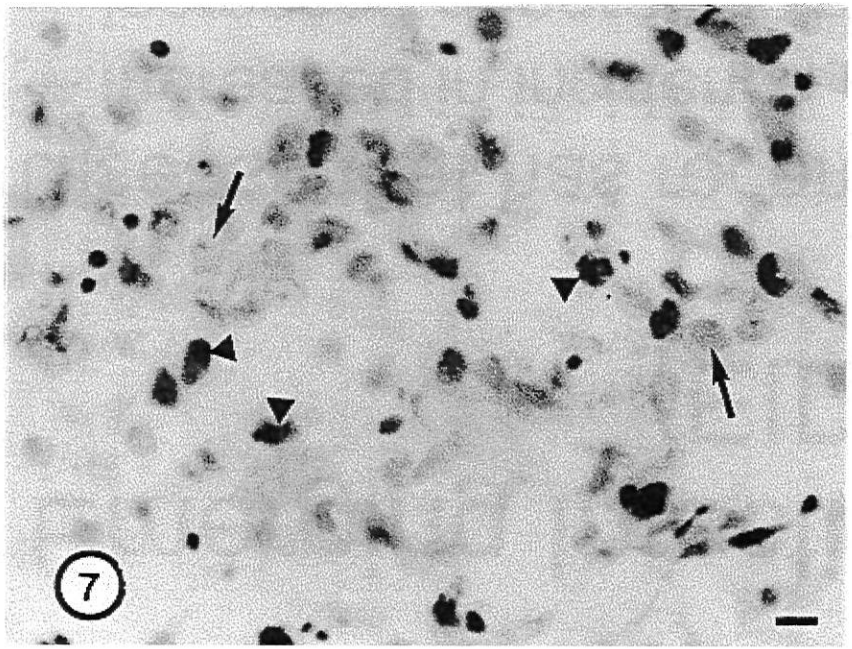
Adapted from Giavannoni, 1991



To see if your PCR works you must separate the DNA on a gel as above







In situ hybridization test – combines histology and PCR probe.

Labelled cells (arrowheads) and non-labelled cells (arrows).

Emerging Infectious Diseases (EID)

- Disease that has recently
  - Increased in incidence
  - Increased geographic range
  - Moved to a new host (host shift)
  - Newly evolved pathogen
    - This is NOT spontaneous generation but refers to the evolution of a pathogen that allows it to infect new hosts or those in which it previously was not highly pathogenic

      – *Daszak et al. 2001*

EID-2

- Similar underlying factors drive emergence of wildlife and human EIDs
  - Ecological change largely driven by anthropogenic environmental changes
  - Also driven by climatic change
  - Other factors
    - *Daszak et al. 2001*

EID-3

- Related to a shift in host-parasite ecology
  - Increase transmission rates between hosts
  - Increase contact with new hosts or species
  - Selection pressures result in a dominance of one or more pathogenic strains adapted to the new environmental conditions

    – *Daszak et al. 2001*

EID-4: Two common underlying causes of emergence

- 1. "Spill-over" and "Spill-back"
  - From wildlife to domestic
  - From domestic to wildlife
    - High population density often allow pathogen to avoid threshold effect
      - Parasites need a certain host density to transmit and cause disease (or large scale disease)
      - *Daszak et al. 2001*

EID-5: Two common underlying causes of emergence…

- 2. Human movement of pathogens to new areas
  - Global transport of domestic animals and products
    - Elephant herpesvirus in zoos, West Nile Virus in US, Crayfish Plague in Europe, Many catastrophic mollusc diseases
  - Pathogen → spread or amplify when naïve host moved to a new area
  - *Bonamia* sp. in *Crassostrea ariakensis* in Bogue Sound, NC
  - Withering Syndrome in abalones

EID-6

- Research into EIDs requires the asking of complex questions and a synergism between researchers from many varied fields such as those here today and their colleagues

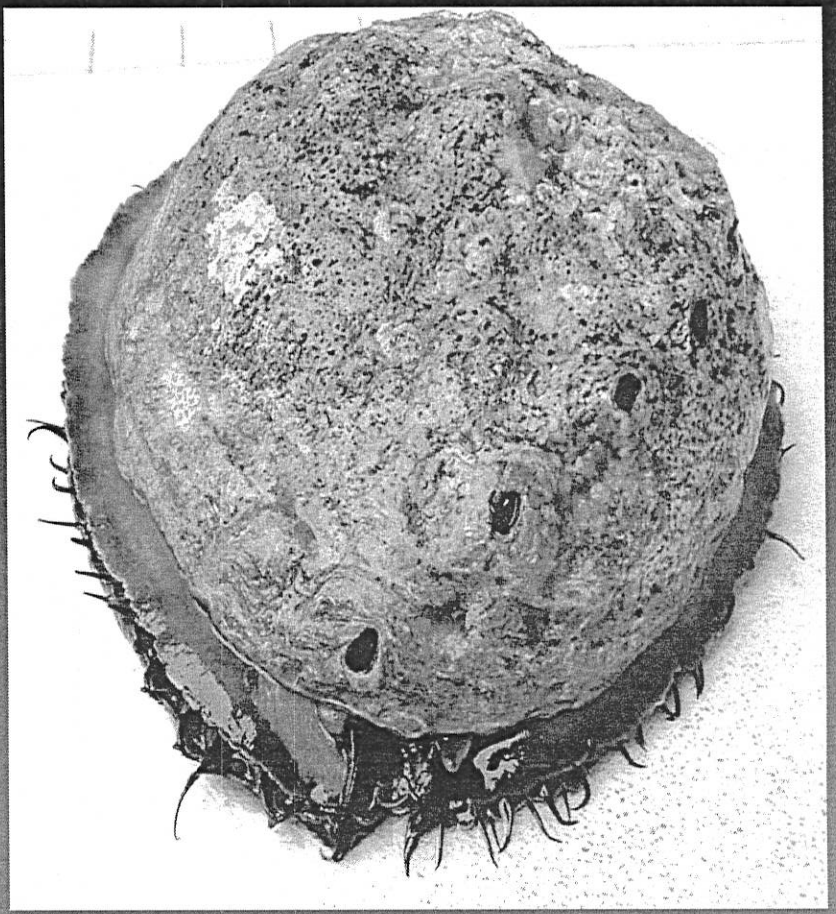
Lessons learned from California, USA:

Withering Syndrome and Sabellid Polychaete Infestations

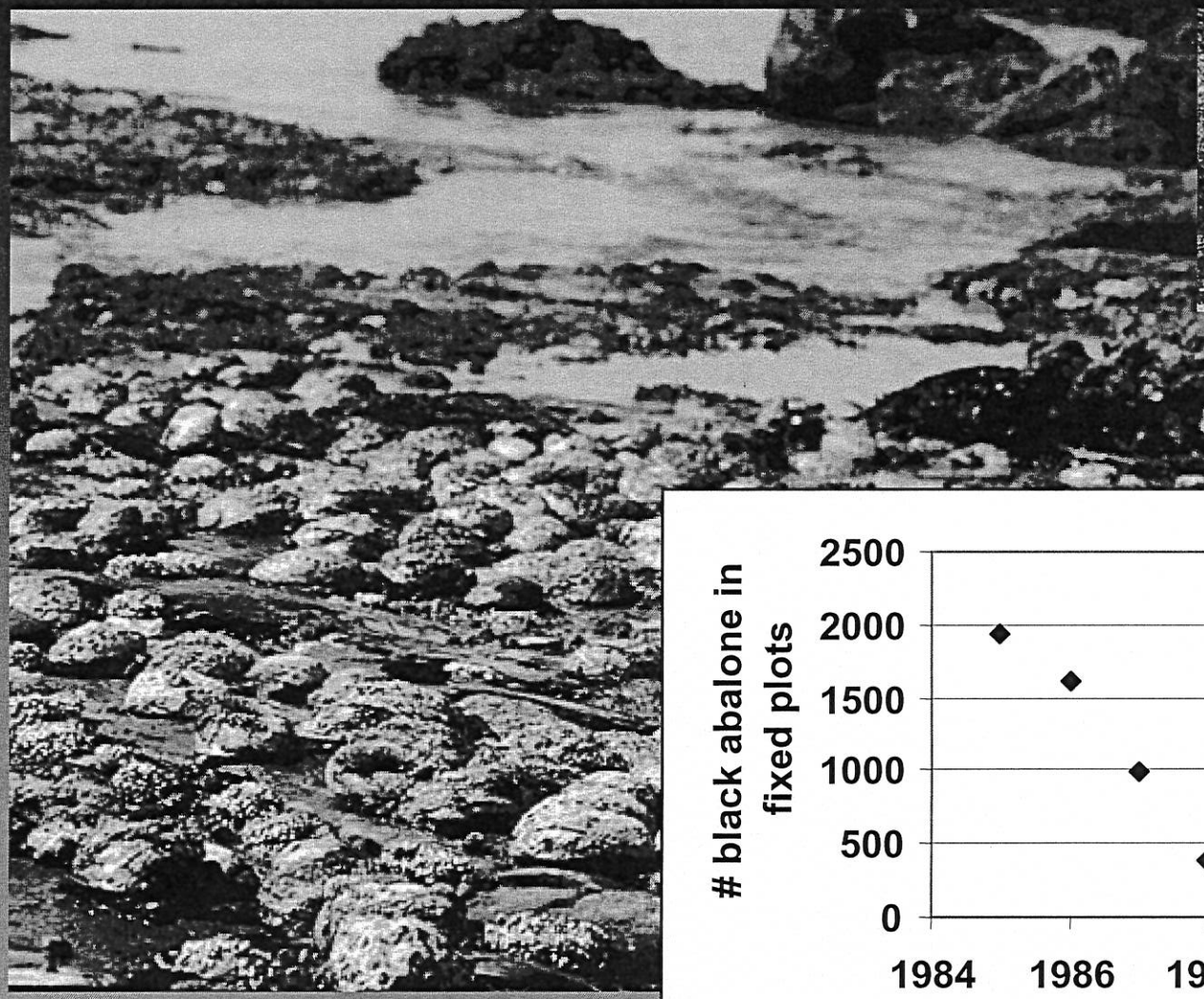
Carolyn S. Friedman
School of Aquatic & Fishery Sciences,
University of Washington
Seattle, WA 98195 USA

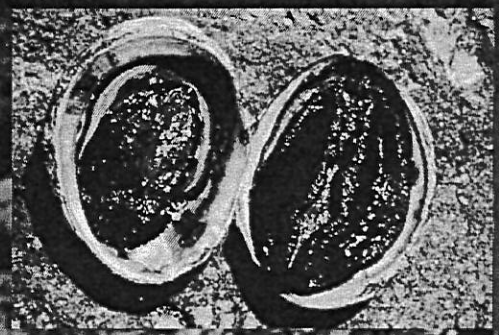
California Abalone

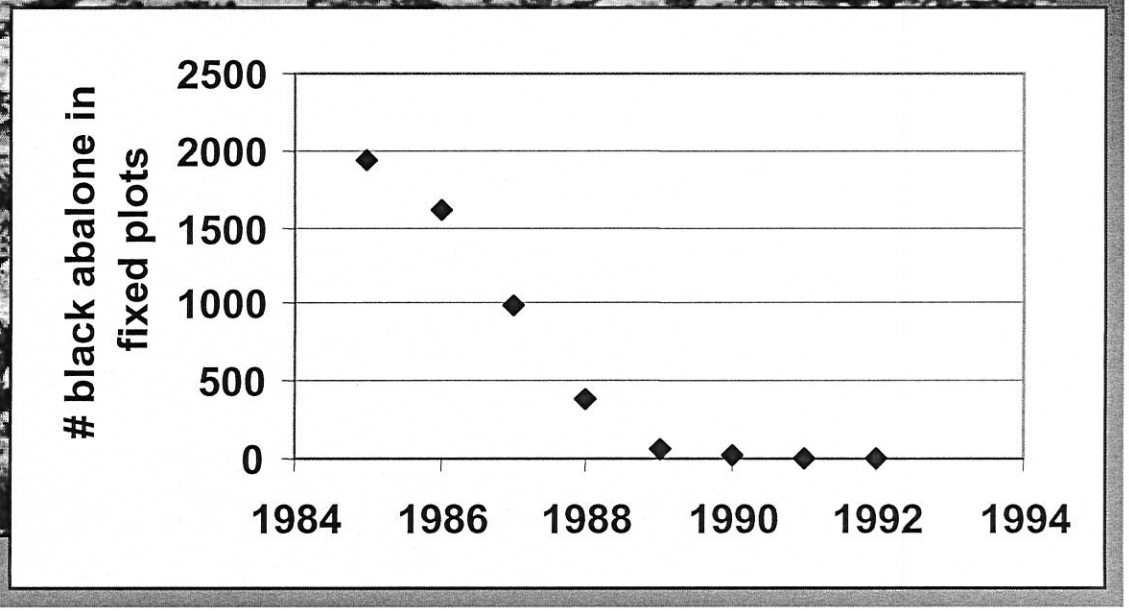
- Historical multi-million dollar commercial and recreational fisheries for 5 species
- By mid-1990s, over-fishing and withering syndrome resulted in population collapse in Southern California
- Healthy recreational fishery north of San Francisco



Haliotis cracherodii on Channel Islands before losses due to WS



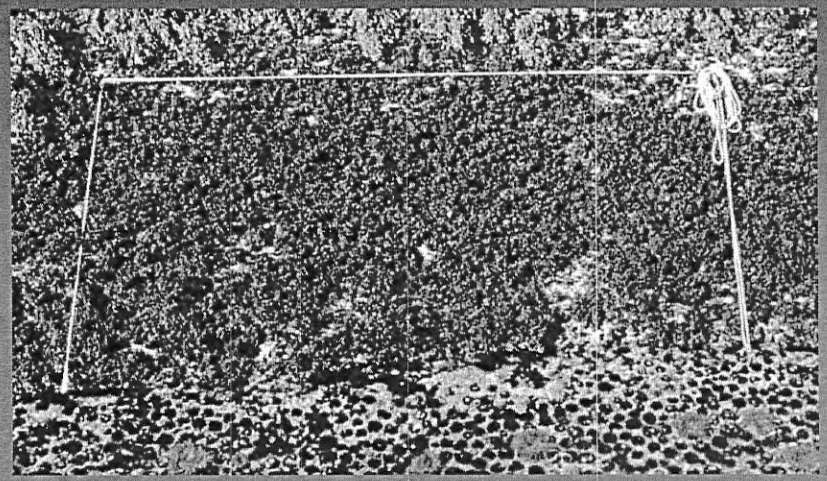




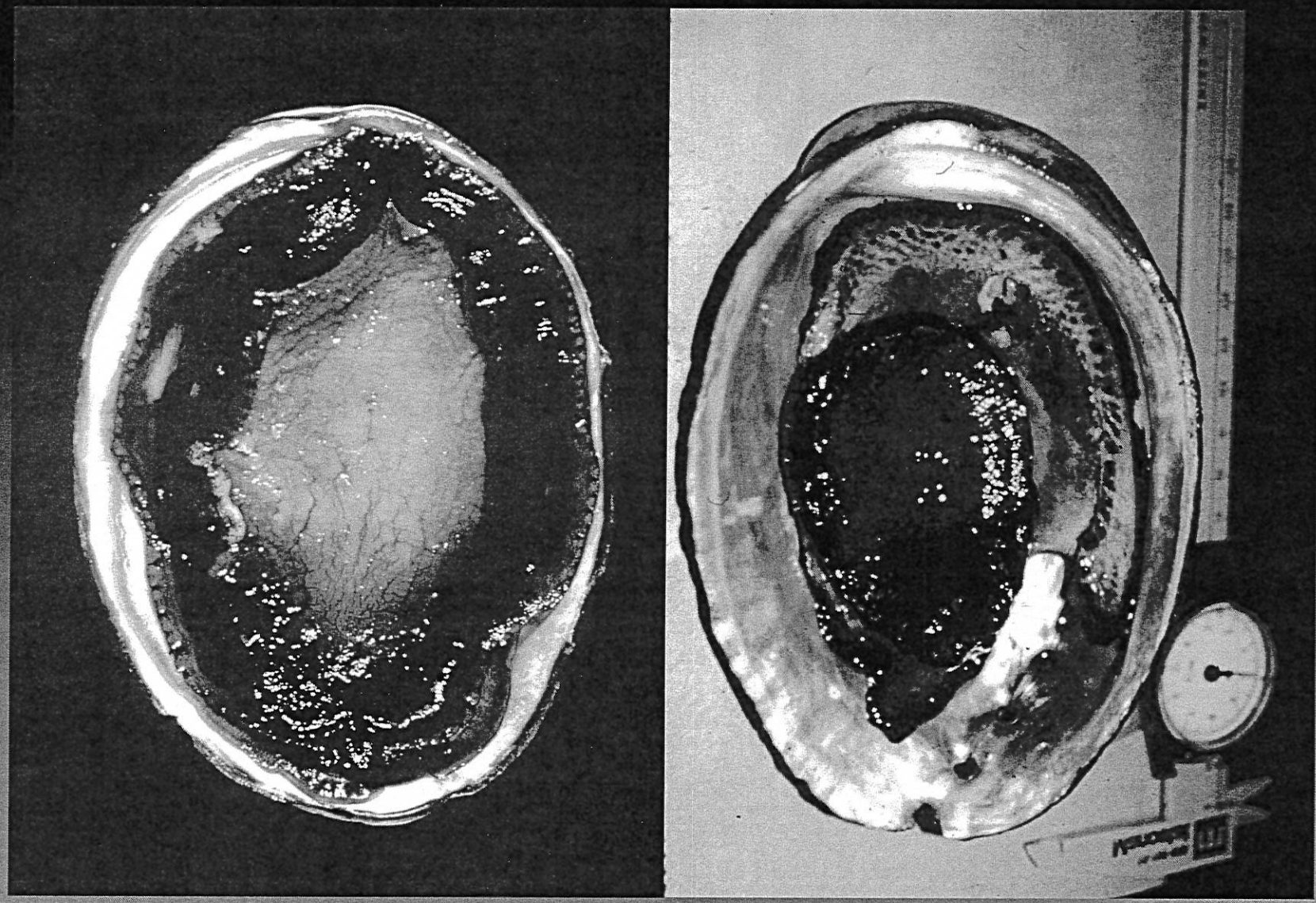
Photos by P. Haaker and J. Dugan

Ecological Impacts of WS

- Change in population structure
- Reduction abalone numbers
- Reduced or eliminated recruitment
- Survival of some abalone
  - Are they more resistant to WS than naïve animals?



Photos by Dan Richards

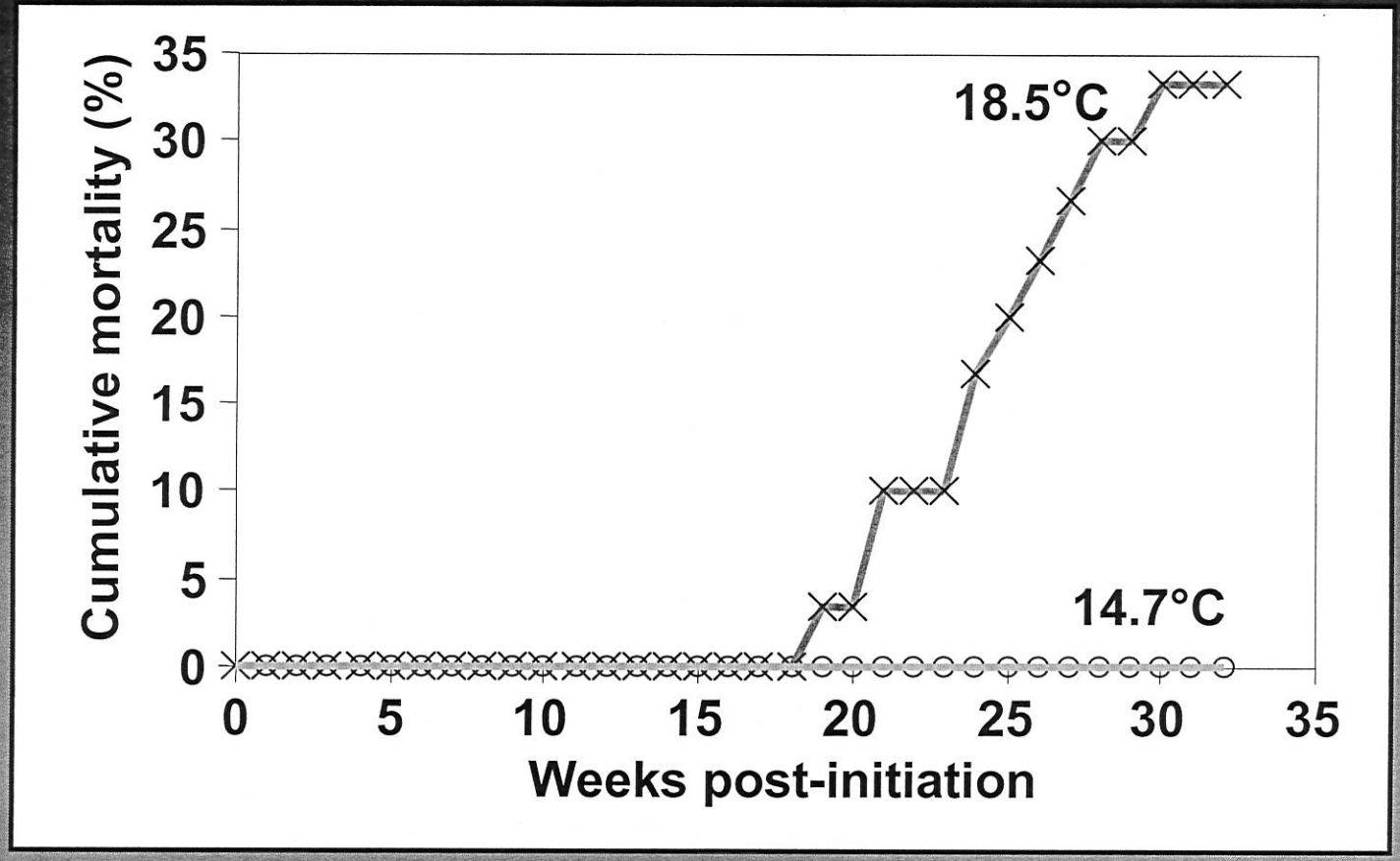


Photos by R. Hedrick and C. Friedman

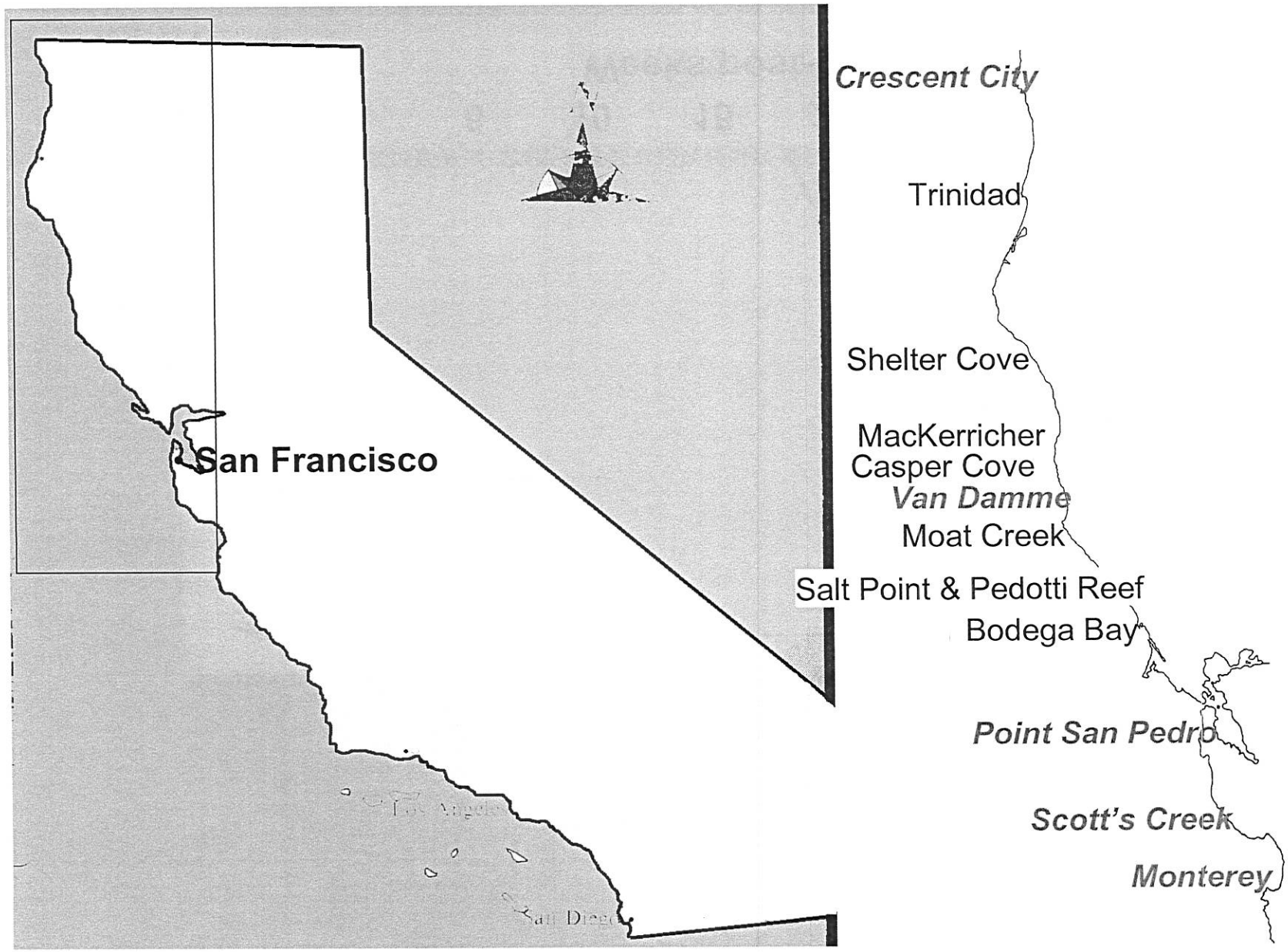
Spread of WS

- Via currents, especially during El Nino years
- Via movement of infected abalone
  - Aquaculture
    - Between farms
    - Potential point source of bacterial release
  - Restoration out plants using infected cultured abalone
  - ? Movement of fished abalone and boats?
  - Others?
- Difficulty in identifying agent
  - Host-parasite relationship
  - Lack of baseline data
  - Lack of sensitive and specific tools

Thermal Induction of WS in Red Abalone



RLO and WS Distribution in California

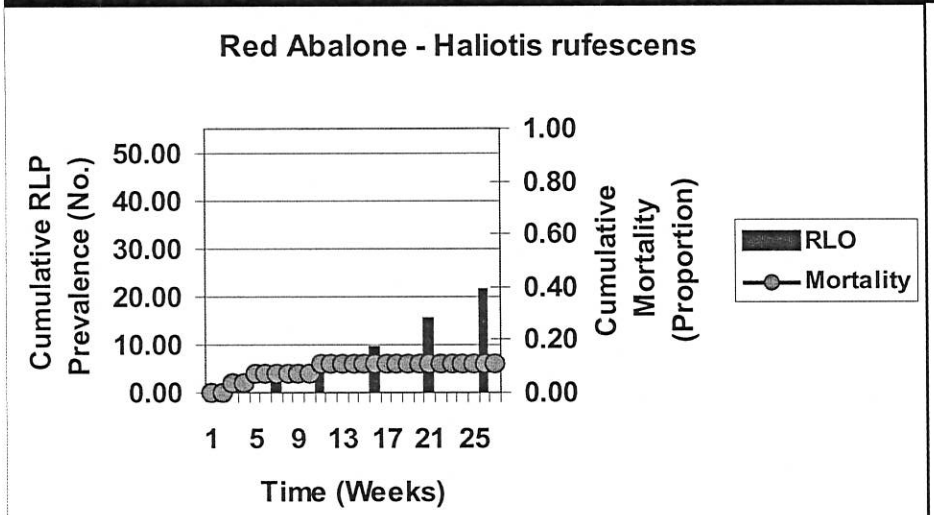


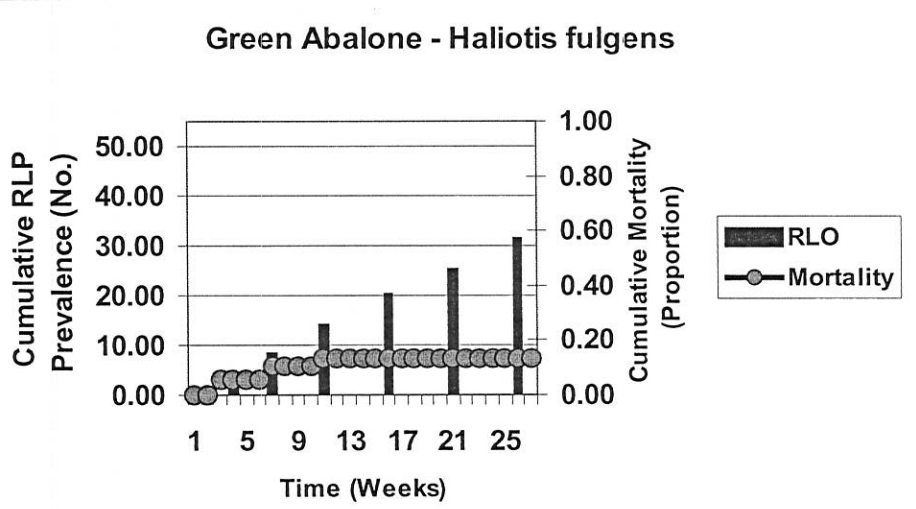
Current *WS* and RLO Distribution

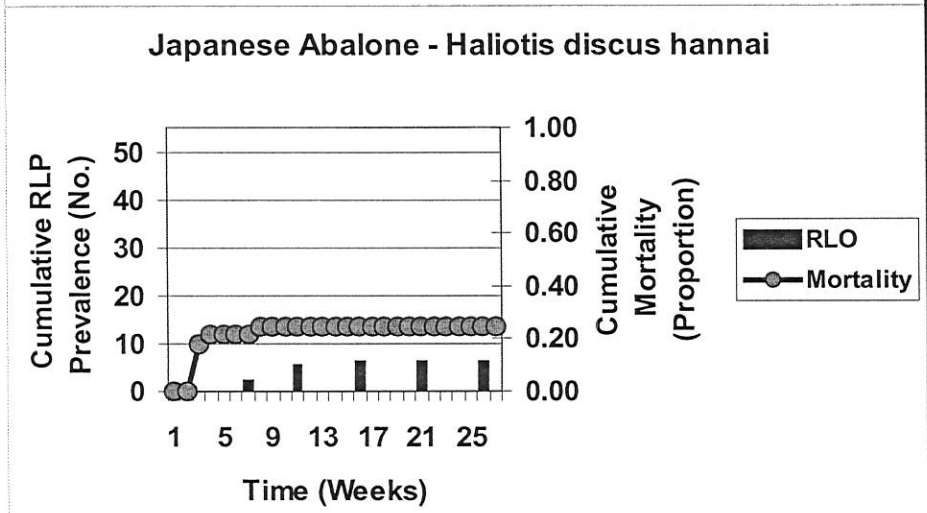
- ***California, USA*** **(~mid-1980s via ENSO or ??)**
- ***Mexico*** **(~early 1990s – natural spread?)**
- **Israel (1990s via imports from CA)***
- ***Chile*** **(1990s via imports from CA)***
- **Iceland (confirmed 2005 via imports from CA)**
- **China and Taiwan confirmed 2006 (suspected via seed from CA)**
- ***Spain*** **confirmed 2005 via imports from Ireland**
  - **Health exam on seed only (NOT brood stock or other adults)**
  - **This paper also suggested France infected but PCR evidence only**
- **Ireland confirmed 2006 via unknown vector**
- **Thailand confirmed 2006 from abalone purchased from Taiwan**

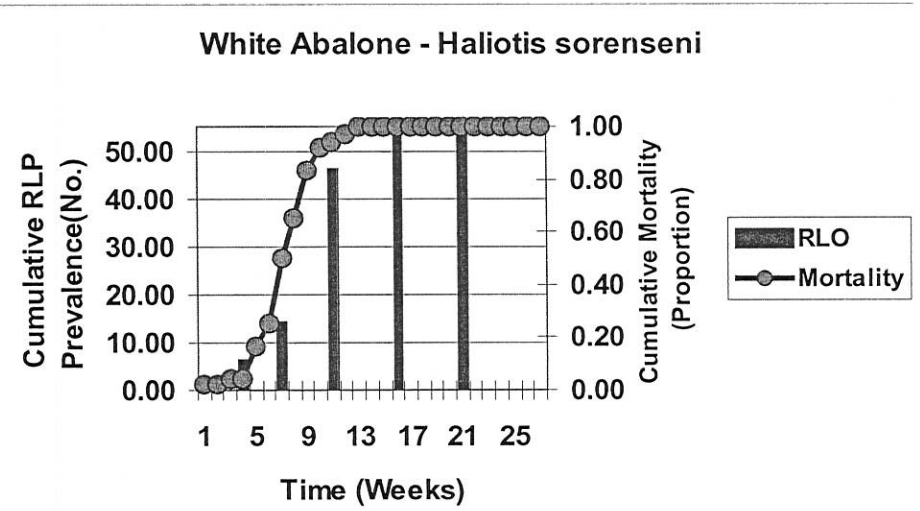
*Prior to identification of cause of WS

Differences in Susceptibility to WS







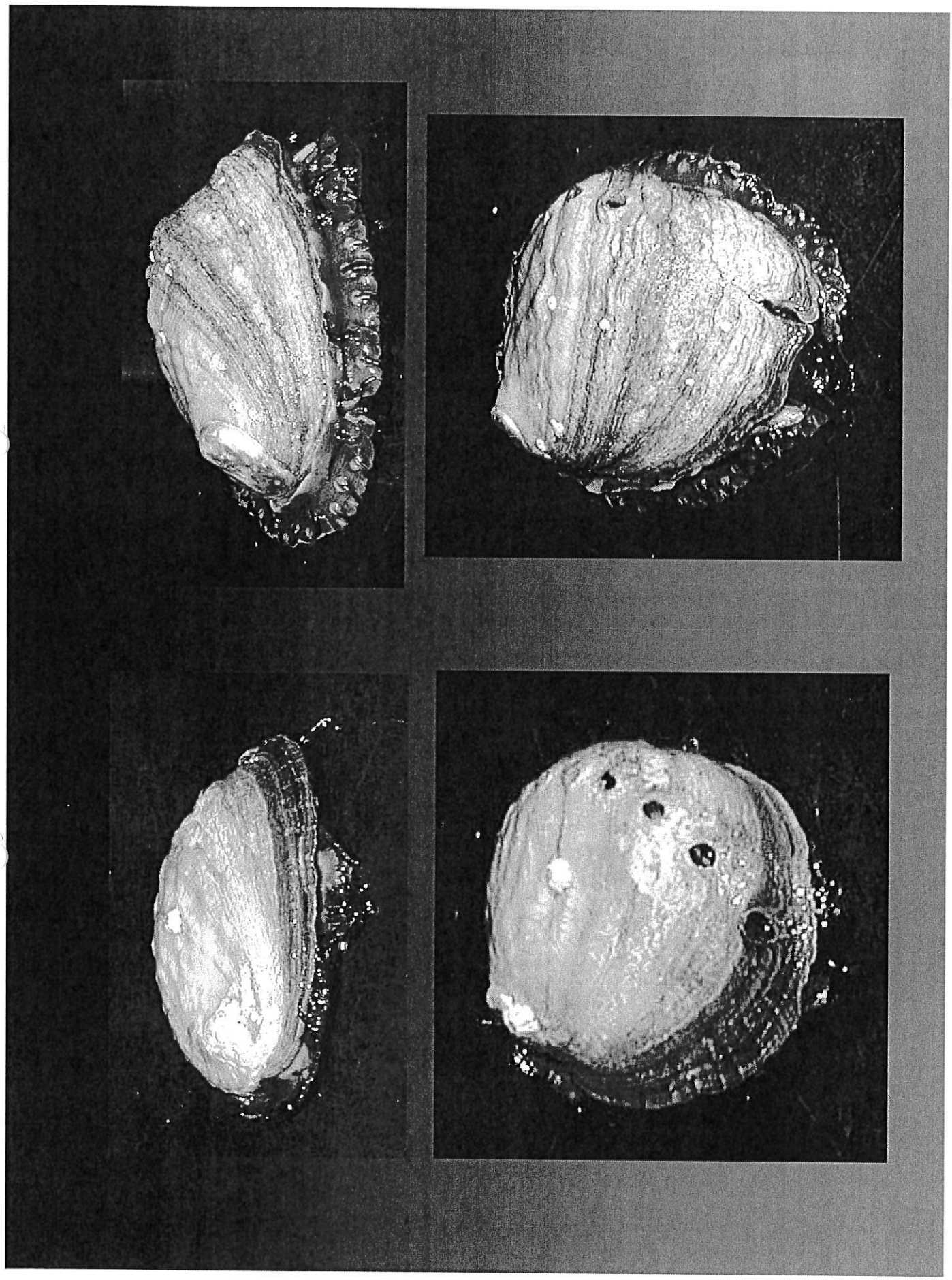


Sabellid Polychaetes 1

- **Previously unidentified Polychaete**
- **Infests various gastropods: Primarily problem in farmed abalone (*Haliotis* spp.)**
- **Also infests *Tegula* spp., limpets, etc.**
- **Introduced into California, USA from South Africa via brood stock to a farm**

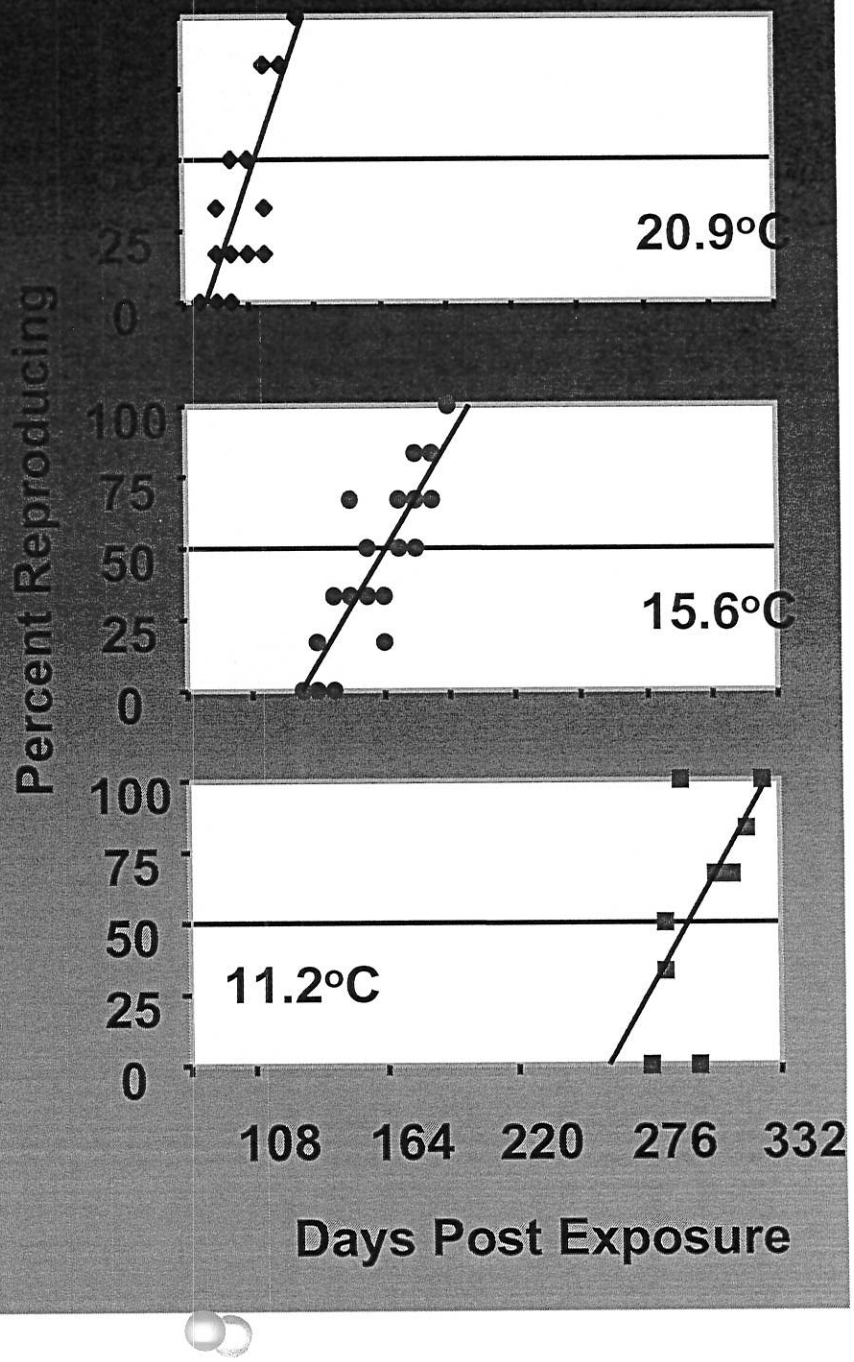
Introduction and Spread

- Lack of health information prior to importation
- Sabellid previously unknown
- Free (no permit, exam needed prior to) movement of animals among registered aquaculturists in state
- Lack of adequate quarantine combined with sabellid biology
- Initial misidentification
- Introduction into main seed hatchery within state
- Lack of understanding of the importance of the sabellid outside areas where it caused severe problems



Sabellid Reproduction

- Worms are simultaneous and functional hermaphrodites
  - It only takes ONE worm to re-establish itself in a farm
- Generation time temperature dependent

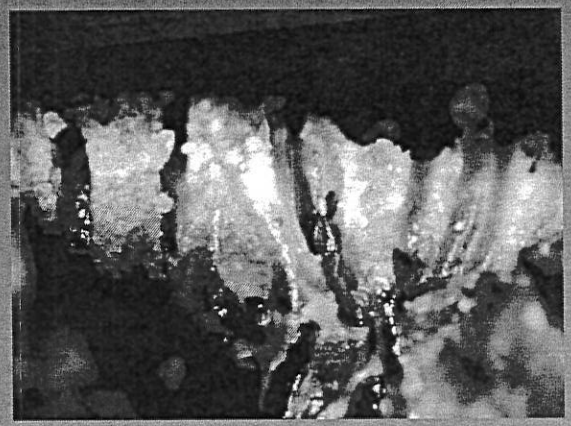


Solutions

- Industry formation of ASWAC – abalone sabellid worm advisory committee
  - Asked CDFG for regulations and guidance
  - Produced educational leaflet with industry and researchers (UC Santa Barbara)
- University and CDFG research
- Sabellid certification protocol/policy
- Sabellid eradication plan developed and implemented
  - 2006 - only ~1-2 farms currently infested out of over 12 farms that were initially infested
    - These 1-2 farms have low level infestations

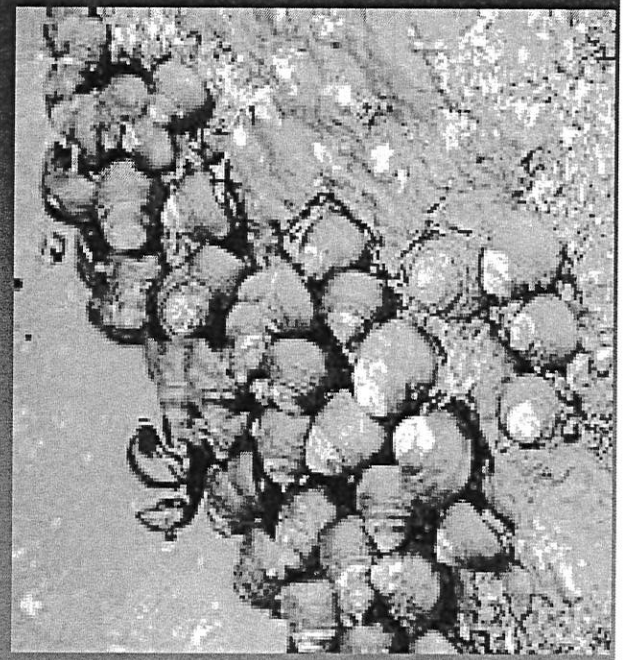
Sabellid Polychaetes: Management

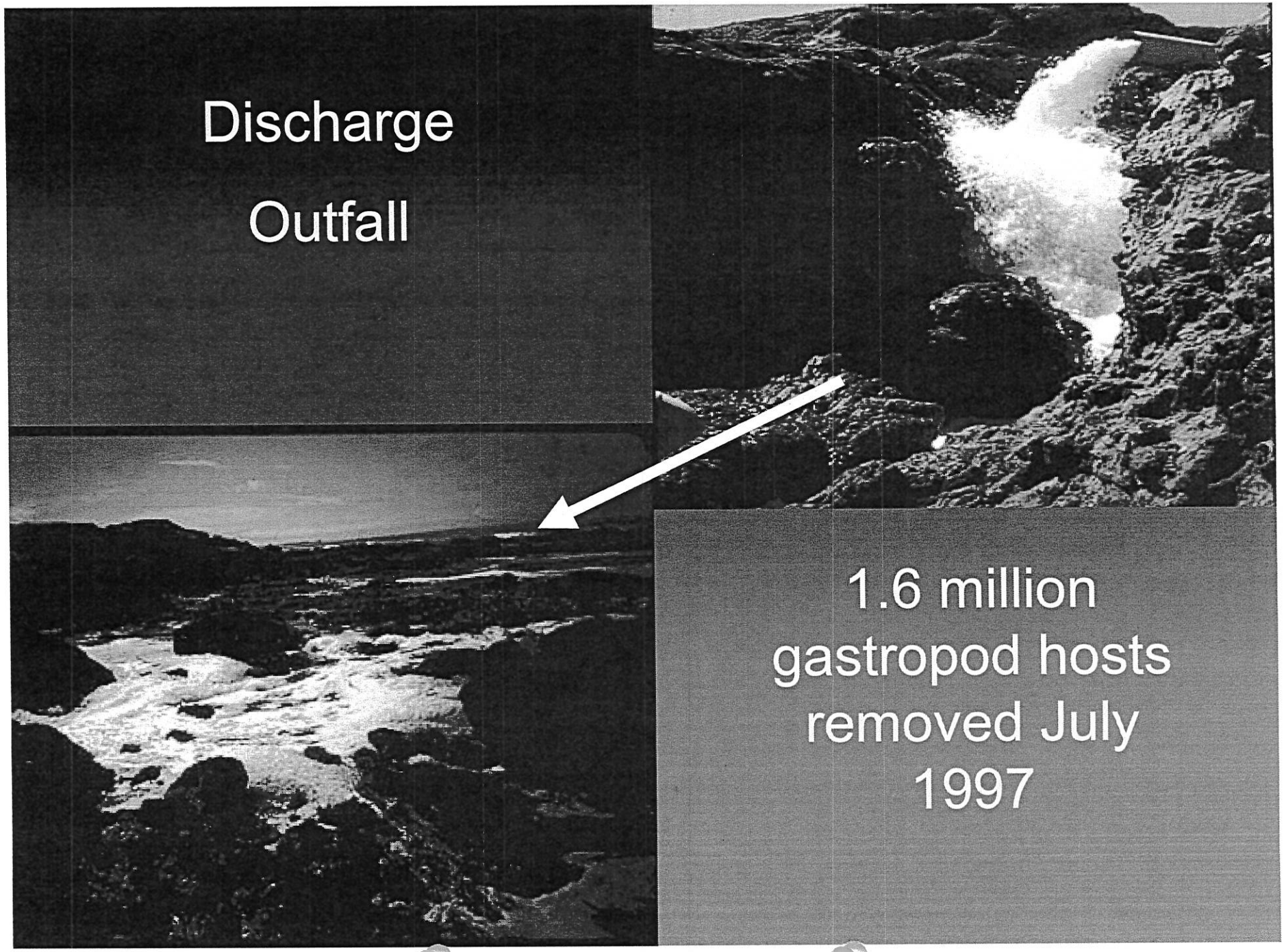
- AVOIDANCE : ***Only Buy Inspected Seed***
- Good Husbandry Practices (Management)
  - Clean Pipes With Hot Fresh Water
  - Eliminate Ability of Abs to Crawl Between Tanks
- Market or Destroy Infested Stocks ASAP
  - Not done routinely and spread from hatchery to ALL farms in state and some were out planted (but no evidence of survival via these out-plants to date)



Establishment in the Wild

- 1996 Kuris and Culver detected pest on *Tegula funebralis*, at abalone farm discharge outfall
- Habitat dense with *Tegula spp.* (snails) and other gastropods (100,000s)
- Sabellids opportunistic in finding alternate hosts
- Mark & recapture study showed transmission in the field (active infestations)




Discharge
Outfall
1.6 million
gastropod hosts
removed July
1997

Intertidal Monitoring

- 1995 DFG/UCSB trained personnel
- 1996 first transect surveys
  - 25% wild *Tegula* infested
- 1996-1999 quarterly surveys, downward trend in prevalence
- 1999 only 4 old infestations
- 2000-2002 multiple surveys, zero sabellids found (2006 also)

May = *FIRST Eradication* of an introduced pest ☺

Geographic Distribution Surveys

- Coast-wide general health assessments of wild abalone
- Years 1998-2006, 28> site locations
- Locations often adjacent to historic point sources
- 60> samples per location
- No sabellids found
- Monitor WS and RLO in northern CA also

Questions?

From: Crane, Mark (LI, Geelong)
Sent: Friday, 22 September 2006 1:12 PM
To: 'Judith.Handlinger@dpiw.tas.gov.au'
Cc: Caroline.McGowan@dpi.vic.gov.au
Subject: RE: Abalone herpesvirus: Expert Panel Recommendations

Follow Up Flag: Follow up
Flag Status: Completed

Thanks, Judy. This will help until the final report comes out.

Mark

MARK CRANE Ph.D.
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-----Original Message-----
From: Judith.Handlinger@dpiw.tas.gov.au [mailto:Judith.Handlinger@dpiw.tas.gov.au]
Sent: Friday, 22 September 2006 13:09
To: Crane, Mark (LI, Geelong)
Cc: Caroline.McGowan@dpi.vic.gov.au
Subject: Re: Abalone herpesvirus: Expert Panel Recommendations

Hello Mark

I have the final version of the cut-down one (as the PPT), and a near-final version of the longer one - I think I have the tidy-up and updates also on my home laptop, but the definitive one was on Jeremy's computer, I think. (Nearly all the maim wording is here, but there could be some extra bits and notes etc that were resolved - I need to check this).

DELETION

Here is the latest that I have on the thumb drive. [s47f]. I will send you my other one on Monday but I presume Jeremy will also send this through to WADA as THE definitive version.

Dr Judith Handlinger
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Department of Primary Industries & Water, Tasmania

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<Mark.Crane@csiro.au>

22/09/2006 10:05 AM

To <judith.handlinger@dpiw.tas.gov.au>
cc <Caroline.McGowan@dpi.vic.gov.au>
Subject Abalone herpesvirus: Expert Panel Recommendations

Hi Judith,

Is it possible to get a copy of the recommendations that were discussed at our meeting on Wednesday/Thursday? I suspect that the FRDC Aquatic Animal Health Subprogram Committees will want to have these to assist them in making decisions about support for research proposals.

Thanks

Mark

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email: mark.crane@csiro.au

Research Priorities

To Do Now:

- Develop & Implement Australia-wide sampling regimes – make bench mark collections, and long term system for collecting apparently diseased samples

A: Diagnostic Tools

- Initially Use Available non-specific tools
- Develop Specific Tools
  - Purification of Virus
  - PCR – polymerase chain reaction
  - Comparison with other Herpes and like viruses

B: Patterns of Disease (EPIDEMIOLOGY)

- Distribution of virus / disease in abalone
- Host range (other species – especially gastropods?)
- Agent Characteristics; (Transmission, Latency, Impact on Larvae, Stability, Disinfection)

RESEARCH FRAMEWORK

To Do Now:

- Further EM to define the actual distribution of the virus within the host, and the range of tissues that can be infected. (To be followed by *In situ* hybridization.
- Evaluate the existing PCR's for oyster virus, on abalone
- Use specific probe for OsHV-1 on experimentally infected animals (temporarily sampled experiments) and possibly link with TEM.
- Improve Disease monitoring surveying protocols (use diver knowledge to direct surveying effort)
- Stability of virus in relation to temperature, disinfection, and detergents.
- Industry Based Monitoring of several infected populations over time to document progression of disease over time and eventual level of survivorship (u/w video and marked populations)
- Quantitative Surveying to prepare for documentation of impact of disease (Inside Disease, through buffer zone and in Distant disease free areas)
- Develop & Implement Australia-wide sampling regimes – make bench mark collections, and system for collecting apparently diseased samples

A: DIAGNOSIS

Purification

- **PCR (most likely to be developed by AAHL)**
- In situ hybridization (using PCR genetic information) – most likely developed in conjunction with IFREMER (e.g. International Scientific Linkages)
- Comparison of the herpes virus with the Taiwan herpes virus
- Collaboration where possible to help define the Chinese virus complex

B: EPIDEMIOLOGY

- Distribution of virus / disease
    - In abalone
    - In other gastropods
    - Age class, species effects (regarding impact on population make-up in impacted areas).
    - Density effects
- Control of disease
    - Transmission [source? Role of mucus?]
    - Experimental exposure of lavae / juveniles.
    - Latency / subclinical infection – does it exist?
        - Whether this is likely to change over time – will it attenuate
    - Stability of virus in relation to temperature, disinfection, and detergents.
- Origin of virus (see above + field studies)

FUNDAMENTAL RESEARCH (longer term)

- Host – virus interaction & mechanisms of disease, given the very short time-frame. (Could lead to tests for previous exposure to a virus – enhanced reactions.)
- Is the HOST changing – will the host become adapted
    - Even if it does, still an issue to live trade if OIE listed.
    - Probably easiest to study in farmed stock, but **related heavily to wild industry future.**
        - How does the host repond?
        - Is there variation between hosts in this response –
            - Epidemiology results
            - Genetic analysis of survivors (locus assisted selection etc, rather than continue Family Line program unchanged)

Comparative virulence ? [Define - too general ??*Virulence of the virus is unlikely to have changed in this time frame. Does this refer to virulence with regard to host density?* NB notes say with regard to Taiwanese virus]

Relationship of Rate of Transmission to Density

From: Malcolm.Lancaster@dpi.vic.gov.au
Sent: Friday, 29 September 2006 10:49 AM
To: Crane, Mark (LI, Geelong)
Subject: RE: abalone herpesvirus sequencing

Follow Up Flag: Follow up
Flag Status: Completed

Sounds good.
Bronwyn Murdoch has arranged a visit to AAHL to go through the em stuff with Alex, I think on Tues week (she is away today).
Malcolm

Mark.Crane@csiro.au

29/09/2006 10:42 AM

To: Malcolm.Lancaster@dpi.vic.gov.au
cc: Mehdi.Doroudi@dpi.vic.gov.au, Hugh.Millar@dpi.vic.gov.au, Sally.Ridge@dpi.vic.gov.au, Peter.Appleford@dpi.vic.gov.au, Serge.Corbeil@csiro.au, Crispian@frdc.com.au
Subject: RE: abalone herpesvirus sequencing

Thanks, Malcolm.

Sounds interesting. The FRDC AAHS met this week and I will be sending out recommendations concerning the draft full proposals next week. DPI Vic and AAHL should get together soon after to discuss.

Cheers for now.

Mark

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-----Original Message-----
From: Malcolm.Lancaster@dpi.vic.gov.au [mailto:Malcolm.Lancaster@dpi.vic.gov.au]
Sent: Monday, 25 September 2006 11:29
To: Crane, Mark (LI, Geelong)
Cc: Mehdi.Doroudi@dpi.vic.gov.au; Hugh.Millar@dpi.vic.gov.au; Sally.Ridge@dpi.vic.gov.au; Peter.Appleford@dpi.vic.gov.au
Subject: abalone herpesvirus sequencing

Mark:

While we were meeting at Spring St on Thursday, Simone Warner was finding out about our GS20 - see below.

Malcolm

----- Forwarded by Malcolm Lancaster/NRE on 25/09/2006 11:18 AM -----

Malcolm Lancaster

22/09/2006 04:14 PM

To: wada@pipeline.com.au
cc:
Subject: abalone herpesvirus sequencing

Harry:

My interpretation of the many meetings held recently regarding the abalone herpesvirus problem is that there was a clear consensus view that the virus needs to be sequenced ie. we need to establish the order of the nucleotide building blocks of the DNA of this virus.
Once DNA sequence information is available, then we can design PCRs to detect low numbers of the virus, do ISH (in-situ hybridisation) to work out where in the abalone the virus goes, and we can compare the Victorian virus to the oyster herpesvirus.

The method proposed by PIRVic Attwood and AAHL Geelong was to perform subtractive hybridisation to obtain viral DNA, clone this DNA in bacteria, and then sequence some of these short sequences of likely viral DNA. Viral purification would be a preliminary step.
This process is likely to take some months, and only yield a part of the total viral DNA sequence.

PIRVic Attwood in conjunction with the DPI's Plant Biotechnology Centre (headed up by Dr German Spangenberg) is now in a position to propose a quicker, state of the art method of sequencing which will enable the entire abalone herpes viral genome to be sequenced and aligned against the published sequence of the oyster herpes virus.
Virus purification is still the preliminary step (this could be done at AAHL Geelong or PIRVic Attwood).
Extracted DNA is then run through the DPI's Genome Sequencer 20 (the only machine of this kind currently in Australia) (a single run is all that is likely to be required) and then the accompanying software produces a consensus genome.
Further analysis of the sequence data will still be required, using one of Attwood's experienced bioinformatics experts, comparing it to a known genome (in this case the oyster herpesvirus genome).

For your consideration,

Malcolm Lancaster
PIRVic Attwood

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From: Crane, Mark (LI, Geelong)
Sent: Monday, 2 October 2006 2:10 PM
To: McColl, Ken (LI, Geelong)
Subject: FW: ab diissection
Attachments: Abalone dissection pictures.pdf

Follow Up Flag: Follow up
Flag Status: Completed

Hi Ken,

FYI

Please send any comments directly to Carolyn.

Mark

MARK CRANE Ph.D.
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-----Original Message-----
From: Carolyn S. Friedman [mailto:carolynf@u.washington.edu]
Sent: Tuesday, 26 September 2006 09:44
To: Judith.Handlinger@dpiw.tas.gov.au; Celia Hooper; Crane, Mark (LI, Geelong); Corbeil, Serge (LI, Geelong); apaul@panaquatic.com
Subject: ab diissection

HI Everyone,

I wanted to get your opinion of tissue samples to take in general and for GN..Can you let me know what you think of the attached file...it's a modification from a guide for WS sampling.

It was nice to see everyone last week.

cheers,

Carolyn
****
Carolyn S. Friedman, Ph.D
Associate Professor
School of Aquatic and Fishery Sciences
University of Washington
Box 355020
Seattle, WA 98195 USA

courier:

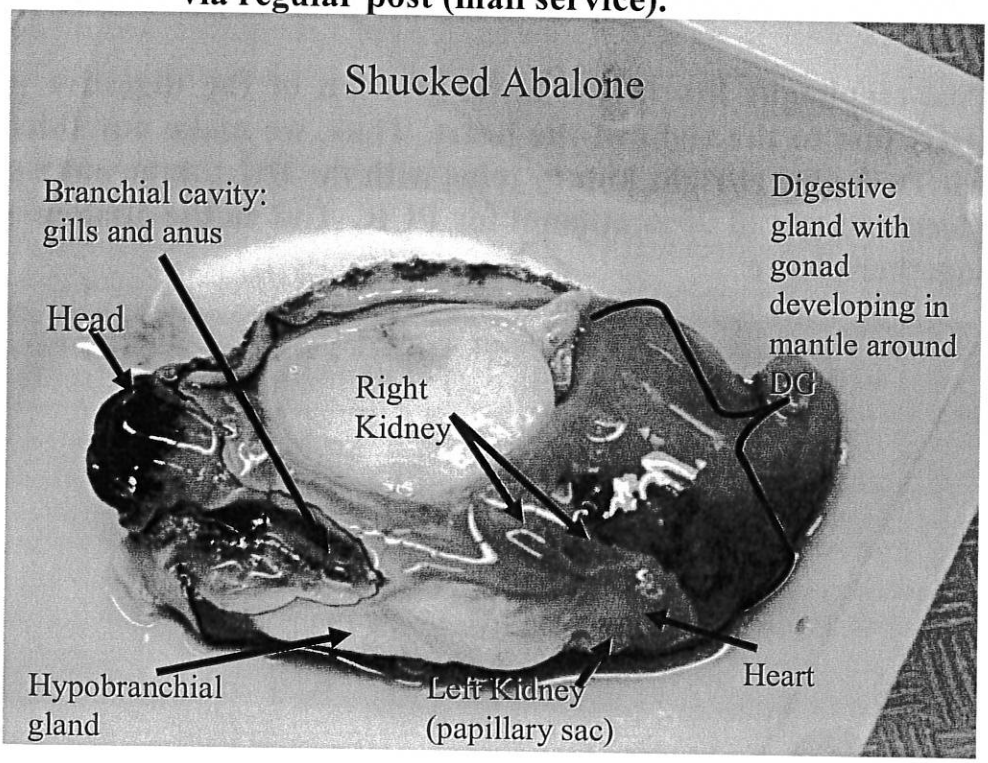
1122 NE Boat Street
Seattle, WA 98105 USA

Ph: 206/543-9519
Fax: 206/616-8689
****

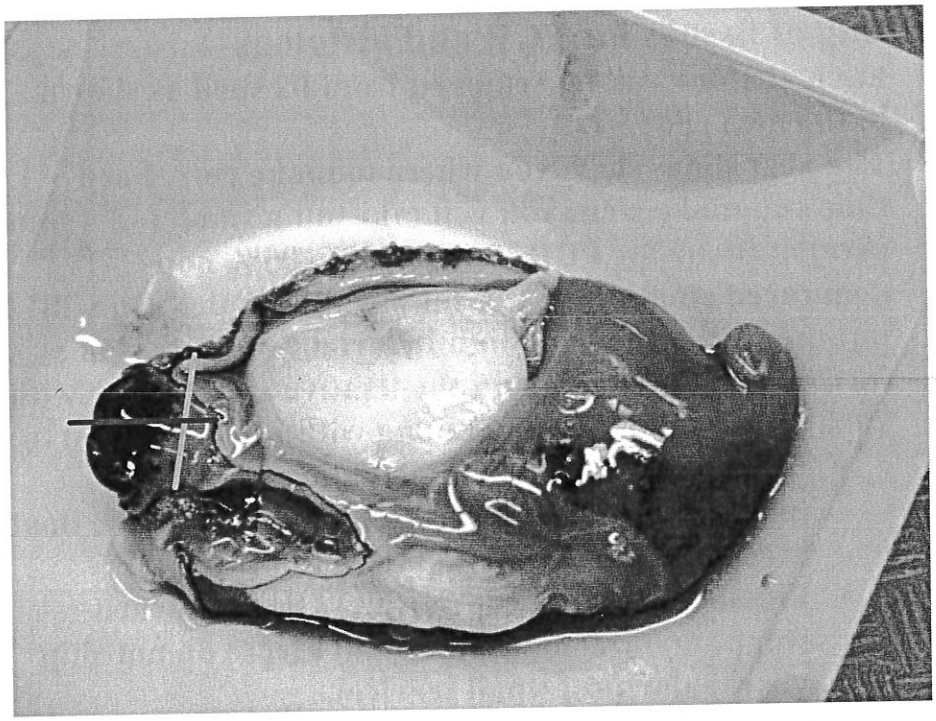
Dear Abalone Sampler, here are 3 pages of sampling instructions:

Splitting of tissues for PCR and histology

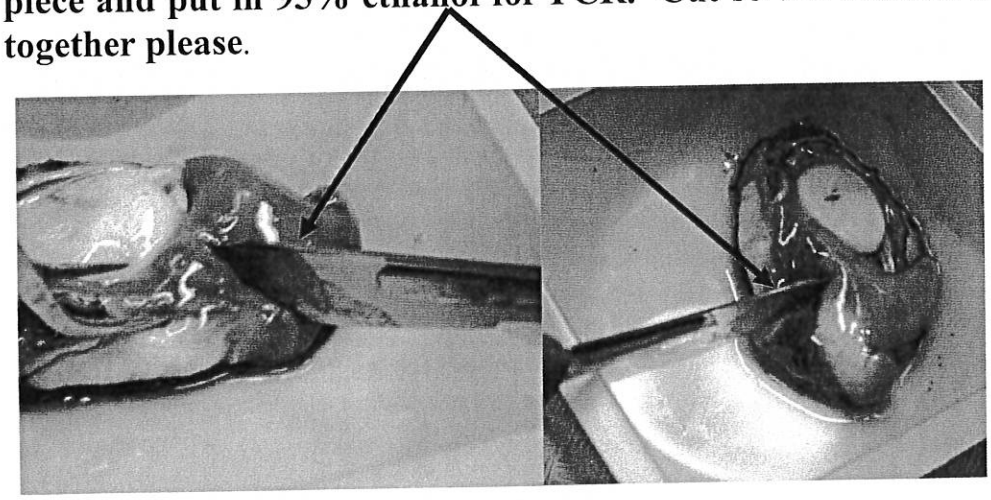
- **Each abalone will be removed from its shell as shown in the next slide.**
- **The next slides show you where to make cuts. Each time you make a cut you will take out a small (~3-4 mm slice) of tissue for PCR (and put into 95% non-denatured ethanol). After taking all samples you will put the rest of the body into Invertebrate Davidson's for 24hr and then pour off the fixative and replace with 70% ethanol for shipping (along with PCR samples) to:**
  - **C. Friedman, School of Aquatic and Fishery Sciences, University of Washington, 1122 NE Boat Street, Seattle, Washington 98105 USA.**
  - **Please note that a courier must be used when shipping hazardous material. Please do not ship via regular post (mail service).**



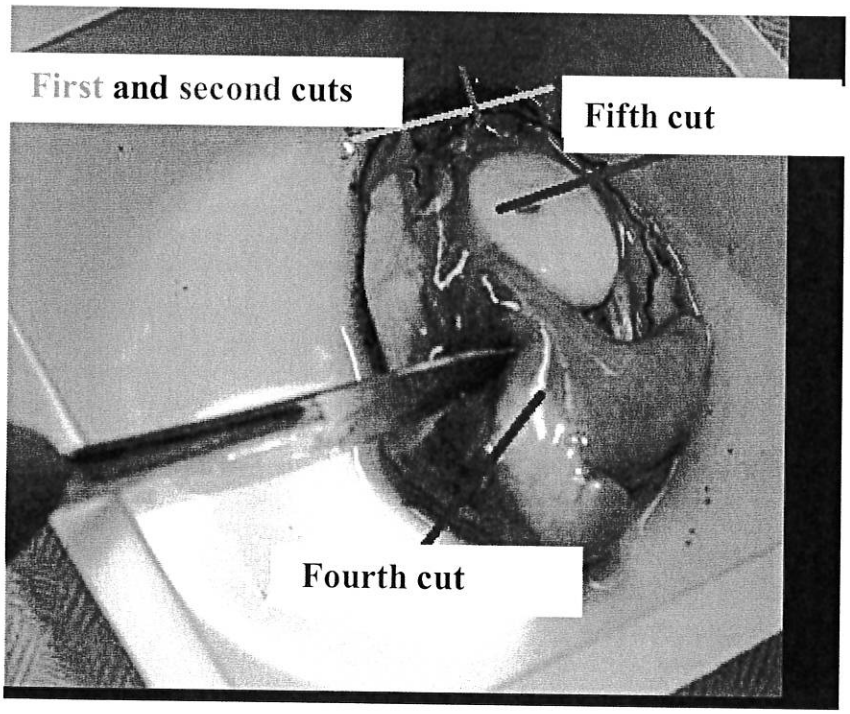
Please make the first and second cuts through the head and place half in 95% ethanol and half in fixative. See below for a side view of cuts one and two.



Post-esophagus lies in the lower portion of the digestive gland (DG) just to the right of the heart. Thus, we make our third cut where the heart/right kidney joins with the DG....take out a small piece and put in 95% ethanol for PCR. Cut so the abalone stays together please.



Then make fourth a cut mid-digestive gland (and also remove a small piece and put in the same PCR tube as before) and a fifth cut through the foot and remove a small piece for PCR. Cut so the rest of the abalone stays together please and put this tissue into Invertebrate Davidson's for 24 hr and then transfer to 70% ethanol for shipping.



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From: Crane, Mark (LI, Geelong)
Sent: Monday, 9 October 2006 11:44 AM
To: [s22] DELETION
Cc: Corbeil, Serge (LI, Geelong)
Subject: TRIM: Abalone herpesvirus research

DELETION

Dear [s22]

The FRDC Aquatic Animal Health Subprogram met on 27 September and one of the issues discussed was, of course, abalone herpesvirus. To up-date you, recommendations from the Subprogram, in consultation with FRDC, were:

A single collaborative project should be developed between AAHL and DPI Victoria that will cover the development and validation of molecular tools (diagnostic PCR and ISH probes) for the sensitive and specific detection and identification of abalone herpes-like virus. While there are many research issues that need to be addressed, this is considered to be the highest priority.

The Principal Investigator for the project should be Mark Crane, who will oversee project activities and manage the project, as FRDC Aquatic Animal Health Subprogram Leader.

A Steering Committee to be established to assist with governance of this project. It is generally accepted that abalone herpes-like virus has become a national, rather than a Victoria-specific, issue and therefore this Steering Committee will have a national focus and made up of representatives or delegates from the following national organisations:

All sectors of the abalone industry
Office of the Chief Veterinary Officer of Australia
FRDC (Crispian Ashby)
FRDC Aquatic Animal Health Subprogram (Mark Crane, Project PI)

The research proposal needs to be approved by this Steering Committee and will be expedited through the approval process within FRDC, out-of-session if needs be, to ensure the earliest possible start.

That was the recommendations. The way I see this committee working is as follows:

The steering committee should not be a cast of thousands but should include one industry representative from the abalone wild-catch sector and one from the abalone aquaculture sector. Can you suggest any other abalone sectors that need to be represented? Also can you suggest possible industry representatives who would be willing to sit on this committee. Once the research project is approved (this can be done out-of-session) I suspect that the committee would meet approximately every 6 months for no more than one day (we could, for example, have alternate meetings at AAHL and Attwood or any other mutually convenient venue) to hear progress reports from the scientists and to review milestone reports prior to submission to FRDC Aquatic Animal Health Subprogram for approval. It should not be too onerous but, in this way, industry will be kept fully informed about the research, will be able to comment on the research and the industry reps on the committee can report back to their constituents.

FRDC has asked me to get this committee together asap so that we can progress this issue promptly. As part of the process to develop the research proposal I need a clear understanding of how much money industry is prepared to contribute and from which sectors that money is coming from.

I trust that you support these recommendations and would really appreciate your assistance with the formation of the steering committee. I've discussed this with Harry Peeters and he mentioned that you have a meeting on 17 October and it might be worthwhile for me to attend so that we can progress this.

Regards

Mark

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From: Crane, Mark (LI, Geelong)
Sent: Friday, 3 November 2006 3:13 PM
To: Corbeil, Serge (LI, Geelong)
Subject: RE: Herpes virus disinfection project.doc
Attachments: Herpes virus disinfection project MCRev 061103.doc

Follow Up Flag: Follow up
Flag Status: Completed

Hi Serge,

I think this is useful - I have made some suggestions - see attachment.

While the project you have outlined will be useful for on-farm application, I feel there needs to be in addition, a more quantitative experiment with some more rigorous science for the student, perhaps the following would be the basic set-up:

Determine LD50 as described (is this by injection or by immersion?).

Treat at least 100X LD50 of the virus with recommended dose of virkon for the recommended time period.

Dilute viral suspension 100-fold to give 1X LD50.

Expose abalone to the suspension of treated virus (by immersion or injection).

Treatments and virus dose could be "checkerboard titrated". For example:

Virkon treatment	5%	2%	1%	0.5%	0%
Viral dose 10X LD50					
3X LD50					
1X LD50					
0					

But this would be 20 treatment groups which is probably too much but you can probably see what I am getting at.

Perhaps when the student is working out the LD50 (virus titration) it could be done in two virus groups: (1) Untreated virus (2) 100X Virus treated with the recommended dose of virkon and then diluted 100-fold prior to doing the titration.

Mark

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email: mark.crane@csiro.au

-----Original Message-----
From: Corbeil, Serge (LI, Geelong)
Sent: Friday, 3 November 2006 09:28
To: Crane, Mark (LI, Geelong)
Subject: Herpes virus disinfection project.doc

Herpes virus disinfection project (undergraduate French student)

Aim:

EstablishDetermine the sensitivity of the abalone herpesvirus, present in the water column and in the abalone mucus, to the disinfectant Vvirkon.

Objectives:

- Determine the LD_{50} of a viral suspension
-Determine the effectiveness of vVirkon to killinactivate the abalone herpesvirus from tanks previously containing infected tanksabalone.
-Determine if the abalone mucus protects the virus from the disinfectant.
-Determine if treatment of infected mucus with a mucolytic agent prior to or simultaneous with disinfection increases the virus susceptibility to Vvirkon.

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Experimental method:

-Prepare a large solutiontock of infectious virus from frozen infected abalone.
-Titer the viral stolutionck by establishing an LD_{50} in abalone.
-Set up small tanks containing sea water (18°C) for infection trials.
-Seed static water with titered virus solutuspension or with mucus obtained from infected, stressed abalone and incubate for 2 hours.
- Empty and spray the relevant tanks (#5 and #6, c.f. below) with a solution of mucolytic agent and incubate for 30 minutes.
-Empty all tanks and spray a 2% vVirkon solution in the inner surface of the tank, incubate for 30 minutes.
-Rinse and refill tanks with sea water (flow through system) and add healthy abalone in each tank.
-Monitor daily (for 15 days) the health status of abalone.
-During the course of the experiment, sample dying abalone and conserve tissues for subsequent histology and PCR analysis. At the completion of the experiment, sample tissues from all remaining live abalone for subsequent analysis.

Experimental groups:

Disinfectant :
-A (Virkon)
-B (Mucolytic agent N-acetyl L-cysteine)

Tanks:
1-2 Free virus + A
3-4 Mucus virus +A
5-6 Mucus virus +B and A
7-8 Free virus alone
9-10 No virus
11-12 Free virus + B
13-14 Mucus virus + B
15-16 Free virus + B and A

Outcomes:

The information obtained through this experiment will shed some light on the potential usefulness of using Vvirkon in the abalone industry setting as part of their biosecurity procedures for the control and eradication of the herpesvirus.

From: Van Driel, Rosey (LI, Geelong)
Sent: Monday, 27 November 2006 12:02 PM
To: Young, John (LI, Geelong)
Subject: RE: Submission for EM

Thanks John,
We are testing for Herpes. I'd worked it out when I started sorting out the samples because they are mostly ganglia.....then Alex rang and confirmed.
Thanks for the contact anyhow!

Cheers,
Rosey

From: Young, John (LI, Geelong)
Sent: Monday, 27 November 2006 11:43
To: Van Driel, Rosey (LI, Geelong)
Subject: RE: Submission for EM

Hi Rosey,

None of the AFDL staff had any notice this stuff was coming so I assumed Malcolm had told Alex. I cannot add anything beyond what is on the paperwork. I suggest you call Malcolm for more information (9217 4200).

Sorry I cannot be more helpful.

John

From: Van Driel, Rosey (LI, Geelong)
Sent: Monday, 27 November 2006 11:01
To: Young, John (LI, Geelong)
Subject: RE: Submission for EM

Hi John,
Are these abalone suspected Herpes or suspected parasite?

Cheers,
Rosey

From: Young, John (LI, Geelong)
Sent: Monday, 27 November 2006 10:30
To: Hyatt, Alex (LI, Geelong); Van Driel, Rosey (LI, Geelong); Crameri, Sandra (LI, Geelong)
Subject: Submission for EM

Hi All,

I have logged a submission from Malcolm Lancaster of gluteraldehyde-fixed abalone samples (06-04039) and am about to place them in the pass box and call the lab. I have left it to you to assign analyses.

Cheers,

John

85

From: Fiona.Gavine@dpi.vic.gov.au
Sent: Wednesday, 29 November 2006 9:34 AM
To: Crane, Mark (LI, Geelong)
Subject: Herpes like virus of abalone and detergents

Follow Up Flag: Follow up
Flag Status: Completed

Mark

I am working with Dr Mehdi Doroudi on the FRDC project that is developing codes of practice for the abalone aquaculture, commercial fishing and processing industries.

We are currently trying to make recommendations about when a detergent is required and when a disinfectant can be used. I have found a lot of general information stating that detergents kill herpes like viruses, but no definitive statements that it kills this one (abalone herpes-like virus). I was wondering if you or your team have done any work on this, or if you could give your opinion on the likelihood that detergents will be effective.

Thank you for your assistance with this matter.

Kind regards

Fiona G

Fiona Gavine
Senior Scientist
Aquaculture Section
Primary Industries Research Victoria
Private Bag 20
Alexandra
Victoria 3714

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Fax: 03 5774 2659