Disease and the Immune Response

1. General Immune Response
   1. Medzhitov & Janeway 2000. Innate immune recognition: mechanisms & pathways
      1. Innate immune recognition
         1. Pathogen-associated molecular patterns (PAMPS, e.g. LPS)
         2. Recognition of altered self or absence of self (viruses)
      2. Protein domains in pattern recognition
         1. C-type lectin
         2. Scavenger receptor cysteine-rich domain
         3. Leucine-rich repeat domain
      3. Function of domains: opsonization, uptake by phagocytes or dendritic cells, trigger signaling pathways
      4. Evidence that TLR can distinguish between types of pathogen
   2. Alavi et al. 2009. Development of an in-vitro assay to examine intracellular survival of *Perkinsus* *marinus* trophozoites upon phagocytosis by oyster hemocytes
      1. *C. virginica* and *C. ariakensis* hemocytes – *Car* not susceptible to mortality from *Pmar*
      2. Range expansion of *P. marinus* (Dermo) associated with global warming and trade
      3. Trohpozoites are killed by oyster hemocytes, which slows proliferation
         1. Some trophozoites survive intracellularly and are able to proliferate once the hemocyte dies
      4. *P. marinus* uses hemocytes as vector to infect other tissues and proliferate
      5. Threshold of number of phagocytosed throphozoites that may be killed by oyster hemolympth 🡪 over threshold (1:16) and trophos overwhelm hemocytes in both species
         1. Resistance of *Car* due to differential pathogenicity of *Pmar* to the 2 spp rather than infectivity
   3. Diacovich & Gorrel 2010. (review) Bacterial manipulation of innate immunity to promote infection.
      1. Bacteria recognized by pattern recognition receptors: TLRs, NCRs (c-type lectin)
      2. Bacteria pathogens in intracellular membrane-bound compartments are in an enclosed space where cell can launch ROS, reactive nitrogen intermediates, and antimicrobial peptides
      3. When intracellular by outside compartments, defense is ubiquitylation, proteasome degradation, autophagy, and inflammasome
      4. Bacteria protesases interfere with TLR pathway and avoid recognition by ubiquitin system
   4. Morga et al. 2009. Infection with the protozoan parasite *Bonamia ostreae* modifies in vitro hemocyte activities of *O. edulis*
      1. Adult *O. edulis* hemocytes incubated with live or dead *Bo* at 2 concentrations, 2h 15C
      2. Hemocytes engulf live and dead *Bo* although more live than dead inside hemocytes – only in large hyalinocytes and granulocytes
      3. Decreased non-specific esterase activity and ROS production, especially with more live *Bo*
      4. Parasite may play active role in incorporation into hemocyte
      5. *Bo* may be able to inhibit hemocyte esterases and ROS production
   5. De Decker et al. 2011. Responses of diploid and triploid *C. gigas* to *Vibrio* infection in relation to their reproductive status.
      1. Exp I: spawning, mature, and non-mature diploids: bath challenge with *V. splendidus* and *aestuarianus*, 18C for 40 h; then induced spawning with thermal stress and observed for 2 weeks
      2. Exp II: diploids and triploids (made 2 ways): injected with vibrios at 4 different months to encompass range of reproductive status; 2 doses (10^5 and 6) for 6 days
      3. Bath infection more natural but also more variable, not as reproducible
      4. Sexually mature oysters more susceptible (bath) and vibrio found mostly in gonads
      5. Decreased resistance during gonad maturation due to energetic and mechanic disturbances of gonad formation
      6. Triploids did not show increased survival, except during reproductive period
2. Oyster genes involved in immunity
   1. Bardiotti et al. 2006 Characterization of an atypical 18 chitinase from C. gigas: role in development and immunity
      1. Upregulated in oocytes and early larvae and hemocytes and 9 & 12 h post-injection with vibrios
   2. Badariotti et al. 2007. Characterization of chitinase-like protein (Cg-Clp 1 & 2) involved in immune of C. gigas
      1. Cg-Clp 2 expressed during metamorphosis in mantle edge and DG; in gonads during reproductive cycle
      2. CLp 1 up-regulated 9 & 12 h post-injections of LPS
   3. Badriotti et al. 2006 Phylogenetically conserved molluscan chitinase-like protein 1, homologue of human Hc-gp 39
      1. Cg-Clp1 expressed moderately throughout embryonic development
      2. Upregulated 10x during metamorphosis
      3. Most abundant in adult mantle edge and DG
      4. Not catalytically active – growth factor involved in tissue remodeling and cell proliferation, growth & immune homeostasis
   4. Wang et al. 2010. Microarray analysis of gene expression in C. virginica reveals oxidative stress and apoptosis
      1. P. marinus induces oxidative stress (ROSs)
      2. To eliminate parasites SOD converts to H2O2, which increases dangerous radicals, leading to apoptosis and production of apoptosis, anti-apoptotics (i.e. metallothionein)
      3. Genes 30 days post-infection
         * 1. Light & heavy infections: 807 genes differentially expressed in light infection, 545 in heavy, overlap of 319 genes. No significant difference in expression profiles between groups but much variability within groups.
           2. Upregulation: oxidative stress & apoptosis regulation (3 metallothioneins), lectins (light infection)
           3. Downregulation: defense, immune & apoptosis response (cathepsin s precursor, interferon-induced proteins…), galectin
   5. Gagnaire et al. 2007. Combination of a pesticide exposure and bacterial challenge: in vivo effects on immune response of C. gigas
      1. 8 pesticides for 7 days; separate challenge with V. splendidus for 168h; pesticide (7d) then Vs (72h)
      2. sampled hemolymph; 19 genes in hemocyte function (qPCR)
      3. pesticide: no mortalities; phagocytosis decreased in challenged; gene expression decreased in challenged
      4. bacteria: dose-dependent mortality (intermediate dose chosen for pesticide + Vs)
      5. pesticide + bacteria: mortality greater in challenged (48 & 72h post-injection); changes in gene expression with treatment
         1. down-regulated (4h): galectin, C-Src kinase, ankyrin, lysozyme, defensin, SOD
         2. up-regulated (24h): ficolin, galectin, LBP/BPI, C-Src kinase, ankyrin ProCL, SOD, TIMP, lysozyme, defensin
      6. decreased bactericidal activities in pesticide exposure
      7. upregulation of defense genes could harm host tissue and pesticides could change regulation of immune response
   6. Anderson et al. 1998. Effects of tributylin and hypoxia on the progression of infections in *C. virginica*.
      1. TBT exposure increased body burden - ~10x in 6 weeks
      2. In hypoxia (3 mg/L), higher TBT accumulation (non sig.) – as O2 decreases, pumping/clearance rates increase
      3. Mortality
         1. No effects of TBT alone
         2. Hypoxia caused higher mortality after 4 wks
         3. TBT+hypoxia had higher mortality for all 6 wks - synergism
      4. Only TBT alone caused decrease in ROS production (at 3 wks)
      5. Lysozyme concentrations non-significantly lower only in TBT+hypoxia
   7. Taris et al. 2009. Transcriptome response of *C. gigas* to infection with *V. tubiashii* using cDNA AFLP DD.
      1. Heat shocked oysters – identified families of high- and low-surviving then exposed to Vt
      2. 92 transcript derived fragments were differentially expressed
         1. cell/organism defense; detoxification/stress proteins; cell cycle; transcriptional regulation; cell signaling; metabolism; ribosomal proteins
      3. the different tolerance families showed different expression of these genes even before bacteria exposure
   8. Gueguen et al. 2003. Immune gene discovery by ESTs from hemocytes of bacteria-challenged *C. gigas*.
      1. Farmed adults (3-4 years)
      2. Injected 4 vibrios into adductor and collected hemolymph
      3. Potential immunity genes
         1. Proteases and protease inhibitors: expressed during humoral immune response
         2. Adhesive proteins: non-self recognition, opsonization, encapsulation, ficolins (animal lectins, first line of defense)
         3. Stress proteins: metal-binding ferritin and metallothionein
         4. Signal transduction: VaV protein (NFkB cascade), homologue of IkB
   9. Labreuche et al. 2006. Cellular and molecular hemocyte response of *C. gigas* to bacterial infection.
      1. Infected with *V. aestuarianus* via injection into adductor (5x10&7 cfu per oyster) and collected hemolymph 1,3,5,8 days post-challenge
      2. Day 1: increase of hemolymph protease activity
      3. Day 3: decrease hemocyte adhesive capacity, increase hemocyte ROS production, decrease EcSOD
      4. Day 5: EcSOD back to normal
      5. Day 8: increase in hemocyte phagocytic activity
      6. Phagocytic activity decreased when production of bacterial proteases at highest level
      7. Decrease in EcSOD while ROS increase – potential bacteria tactic of causing over-production of ROS so that host enters oxidative stress
   10. Gueguen et al. 2003. Immune gene discovery by ESTs generated from hemocytes of bacteria-challenged *C. gigas*.
       1. Injected adductor with mix of 4 vibrios: *anguillarum, metshnikarii, tubiashii,* s322; collected hemolymph after 12 hours
       2. Immune-induced proteins in Northern blot: metalloproteinase, transglutaminase, galectin
   11. Sauvage et al. 2010. QTL for resistance to summer mortality and OSHV-1 load in *C. gigas*.
       1. Determined family-specific mortality for OSHV-1 July-October
       2. Found QTL involved in resistance to summer mortality (large effect QTL)
   12. Gonzalez et al. 2005. Evidence in oyster of a plasma extracellular superoxide dismutase which binds LPS
       1. *C. gigas* SOD has 20% amino acid identity with extracellular human and nematode SODs = CgEcSOD
       2. shows activity in dismutation of superoxide anion
       3. only expressed in hemocytes
       4. binds LPS (lipopolysaccharides) and Lipid A (embedded in outer membrane of bacteria)
   13. Zhang et al. 2011. A *C. gigas* toll-like receptor and comparative analysis of TLR pathway in invertebrates.
       1. Challenged adult C.g. with *V. anguillarum*  in adductor – sampled 0-48h
       2. Cg-Toll1 expression: hemolymph>muscle>mantle>gill>DG>gonad
       3. Cg Toll expression hemolymph post-injection: elevated at 3 h, peaked at 12 h, always significantly > control group
       4. Most similar to other molluscs when compared to *Drosophila* and *C. elegans*
   14. De Decker & Saulnier 2011. Vibriosis induced by experimental cohabitation in *C. gigas*: Evidence of early infection and downregulation of immune genes.
       1. Hemocyte-bacterium interaction using Cg-BPI, EcSOD, IkB, TIMP
       2. Injected with mix of *V. aestuarianus* and *splendidus*
       3. At 24h post-injection sacrificed and placed valve + flesh in cohabitation tank with naïve oysters for 48h – samples taken 2-48h and 7 & 19 d post-cohabitation
       4. Cohabitants with vibrio-injected oysters
          1. Significant mortality
          2. Vibrio detected in hemolymph and organs of cohabitants within 2 hours
       5. Evidence of avirulent vibrio infections and heterogeneity in susceptibility to vibrio
       6. Avirulent and virulent vibrios transmitted horizontally in water
       7. No virulent vibrios in survivors of cohabitation trial at 7 & 19 d
       8. Avirulent vibrios caused increased expression of all 4 genes in controls at 2 h post-cohabitation, gone by 6h
       9. Virulent vibrios caused decreased expression at 2 h post cohabitation – strategy of pathogen? Also gone by 6h
   15. Seo et al. 2010. Multiple antibacterial histone H2B proteins are expressed in tissues of American oyster *C. virginica*
       1. Extracted adult tissues for antibiotic isolation
       2. Cv-H2B-1 and -2 are most active against gram negative bacteria (more common oyster pathogens)
       3. Strong activity against *v. vulnificus* of all 3 antimicrobial histone H2 proteins
       4. Very high levels of antimicrobial histones present
   16. Morga et al. 2011. Molecular responses of *O. edulis* haemocytes to an in vitro infection with *Bonamia ostreae*.
       1. Took hemolymph from adult oysters
          1. Hemocytes + parasites 2h – SSH
          2. Hemocytes + live *Bo*, hemocytes + dead *Bo*, 2h – expression level of genes based off of SSH
       2. ID of immune related genes
          1. Galectin: closely related to other molluscs
          2. Interferon regulatory factor-like – related to IRF-1 and -2
       3. Gene expression
          1. + live *Bo* – increase omega glutathione s-transferase (OGST), SOD, TIMP, Gal, IRF, cytochrome oxidase III, filamin; no change in cyt P450 or hsp90
          2. + dead *Bo* – increase cytP450, IRF; decrease Gal, OGST
       4. TIMP: typically part of invertebrate host response
       5. OGST and SOD: increased maybe because increase in cytotoxic compounds during immune response
       6. Filamin: increases cytoskeletal polymerization to facilitate *Bo* internalization
       7. IRF: diverse immune functions
       8. Galectin: lectins are pattern recognition receptors, recognize and opsonizatoin of pathogen
   17. Tirapé et al. 2007. Expression of immune-related genes in C. gigas during ontogenesis.
       1. Non-pathogenic bacteria – exposed embryos through spat for 24h
       2. No transcript at any stage for def, def2, ficolin 3
       3. Expression (cPCR) in oocytes, 2-4 cell, trocophore & spat: a-2 macroglobulin, MMP, Drac3, tal, timp, galectin8
       4. Expression (cPCR) in all stages: MyD88\*, ECSIT\*, TRAT3\*, rel\*, LBP/BPI, EcSOD, Ring3, Lyn, vav
          1. \*signal transduction
       5. in spat EcSOD and tal expressed on hemocytes attached to blood vessel endothelium, EcSOD in circulating hemocytes and infiltrating in gills, mantle, DG
       6. stage-specific upreg of genes in response to bacteria
          1. no change in EcSOD, gal8, or tal
          2. LBP/BPI upreg in veligers
          3. Timp upreg in D-hinge and veliger (different time points)
          4. Drac3 upreg in D-hinge, veliger, and pediveliger although at different time points
          5. MyD88 upregulated in D-hinge and veligers
       7. Oocyte and 2-4 cell expression probably maternal effects
       8. Differential gene expression may explain variation in disease susceptibility at different stages of development
   18. Yu et al. 2011. Polymorphisms in a serine protease inhibitor gene and its association with disease resistance in *C. virginica*.
       1. Find SNPs linked to Dermo resistance in coding of serine protease inhibitor – 10 individuals from 3 populations
       2. Genotyped SNP before and after disease-caused mortality
          1. Allele shift C>A
       3. Higher frequency of C in resistant wild populations and resistant strains; allele may not cause resistance, may be linked
   19. Hoh et al. 2010. Presence and characterization of multiple mantle lysozymes in *C. gigas*
       1. Extracts of mantl tissue showed antibacterial activity to most Gram-positive and no Gram-negative
       2. From oysters tested – variability in antibacterial activity
       3. Lysozyme CGL-3 expressed more highly in mantle than gill, DG or hemolymph
       4. Mantle probably has multiple lysozymes – peaks of antibacterial activity at 2 different pH
          1. CGL-1 and CGL-3 are humoral defense factors
   20. Schikorski et al. 2011. Experimental ostreid herpesvirus 1 infection in *C. gigas*: kinetics of virus DNA detection by qPCR in seawater and oysters
       1. Adult – carriers infected with OSHV-1 by injection, then cohabitation for 48h
       2. Sampled 0-144h post cohabitation (22C)
       3. Mortality of cohabitated slower and less than injected
       4. Amount of virus in water stable to 48h during cohabitation then fluctuated
       5. Significant correlation between mortality and amount of virus in hemocytes, gill, mantle, adductor, but not DG
       6. Highest virus amount found in hemocytes
       7. Maximal virus amounts at 96h
   21. Bezemer et al. 2005. Breeding for QX disease resistance negatively selects one form of defensive enzyme, phenoloxidase, in Sydney rock oysters
       1. 3 populations: wild type (QX naïve), QXR4 (4th generation bred for resistance), CR (wild oysters have survived QX outbreaks since 1976)
       2. 5 forms of phenoloxidase – frequencies differed among populations
       3. outplanted – phenoloxidase activity decreased before *M. sydnei* detected
          1. phenoloxidase form b much less frequence in survivors in QXR4 and wild type
          2. b also underrepresented in CR oysters
       4. breeding for QX resistance negatively selects for b
       5. phenoloxidase activity could be biomarker of disease onset
3. Other invertebrates and Immunity
   1. Pinsino et al. 2007. Coelomocytes and post-traumatic response in the common sea star *Asterias rubens*.
      1. 4 cell types in coelomic fluid: phagocytes, white & red amoebocytes, vibratile cells, and hemocytes
         1. phagocytes are most abundant in freely circulating cells, ~95%
      2. Post-stress in sea stars: increase # of circulating coelomocytes at 4.5 and 6 h
         1. Either rapid division of circulating stem cells or recruitment from epithelial tissue
         2. Show recruitment to site of injurty
      3. Increase in Hsp70 (protein) expression at 1 h post-trauma, peaking at 6 h, and still elevated at 24 h
   2. Parry & Pipe 2004. Interactive effects of T and Cu on immunocompetence and disease susceptibility in mussels.
      1. At 2 different T (10 & 15 C) Cu (0.02 and 0.05 ppm) then Vt and Cu + Vt
      2. Cu then Vt:
         1. fewer hemocytes at low T (10C)
         2. low Cu increased hemocytes, high Cu decreased
         3. increased phagocytosis by just increasing T to 15C
         4. phagocytosis increased with low Cu or Vt and then decreased at higher levels of both
      3. Cu + Vt
         1. 0.02 ppm Cu: number of circulating hemocytes decreased – hemocytes may be migrating to tissues
         2. 0.05 ppm: increase number of hemocytes
         3. at 15 C, increased SOD inhibitable superoxide production
   3. Tonteri et al. 2010. Beyond MHC: signals of elevated selection pressure on Atlantic salmon
      1. 8 populations of salmon with different tolerance levels to parasite *Gyrodactylus salaries*
      2. 94 loci are linked to MHC or immune relevant genes of unknown function
      3. tested links between allele distribution and mortality due to *Gs*, salinity, T, lat & long
      4. significantly more immune-relevant loci showed signatures of selection compared to those with no obvious immune function link
      5. alleles of immune-relevant genes are more linked to latitude and T – differing selection pressures could indicate pathogen-driven selection (more pathogens in warmer latitudes)
      6. variability of non-MHC immune genes also important for pathogen response in variable T environments
   4. Canesi et al. in press. Effects of vibrio challenge on digestive gland biomarkers and antioxidant gene expression in *M. galloprovincialis*
      1. Wild adults exposed to *V. splendidus* and *anguillarum* (injection of heat-killed)
      2. Sampled DG 3, 6, 24 hour post-injection
      3. Decreased lysosomal membrane stability in vibrio-inj. At 6 & 24 h; increased lysosomal lipofuscin content and antioxidant enzyme activity (enzyme activity and gene expression)
   5. Venier et al. 2011. Insights into the innate immunity of *M. galloprovincialis*.
      1. In silico search to make microarray – bacterial challenge and application
      2. At 3h post-challenge, upregulated genes outnumber down-regulated, but numbers even out by 48 h
      3. Downreg of AMP, acute phase response proteins, and macrophage migration inhibitory factor – possibly due to toxicity of live bacteria
      4. Upreg of allograft-inflammatory factor, SOD small HSP20, plasminogen, recognition receptors, intracellular signaling, cytoskeleton remodeling/motility
      5. At 48 h, increase in protease inhibitors, TNF signaling
      6. Need to consider other genes involved in response, not just immune genes
      7. 3h = mounting inflammatory response
      8. 48 h = general stress condition
4. Immunity and the Environment
   1. Dionne et al. 2007. Clinal variation in MHC diversity with termperature: evidence for the role of host-pathogen …
      1. Role of T in maintenance of MHC class IIB diversity along a latitudinal gradient in Atlantic salmon
      2. MHC allelic richness increased significantly with T (T corresponds to latitude) = declining allelic richness (Ar) with latitude
      3. Msat Ar did not vary with T
      4. MHC amino acid diversity higher for pathogen binding region than non-PBR
      5. MHC aa at PBR showed logarithmic increase with T (no trend in non-PBR)
         1. MHC aa diversity at PBR shows logarithmic increase with bacterial diversity in rivers
         2. Logarithmic increase of diversity = population is reaching plateau of MHC diversity
      6. Dn/ds of PBR>non-PBR – positive selection on PBR
      7. T influences pathogen diversity and virulence -> clinal change in pathogen-driven balancing selection intensity -> host shows local adaptation -> diversity gradient in MHC genes
   2. Ivanina et al. 2011. Interactive effects of Cd and hypoxia on metabolic responses and bacterial loads in *C. virginica*.
      1. Adult *C. v.*
      2. Cd added at 50 ug/L (normal estuarine range is 15-80) – 30 d acclimation then 2 weeks in normoxia or hypoxia (5% O2)
      3. Higher Cd accumulation in hypoxia
      4. Bacterial communities in different treatments
         1. Cd + hypoxia – more *V. vulnificus*
         2. Cd + normoxia – more *V. parahaemolyticus*
      5. Acute hypoxia (up to 24h) – reduced O2 consumption rates
      6. O2 consumption not different after 2 weeks and no Cd effect
      7. No accumulation of anaerobic end products
      8. Cd + normoxia:
         1. lower HIF1a expression than control (in HP)
         2. downreg hexokinase expression (not seen in hypoxia); increased HK enzyme activity (not in hypoxia)
         3. increase adolase expression and enzyme activity
         4. increase citrate synthase enzyme activity
      9. Hypoxia:
         1. decreased expression of HIF1a (not in Cd)
         2. increase citrate synthase enzyme activity
         3. decrease gene expression cytochrome c oxidase, increased enzyme activity
      10. Cd + hypoxia:
          1. upreg of PHD-2 expression
          2. downreg of adolase and enzyme activity
          3. decrease citrate synthase expression
      11. elevated enzyme activity of CS and COX indicates tissue aerobic capacity is upregulated to compensate for reduced O2 and allows SMR to not change
      12. no SMR increase in Cd exposed maybe because oysters from northern populations which are less sensitive to Cd
      13. Cd may inhibit HIF1a at transcriptional level
      14. Lack of correlation between gene expression and enzyme activity may be post-translational regulation
   3. Kuchel et al. 2010. Immunosuppressive effects of environmental stressors on immunological functions of *Pinctada imbricate*.
      1. Adult Akoya pearl oyster
      2. Stressors: low salinity (25 ppt), mechanical agitation, air exposure
      3. Change in number of granulocytes with salinity
      4. Phagocytosis inhibited by all 3 stressors
      5. Increase in total protein in mechanical stress and salinity
      6. Acid phosphatase activity (lysosomal enzyme in phagocytes of bivalves) increased in air exposed
      7. Moderate stresses can have significant impacts on the immune system
5. Bacterial Response
   1. Labreuche et al. in press. V. aestuarianus zinc metalloprotease causes lethality in C. gigas
      1. Bacteria in hemolymph of diseased oysters in France
      2. Protease activity of extracellular products is zinc-dependent
      3. Expression of metalloprotease significantly reduces hemocyte phagocytic activity
   2. Lipp et al. 2002. Effects of global climate on infectious diseases: the cholera model. (*Vibrio cholerae*)
      1. Sever weather events (e.g. ENSO) increase enteric disease
      2. Climate also influences other nonenteric vibrios, in terms of abundance and ecology
         1. Associated with warm waters and moderate salinity so change could affect distribution
         2. Also have ecological relationships with plankton and hosts
      3. Diseases have distinct seasonal patterns of detection/isolation of pathogen and prevalence of disease
      4. Strains can be clinical (toxigenic) or environmental (nontoxigenic) depending upon: acquisition of virulence genes from environment which is affected by seasonal environmental factors
      5. Vibrios do well at higher T and can survive at very low salinities; also associate with zooplankton
   3. Duperthuy et al. 2010. The major outer membrane protein OmpU of *V. splendidus* contributes…
      1. Knock-out OmpU greatly reduces oyster mortalities; wild type not cleared as readily from immune system
      2. OmpU protein is essential in V.s. virulence to C.g. and is resistant to antimicrobial peptides/protiens
      3. Dual role of OmpU: 1. Resistance to host defenses, 2. Host recognition.
   4. DePaola et al. 1998. Phages infecting *V. vulnificus* are abundant and diverse in oysters.
      1. Phages virulent to *V.v.*  apparent in oyster tissues throughout the year in Gulf of Mexico
      2. 6 distinct phage morphologies belonging to 3 bateriophage families
      3. 5 *V.v.* strains have 16 morphotypes, phages have differing degrees of effectiveness
   5. Elston et al. 2008. Re-emergence of *V. tubiashii* in bivalve shellfish aquaculture.
      1. Originally bacillary necrosis, became *V. t.* in 1984.
      2. Different isolates had different degrees of pathogenicity
      3. Larvae cease swimming and remain closed
      4. Toxigenic effects include (all stages of metamophosis and juveniles): loss of velar cilia, exfoliation of velar epithelium, failure to swim
      5. Total marine agar culturable bacteria greater in cooler T, associated with upwellings in late June-Aug (nutrients) followed by warmer T
      6. Some pathogenic isolates do not have genes for extracellular protease or hemolysin