1. Changing environment
   1. Ocean acidification
      1. DIC
         1. Anthropogenic DIC input has contributed to increased corrosivity of Puget Sound waters (Feely et al. 2010)
      2. TA
         1. Higher TA of PS waters compared to open ocean make relative contribution of increased DIC to OA less (Feely et al. 2010)
      3. Omega
         1. In both winter and summer, PS waters are undersaturated wrt aragonite (Feely et al. 2010)
         2. Waters in the CCS are become increasingly less saturated wrt aragonite (frequently under 2) compared to pre-industrial; also strong seasonal influence – especially low in summer and winter (Hauri et al. 2009)
         3. Formation is thermodynamically favored when available Ca and CO3, but increased CO2 decreases availability of CO3
         4. Exposure to low saturation states during spawning result in decreased production later (Barton et al. in press)
      4. Carbonate chemistry in the near-shore environment
         1. CO2 radiative forcing will decrease early season wind stress and upwelling and increase peak- and late-season in northern CA Current (Diffenbaugh et al. 2004).
   2. Contaminants
2. Acclimatization and Adaptation
   1. Physiology
      1. Energy
         1. Decreased expression of ATPases in larval urchins could signal shift to more efficient transporters (Todgham & Hofmann)
      2. Intracellular and extracellular pH
         1. No modulation of pHe in C. gigas exposed to pH of 7.6; also metabolic differences in gill and mantle (not other) tissues (Lannig et al. 2010)
         2. Intracellular pH is 0.5-0.8 units lower than extracellular; intracellular has the largest amount of non-bicarbonate buffers (Portner et al. 2004)
         3. Frequently causes metabolic depression (Portner et al. 2004)
         4. Some organisms remove fixed number of H+ from their calcifying fluid while others maintain a certain internal:external H+ balance; OA will be more negative for species that have weaker control over calcifying compartment pH (Ries 2011)
      3. Acid-base balance
         1. Cuttlefish are able to regulate pHe and pHi to an extent by increasing [HCO3-] (Gutowska et al. 2010)
         2. Limpets are able to compensate and maintain balance by increasing hemolymph concentrations of Ca and HCO3 (Marchant et al. 2010)
         3. Adult sea urchins buffer by increasing HCO3 in coelomic fluid with evidence of test dissolution (Miles et al 2007)
         4. Velvet swimming crab compensates for low pH (efficiently) by shell dissolution, evidenced by increased [HCO3-] and [Mg2+] – increased hemolymph magnesium could have a narcotic effect (Spicer et al. 2007)
         5. Increased NH4+ excretion in blue mussels may indicate attempt at proton removal and also increased energy loss through upregulated protein metabolism (Thomsen and Melzner 2010)
         6. Urchin larvae upregulate H+ transporters under high CO2; also down-regulation of Na+ transporters, which is tightly linked to acid-base regulation (Todgham & Hofmann)
      4. Immune
         1. Sea star decreased activity of hsp70 and mapk-p38 as well as lowered phagocytic activity of coelomocytes and reduced number of coelomocytes after 1 week, maintained through 6 mos at low pH (Henroth et al. 2011)
   2. Range shifts
   3. Fitness
   4. Adaptive potential
      1. Controlled crosses of corals and raised at elevated T – found additive genetic effects and high heritability for responsiveness to settlement and gene expression of βγ-crystallin = potential adaptive responses to selection (Meyer et al. 2009).
      2. Qst (measure of quantitative trait) greater than Fst in sunflowers = adaptive differentiation in flowering time; due to paralogue-specific gene expression divergence (Blackman et al. 2011)
3. Calcification/Shell Mineralization
   1. Shell protein content
   2. Intracrystalline fraction = closed system (i.e. maintains integrity of amino acid content); taxon-specific differences in intracrystalline amino acid content. Measured by reverse-phase HPCL with fluorescent detection (Penkman et al. 2008).
   3. *L. elliptica* increased expression of calcifying gene CHS (chitin synthase) in response to decreased pH 7.78 (Cummings et al. 2011)
   4. no change in barnacle calcification after 104 days, but increased mortality so perhaps energy needed to maintain calcification = costly (Findlay et al. 2009)
   5. smaller C. gigas at higher pCO2 (kurihara et al. 2007)
   6. both temperature and pCO2 affected mortality in juvenile Arctic pteropods; however, pCO2 only had an effect on shell growth and integrity after 29 days (Lischka et al. 2011)
   7. early sea urchin larvae increase expression of biomineralization genes under low pH (Martin et al. 2011)
   8. downregulation of genes implicated in calcification in sea urchin larvae (Todgham & Hofmann )
4. Maternal Effects
   1. Larvae from selectively bred oysters and from oysters acclimated to high pCO2 are more tolerant of high pCO2 (grow larger) (Parker et al. 2011)
5. Oxidative Stress
   1. Glutathione-s-transferase in C. virginica changes expression in response to metals that produce ROS (Chapman et al. 2011)
   2. 3 hypotheses for how elevated pCO2 can cause oxidative stress (Tomanek et al. 20110
      1. CO2 reacts with peroxynitrite (a reactive N species), which would oxidize multiple cellular compounds and directly increases ROS production
      2. High CO2/low pH could impact mitochondrial function and/or non-enzymatic production of free radicals – oysters not able to regulate pH well so subject to acidosis, which could affect intracellular ETC or release of chelated transition metals from intracellular stores
6. Metabolic Rate
   1. Bivalve *Laternula elliptica* had increased (> 2x) basal metabolic rate at lower pH (7.78) (cummings et al. 2011)
   2. OA causes developmental delay and decreased scope for growth in purple urchin larvae – indicative of impacted energy budget from abiotic stressor (Stumpp et al. 2011)
   3. Blue mussels increased metabolic rate after 4 months at elevated pCO2 coupled with decreased growth (Thomsen and Melzner 2010)
7. Immune Suppression
8. Multiple Stressors
   1. Abnormal larvae of S. glomerata and C. gigas at higher pCO2, but increased temperature caused more abnormal larvae for S. glom than gigas; interacting effects caused decreased size in both species (Parker et al. 2010)
9. PCP
   1. Increases incidence of intraepithelial oviduct cysts in ewes and also disrupts endocrine system (decreases thyroxine concentration and increases insulin) – disruptions to endocrine can inhibit ability to deal with environmental stress (Rawlings et al. 1998)
   2. Pacific oysters have a limited ability to metabolize PCP (11%) and are able to get rid of it via depuration; most PCP is absorbed in the viscera (Shofer & Tjeerdema 1993)
   3. Oxidative phosphorylation uncoupler (Winbech 1954)
10. Mitochondrial Phosphorylation
    1. From glycosylation and Kreb cycle, products are transported to ETC in mitochondria. Via redox reactions, electrons are transferred down chain to efficiently produce energy.
11. Molecular Chaperones
    1. Hsp70 in C. virginica responds to both temperature and pH, but response to pH is relatively weak (Chapman et al. 2011)
    2. Downregulation of multiple molecular chaperones at high pCO2 for urchin larvae (Todgham & Hofmann)
12. Statistics
    1. ANOVA
       1. Multiple t-tests increase risk of type I error (detecting a difference when there isn’t one). Compares variability within and between groups. When levels of a factor are specifically chosen (not at random), these effects are *fixed*; if effects are chosen at random then null hypothesis needs to be stated more generally. Assumes that population variances are equal and have that samples have been chosen randomly – in general, ANOVA is robust to deviations from these.
    2. Tukey’s HSD
       1. Used in replacement of doing multiple t-tests. Calculates a *q* statistic by dividing the difference between 2 sample means by the SE. If sample sizes are unequal then use Tukey-Kramer procedure (different SE). Not robust to differences in variances, so use the Welch approximation of SE, but each sample size should be at least 6 (this moderates Type I error rate).
    3. GLM
       1. Variance is not always constant and errors are not normally distributed
       2. Linear model: equation that contains mathematical variables, parameters and random variables and is linear in parameters and random variables
       3. 3 properties: error structure, linear predictor, link function
          1. error structure: poisson for count data; binomial for data on proportions; gamma for constant coefficient of variation; exponential for time to death/survival analysis
          2. linear predictor: has one term for each parameter
          3. link function – want one that produces minimal residual deviance: log link for count data, explanatory variables with multiplicative effects; logit link for proportion data; probit for bioassay; complementary log-log for dilution analysis, binary, proportional response
    4. T-test
       1. Testing the difference between the means of 2 groups: the difference between the means divided by the standard error of the difference between the sample means (transformed to the square root of 2 times the pooled sample variance divided by the sample size). Assumes that variances are equal. When variances (and/or sample sizes) are not equal, simply replace the pooled variance with the SE of the difference between the means.
    5. Chi square
       1. Used for testing differences in sample variance. Assumes that sample observations are normally distributed. Can be used to construct CIs and test hypotheses about the population variance.
    6. PCA
13. Housekeeping gene
14. Histology
    1. Invert Davidson’s (Shaw & Battle 1957)
    2. Ehrlich’s haematoxylin and eosin
    3. Metaplasia
    4. Hemocyte influx
15. Next-generation sequencing
    1. SOLiD: Sequencing by Oligonucleotide Ligation and Detection
       1. Individual fragments from the library attach to beads
       2. Beads have universal adapter sequence attached
       3. Emulsion PCR is performed on individual beads
       4. PCR’d beads are covalently bound to a glass slide
       5. Primers attach to universal adapter
       6. Fluorescently labeled probes ligate to sequencing primers; the colors of the probes are specific to dinucleotide sequences
    2. Assembly to a backbone
    3. RPKM
       1. (number of reads per contig)x(1000/length of contig)x(1x106/total number of mapped reads in the assembly)
    4. Qvalue (Storey 2002)
       1. Positive false discovery rate (pFDR): fixes rejection region then estimates alpha (probability of type I error); has greater power because rejects a greater number of hypotheses while controlling the same error rate; tests multiple hypotheses at once
       2. Q-value is a p-value for pFDR; gives hypothesis testing error measure for each observed statistic with respect to pFDR; minimum pFDR that can occur when rejecting a statistic with a certain value for the set of nested rejection regions
16. Proteins
    1. SDS-PAGE
    2. Western blots
17. Heat shock
    1. After OA exposure, C. gigas from higher pCO2 elevated SMR more at 20 and 25°C (acclimation T = 15) than controls (Lannig et al. 2010)
18. Selective Breeding
19. Reproduction
    1. Gametogenesis
    2. Gonad-somatic index
    3. Glycogen
    4. Protein
    5. lipids
20. HRM
    1. 454 sequence discovered >33,000 SNPs; 65-75% of 48 tested were polymorphic in wild populations. Using cDNA to find SNPs in non-models means that can find causal genes underlying complex traits (Wang et al. 2009)
    2. Distinguishes genotypes with high accuracy. Using dye LCGreen is low cost and time saving. (Lieuw et al. 2004)
       1. Benefits of small amplicons: simple assay design; Tm differences among genotypes increases; shorter cycling times because of low denaturation T and increased specificity; no T holds for polymerase extension
       2. Disadvantaes: narrows choice of primers; less amplicon carry-over contamination control; primer dimer confusion
21. Cervus
    1. Uses likelihood to assign offspring to parents
    2. Uses simulations to assess confidence of assignments
       1. Are loci suitable for parentage analysis?
       2. Find critical values of likelihood ratios so that confidence can be determined with real data
22. Apparent Positive Effects of OA
23. Sweepstakes Selection
24. Immunofluorescence
    1. Vibrio-specific antiserum attached to fluorescing molecule – apply to sections and visualize (Elston & Liebovitz 1980)
    2. Deparaffinize sections and rehydrate through graded ethanol series. Equilibrate in PBS, block for 30 minutes. Incubate for 30-60 min in rabbit or mouse antiserum diluted to either 10-2 or 10-3 in PBST/BSA or in undiluted hybridoma culture medium (RPMI-20). Wash sections with PBST and incubate with conjugated, species immunoglobulin-specific secondary antibodies for 30 minutes. FITC-conjugated secondary antibodies diluted to 10 µg Ig mL-1 with PSBST/BSA. FITC-stained slides washed with PBST, counterstained 30s with 0.01% (w/v) Evan’s blue, mounted in buffered glycerol, and observed with an epifluorescence microscope. (Dungan & Roberson 1993)
       1. Changes from clegg 1998: primary hsp-70 antibody diluted 1:50 and secondary fluorescent isothiocyanate-labeled rabbit antirat serum (Sigma) was diluted 1:250. Stained tissues viewed with both interference contrast and epifluroescence optics using an Olympus BH-2 microscope.
25. Measuring Ne
26. Miscellaneous
27. Homologous: trait is shared and used for similar purpose because it evolved from a common ancestor. Paralogs arise from a gene duplication event that can be after speciation (salmonids) or pre-speciation (chimps and humans).