Ocean Acidification and Other Stressors

1. OA by itself
   1. Gutowska et al. 2010. Acid-base regulatory ability of the cephalopod in response to environmental hypercapnia.
      1. 48h exposure to low pH (7.1)
      2. immediate response: cuttlefish blood pCO2 increased with water pCO2, drop in blood pH (only 0.2 units compared to water’s drop of 1 unit), small decrease in pHi
      3. after 6h: blood [HCO3-] ~ 4x that of water
      4. acid-base regulatory ability is energetically costly
   2. Beniash et al. 2010. Elevated level of CO2 affects metabolism and shell formation in *C. virginica*
      1. Bubbled CO2 and ambient air: 380 ppm (8.2), 3500 ppm (7.5)
      2. Juveniles exposed 20 weeks, adults 2 weeks
      3. Hypercapnic juveniles: increased mortality, decreased dry shell mass and soft tissue mass, decreased fracture toughness, thicker calcitic laths, 2x standard metabolic rate
      4. Adult carbonic anhydrase expression: greater in mantle than gills, hypercapnia caused increased expression in mantle and decrease in gills
         1. Carbonic anhydrase converts CO2 into bicarbonate for mineralization
      5. Higher costs of basal metabolism in high CO2, energy taken away from other processes
      6. Reduced shell growth in seawater saturated wrt calcite because 1) CaCO3 less available and 2) overall decrease in somatic growth
   3. Fabry et al. 2008. Impacts of ocean acidification on marine fauna and ecosystem processes.
      1. Decreasing pH has impacts on biodiversity, trophic interactions and other ecosystem processes
      2. With increase in pCO2, dissolved CO2 more readily diffuses across animal surfaces and reaches equilibrium intra- and extracellular space
      3. CO2 reacts with internal fluids, decreasing pH with limited physiological counteraction
         1. Passive buffering of intra- and extracellular fluids
         2. Transport and exchange of ions
         3. Transport of CO2 into blood (respiratory pigments)
         4. Metabolic suppression
   4. Todgham and hofmann 2009. Transcriptomic response of sea urchin larvae to elevated CO2.
      1. pH: 380 ppm (8.01), 540 (7.96), 1020 (7.88)
      2. no obvious mortalities or abnormalities at any pH treatment
      3. subtle but significant transcriptomic change in elevated vs. 380 ppm
         1. 1057 genes on microarray
         2. 540 ppm: 83 genes significant differential expression (less transcript of biomineralization, cellular stress response, energy metabolism, translational control, ion regulation, acid-base balance, cell cycle, development)
         3. 1020 ppm: 178 genes expressed differentially (apoptosis, cellular stress response, metabolism)
   5. Waldbusser et al. 2010. Biocalcification in *C. virginica* in relation to long-term trends in Chesapeake Bay pH
      1. From 1983, decrease in seasonally average daytime pH in Ches Bay surface waters
      2. Low salinity and T, juvenile calcification rates dropped with decreasing pH
      3. At higher salinity and T, calcification rates did not drop until abrupt decline at lowest pH
   6. Watson et al. 2009. Early larval development of the Sydney rock oyster under predicted CO2.
      1. pH; 8.1, 7.8, 7.6
      2. larval shell size decreased with decreasing pH
      3. at 7.8 & 7.6 abnormal shell growth – pitted & deformed shell deposition
      4. foresee problems for development D-veliger to umbonate with decreasing pH: 1. Problems with shell deposition, 2. Retarted periostracum formation, 3. Increased shell dissolution.
   7. Morita et al. 2009. Ocean acidification reduces sperm flagellar motility in broadcast spawning reef invertebrates.
      1. Sperm flagellar motility regulated by increase in internal pH
      2. pH: 6.6, 7.3, 7.6, 7.7, 7.8, 8.0
      3. coral (*A. digitifera*) and sea cucumber (*Holothuria spp*.)
      4. in both, significant decrease in motility with even small reductions in pH
   8. Riebesall et al. 2000. Reduced calcification of marine plankton in response to increased CO2.
      1. Using acid & base, pH = 2800 – 750 ppm
      2. Both spp of coccolithophorid (*Gephyrocapsa oceanica* and *Emiliania huxleyi*) increased carbon fixation and decreased calcification
      3. Malformed and incomplete exoskeleton increased with increasing CO2
      4. Reduced calcification of these spp would result in more CO2 back to atmosphere while coccoliths decrease their ability to function
   9. DuPont et al. 2010. Near future ocean acidification increases growth of lecithotrophic larvae and juveniles of the sea star *Crossaster papposus*
      1. Newly fertilized sea star eggs
      2. pH: 372 ppm (8.1), 930 ppm (7.7); 2 egg densities
      3. difference in rate of growth and developmental progression: faster in acidic conditions
      4. no negative effect on survival or skeletogenesis in t=28 days
      5. positive direct effect on metabolism
      6. probably negative consequences later in life, but effects of OA are species-specific
      7. negative effects of increased growth: synchrony with food, response to temperature
   10. Ries et al. 2010. Marine calcifiers exhibit mixed responses to CO2-induced ocean acidification.
       1. 18 calcifying spp, 60 days
       2. pCO2 (omega for aragonite): 409 (2.5), 606 (2), 903 (1.5), 2856 (0.7)
       3. net rate of calcification = total calc.-total dissolution
       4. 10 spp decreased calcification with increasing pCO2 (\*=dissolution of shell at highest CO2): temperate corals, pencil urchins\*, hard clams\*, conchs\*, serpulid worms, periwinkles\*, bay scallops, oysters, whelks\*, soft clams\*
       5. spp where calc. increased to intermediate CO2 then decreased at highest: limpets, purple urchins, coralline red algae, calcareous green algae
       6. net calc. greatest at highest CO2: crabs, lobsters, shrimps
       7. blue mussel showed no response
       8. Reasons for spp doing better at high CO2
          1. regulation of pH at site of calcification by turning bicarbonate into carbonate ion – reduces [H+] in calcifying compartments; dissolved inorganic C increases with CO2 and organisms that can regulate pH can make use of it for calc.
          2. protective external organic layer: accretion of shell/skeleton totally covered by external organic layer show more resilience to increasing pCO2
          3. CaCO3 polymorph: organisms that utilize more soluble forms (aragonite, high Mg calcite) more susceptible; effect only seen at highest pCO2 (dissolution)
          4. Fertilization and photosynthesis: evidence that direct use of CO2 for photosynthesis mitigates adverse response (algae, corals + symbionts); hypothesis that increasing CO2 increases photosynthesis which increases the amount of energy available to regulate pH, but this effect disappears >1000 ppm
   11. Gutowska et al. 2008. Growth and calcification in the cephalopod *S. officinalis* under elevated seawater pCO2.
       1. Cuttlebone: internal aragonite shell for structure and buoyancy
       2. Hatched & raised eggs in lab for 6 weeks; exposed juveniles for those 6 weeks 4000 and 6000 ppm
       3. No difference in soft tissue growth between treatments; all groups followed typical cephalopod growth; no O2 consumption different with acute exposure; 6000 ppm exposed incorporated significantly more CaCO3 in cuttlebone than control
       4. *S. officinalis* has high ion-regulatory abilities
   12. Green et al. 2009. Death by dissolution: sediment saturation state as mortality factor for juvenile bivalves.
       1. Lab: mortality of all size classes of clam at lowest saturation state (0.4) and smaller size classes also mortality at intermediate omega (0.6)
       2. Field: buffered sediment with crushed clam shells, increasing alkalinity and pH and aragonite saturation state (still undersaturated at 0.53)
       3. All bivalves reared in sediment with omega<1 showed signs of shell dissolution, but death by dissolution is size dependent
   13. Kroeker et al. 2010. Meta-analysis reveals negative yet variable effects of OA on marine organisms
       1. Effects of altered seawater chemistry on marine organisms with response variables: survival, calcification, growth, photosynthesis, reproduction
       2. Criteria for inclusion: <0.5 pH unit decrease (IPCC projection) by HCl or CO2
       3. From 251 unique experiments OA has a significant effect on survival, calcification, growth & reproduction, but no effect on photosynthesis
          1. Most pronounced on calcification and survival
          2. Heterogeneity in calcification and growth (varied by stage)
       4. Molluscs had greater negative effect in larvae vs. adults
       5. Results differed due to pH manipulation and experimental duration
       6. Calcifiers more sensitive to OA
       7. Mobile organisms with developed intra/extracellular pH regulation will be more resilient to OA
   14. Lannig et al. 2010. Impact of OA on energy metabolism of *C. gigas* – changes in metabolic pathways and thermal response.
       1. Wild adult Cg; 55d exposure
       2. pCO2 from gas mixture – all tanks bubbled with ambient or mixed gas; control = 8.07, OA=7.68
       3. early exposure = delayed behavioral defense, but normalized over 55 days
       4. hypercapnia changes in hemolymph: increased PeCO2, decreased hemolymph pH (~0.5 units), increased [HCO3-], changes in [Na], [K], and [Ca], decreased PO2, decreased body condition index
       5. alanine and ATP in mantle decreased – stimulates gluconeogenesis because OA inhibits glycolysis
       6. increase in succinate in gills and hepatopancreas – anaerobic metabolism
       7. stronger rise of standard metabolic rate with warming T
       8. pHe affects metabolic rate proportionally
   15. Thomsen & Melzner 2010. Moderate seawater acidification does not elicit long-term metabolic depression in *Mytilus edulis*.
       1. Wild mussels, 8 weeks; aeration of CO2-enriched air
       2. pCO2, Pa(ppm): 39(380), 113(1120), 243(2480), 405(4000)
       3. with increasing pCO2, decreases in shell length, shell mass, NH4 secretion
       4. no effect on somatic growth
       5. peak O2 consumption at 243 kPa, then decrease
       6. longer-term acclimation to increasing pCO2 evidence: increased metabolic rates in moderate hypercapnia
       7. increasing O:N until 405 kPa – lower O:N = decrease in growth due to unfavorable protein metabolism
       8. protein degradation probably promotes pHi regulation through HCO3- production
   16. Cummings et al. 2010. OA at high latitudes: potential effects on functioning of the Antarctic bivalve *Laternula elliptica*
       1. Infaunal suspension feeder, aragonite shell
       2. Wild collected adults
       3. pH (ppm): Antarctic control 7.9 (430), OA in 2100 7.78 (735), Antarctica 20,000 years ago 8.32 (187)
       4. bubbled CO2 to bring pH down a lot then mixed with ambient H2O
       5. all clams first acclimated to 8.32 then lowered over 24h
       6. sampled at days 0,21, 120
       7. no mortality over 120 days, normal feeding behavior observed, no size difference between treatments
       8. Hsp70 (mantle, 21d): lowest in 7.99, elevated at 8.32 and 7.78
       9. Chitin synthase: increased with decreasing pH (high at 7.78)
       10. O2 consumption (day 120): increased at 8.32 and 7.78
       11. Physiological condition: no difference between treatments but overall increase from day 0 to 120 in all, smallest magnitude of change at 8.32
       12. Low pH; increases stress and basal metabolic rate (same at 8.32)
       13. Long-term costs of decreased pH stress on metabolism and calcification
       14. Optimally adapted to current environment
   17. Marchant et al. 2010. Short-term exposure to hypercapnia does not compromise feeding, acid-base balance, or respiration of *Patella vulgate* but surprisingly is accompanied by radula damage
       1. Limpet *Patella vulgata*
       2. pH: 8.2, 7.6 for 5 days
       3. ability to completely regulate hemolymph pH – although evidence of compensation via increased dissolution of bicarbonate
       4. hypercapnia: increased extracellular [Ca], shell dissolution, increased radular damage
       5. no change: hemolymph protein content, [Mg]e, O2 uptake, feeding rates
   18. Hendriks et al. 2010. Vulbernability of marine biodiversity to ocean acidification; a meta-analysis
       1. Only CO2-acidified; growth, mortality, metabolism, fertility, calcification
       2. Effect size, s, = ratio of treatment over control response
       3. Metabolic rates: increased in autotrophs, decreased in heterotrophs
       4. Growth: increased in photosynthetic organisms, decreased in sea urchins, nematodes, bivalves
       5. Survival: overall decreased, but increased in bivalves
       6. Decrease in reproduction and calcification rates
       7. Threshold pCO2 before decrease in response, but different for each response measured
          1. Fertility – 2016 ppm
          2. Calcification – 731 ppm
   19. Miles et al. 2007. Effects of anthropogenic seawater acidification on acid-base balance in sea urchin *Psammechinus miliaris*.
       1. adults at ambient pH 7.95 and pH acidified with gas mix: 5.6 (6.16) (CO2 seep), 6.5, 7.5 (IPCC 2001)
       2. sample coelomic fluid every 24 h for 8d: coelomic CO2 (CCO2), coelomic pH, [Mg+2] for test dissolution
       3. coelomic pH: decreased in all acidified
       4. CCO2: increased causing respiratory acidosis
       5. [Mg+2]: increased at pH 6.16 on day 3-4, increased at pH 6.63 and 7.74 day 7-8
       6. [HCO3-]: increased in pH 6.16 day 4 corresponding with pH coelomic decrease of 0.1 units (similar compensation days 5-7 but all dead day 7); better control at pH 6.63 but lost control at day 8; even better pH control at 7.44 but lost at day 8
       7. can compensate moderate changes in pCO2 but nothing extreme
       8. internal buffering with [HCO3-] not complete – significant reduction in pH of coelomic fluid over 8 days
       9. urchins in water may not need to dissolve tests for access to [HCO3], would be last resort
   20. Kurihara et al. 2007. Effects of increased seawater pCO2 on early development of the oyster *C. gigas*
       1. Bubbled with air (348 ppm, pH 8.2) or CO2 + air (2268 ppm 2300 projection, pH 7.4)
       2. Eggs fertilized in treatment H2O in petri dish
       3. Formalin fixed at 2,3,8,24, and 48 hpf
       4. Omegas
          1. Control calcite = 4.54, aragonite = 3
          2. Treatment calcite = 1.02, aragonite = 0.68
       5. No differences in percent reaching development stage until 24 hpf
          1. D-hinge by 24h: 65% control, 4% OA
          2. 48 h: 68% control, no change OA
       6. abnormal shape in OA: 80% at 24h, 91% at 48 h
       7. OA larvae smaller than control, completely lacked shells, fewer had full shells
       8. Smaller larvae may be less able to get adequate food
   21. Beesley et al. 2008. Effects of CO2-induced seawater acidification on the health of *M. edulis*.
       1. Mussel shells = 83% aragonite
       2. Adult mussels in bubbled CO2 for 60 days: 6.5 (seep), 7.6 & 7.8 (IPCC 2001 scenarios), 8.0
          1. Real values: 340 ppm (pH 8.01), 545 ppm (7.84), 678 (7.77), 1875 (736)
          2. 1875 ppm was pH 6.5 in header tank, presence of mussels (dissolution) may buffer pH change
       3. decreased pH caused decreased lysosomal neutral red retention time (damage) – leaky lysosome
       4. histology: no significant impact on digestive tissues (including parasite load), reproductive tissues, gills
       5. leaky lysosome could lead to increased permeability to substrates, activation of cell death, disruption of normal lysosomal function, cytolytic damage, immunity (downstream of phagocytosis, endocytosis, autophagy)
   22. Spicer et al. 2007. Influence of CO2-related seawater acidification on extracellular acid-base balance in the velvet swimming crab *Necora puber*
       1. Adult crabs for 16 days in bubbled CO2: 7.96 (control, 0.08 kPa), 7.31 (0.25 kPa), 6.74 (1.10), 6.05 (6.04)
       2. Significant effect of time and pCO2 on hemolymph pH, pCO2 and [HCO3-] – most significant for lowest pH
       3. Small but significant increase in hemolymph [Ca] and [Mg] over first 2 days but then no change
       4. Initial big decrease in hemolymph osmolality, recovered after 2 days but still < control
       5. Marked acidosis only at lowest pH, 100% mortality by day 5
       6. pH compensation in 7.3 and 6.7 by accumulating HCO3-
       7. evidence for dissolution of exoskeleton
   23. Pörtner et al. 2004 (review). Biological impact of elevated CO2 concentrations: lessons from animal physiology and earth history
       1. Buffering accomplished mostly by HCO3- and other buffers – more efficient intracellularly than extracellularly
          1. Invertebrates and vertebrates extracellular pH is 0.5-0.8 units > intracellular – more HCO3- extracellular
       2. Membrane carriers transport H+ and/or HCO3- - accumulate HCO3– to compensate pH drop
       3. Seawater fishes use Na/H exchangers, mostly in gills
       4. Marine invertebrates have membrane proteins involved in pHi homeostasis: v-type H+-ATPase, Na/H- and Na-dependent Cl/HCO3 exchange
       5. Universal response to a change in pH is HCO3 accumulation; more effective intracellular than extracellular
       6. Organism sensitivity dependent on : level of organization, activity level, mobility, and reactivity
       7. CO2 tolerant species (worm)
          1. Drop metabolic rate, probably from decreased pHe
          2. Metabolic imbalance – shift amino acid metabolism maybe to produce more HCO3, but could decrease protein synthesis over time
       8. CO2 intolerant species (squid)
          1. High activity necessitates high O2 demand – compromised by low pH
       9. Acute CO2 toxicity will affect few species
       10. Long-term steady-state increases in CO2 could be tolerated by low activity organisms or pre-adapted to environmental change
       11. True long-term (evolutionary) effects still unknown
   24. Havenhand et al. 2008. Near-future levels of ocean acidification reduce fertilization success in sea urchin
       1. CO2-induced acidification: 8.1, 7.7 (1000 ppm)
       2. *Heliocidaris erythrogramma*
       3. Sperm swimming speed decreased 11.7% and motility decreased 16.3% at lower pH
       4. Model: acidification-induced decrease in fertilization success is 24.9%
       5. Fewer larvae developed at 7.7
   25. Havenhand & Schlegel 2009. Near-future levels of ocean acidification do not affect sperm motility and fertilization kinetics of *C. gigas*
       1. Bubble CO2: 8.15, 7.82
       2. Sperm diluted in treatment H2O before use in motility (n=16 oysters)
       3. Fertilized in treatment – assessed cleavage at 1 hfp
       4. Non significant differences for speed, but variable responses
       5. No differences in fertilization success
       6. Effects on sperm does not affect fertilization success
   26. Berge et al. 2006. Effects of increased sea water concentrations of CO2 on growth of *M. edulis*
       1. Exposed adults for 44 days to acidified water with CO2 to 6.5 then mixed: 6.7, 7.1, 7.4, 7.6, 8.1
       2. Mortality began at pH 6.7 at day 23, others at day 37
          1. Day 37 coincided with increased environmental T in incoming water
       3. Larger-shelled mussels had higher mortality at 6.7
       4. Lack of growth at 6.7, lower at 7.1
       5. Lack of growth confounded by low food supply
   27. Kurihara & Shirayama 2004. Effects of increased atmospheric CO2 on sea urchin early development
       1. *Hemicentrotus pelcherrimus* (all experiments), *Echinometra mathaei* (only fertilization bioassay)
       2. Bubble air with different CO2 and HCl acidification: 365 ppm (pH 8.01), 500 (7.77), 1000 (7.61), 2000 (7.38), 5000 (7.03), 10000 (6.83)
       3. Eggs in treatment water then added sperm
       4. Incubation up to 3 days in static, closed culture – caused slight acidification of ~0.1 units in all except 5000 and 10000 ppm
       5. Fertilization
          1. *Hp* rate decreased with increasing pCO2: decrease slight until 500 ppm then dramatic
          2. *Hp* with HCl: not as dramatic
          3. *Em* rate decreased linearly and more than *Hp* with pCO2; with HCl rate decreased only after pH 7.3
       6. Early cleavage – decreased with pH in both
       7. Pluteus – overall length, post-oral arm length, and body length decreased with pH; all different at all pCO2 but not for HCl
   28. Kurihara 2008. (review) Effects of CO2-driven ocean acidification on the early developmental stages of invertebrates
       1. CO2 can easily enter gametes and lower pHi – could prevent fertilization and development
       2. Expression of spicule elongation gene SM50 and direction of crystal growth SM30 did not change at 1000 and 2000 ppm even though morphology affected (Kurihara et al. unpubl, data)
       3. Urchin at 1000 ppm (pH 7.8) for 10 months delayed gonad development and spawning period was ~1/2 that of control (unpubl data)
       4. OA has negative impacts on adult and larvae of corals, molluscs, echinoderms, and crustaceans
   29. Dupont et al. 2008. Near-future level of CO2-driven ocean acidification radically affects larval survival and development in the brittlestar *Ophiothrix fragilis*
       1. Controlled pH with CO2, 25 days: 8.1, 7.9, 7.7
       2. Significant mortality increase after 7d at 7.9 and 5d at 7.7
       3. Normal development at control pH
       4. Differences at low pH
          1. None reached 8-arm pluteus stage
          2. High proportion were abnormal or asymmetric
          3. Took longer to reach developmental stages
       5. Growth proportions: all same at day 1, by day 4 pH 7.7 significantly different from others
       6. More abnormal and asymmetric at low pH (none in control)
       7. 100% mortality at day 8 for low pH
   30. Michaelidis et al. 2005. Effects of long-term moderate hypercapnia on acid-base balance and growth rate in *M. edulis*
       1. Juveniles and adults, 3 months at pH 8.05 or 7.3 (+CO2)
       2. Hypercapnia
          1. Slower shell growth; pHe dropped 7.55 to 7.36 within 2 days and stayed low; increase in hemolymph pCO2 within 4 days; linear increase in [HCO3]e and [Ca] in first 4 days then steady
       3. Same dry-weight-shell-length relationship: reduced shell growth = total reduced growth
       4. Immediate respiratory acidosis for first 0.5-1 day, then compensated by accumulation of HCO3 to prevent further decrease in pHe
       5. pHi decreased within first day but then returned to control values – more evident in mantle and gills than muscle (better at buffering)
       6. significant reduction in O2 consumption: may be because of low pHe; survival under stress
       7. shell dissolution probably source of HCO3
       8. no change in hemolyph pO2
       9. increased ammonia excretion – could be preferential amino acid met/catabolism
2. Multiple Stressors
   1. Crain et al. 2008. Interactive and cumulative effects of multiple human stressors in marine systems
      1. Overall synergistic effect of stressors
      2. Cumulative effects worse than single
      3. Consecutive stressors may cause organism to be less responsive to subsequent
3. OA and Temperature
   1. Zippay & Hofmann 2010. Effect of pH on gene expression and thermal tolerance of early life history in red abalone
      1. pH: 380 ppm (8.04-8.07), 570 (8-7.94), 990 (7.91-7.84)
      2. 6 days, 4 developmental stages (pre-competency), thermal stress for 1 hour and measure survivorship
      3. effect of 990 ppm on survivorship in temperature stress: pretorsion larvae influenced by low pH, no effect of pH on gene expression of biomineralization genes tested
   2. O’Donnell et al. 2009. Predicted impact of OA on marine invertebrate larvae: elevated CO2 alters…
      1. *Strongylocentrotus franciscanus*
      2. Designer gas: 540 ppm (7.98), 970 ppm (7.87), 380 ppm (8.04)
      3. Fertilized red urchin eggs into different CO2, at 93 h post-fertilization heat challenge for 1 hour (at this stage is echinopluteus and skeleton begun to form)
      4. High CO2 changes expression profile of hsp70
   3. Parker et al. 2010. Comparing the effect of elevated pCO2 and temperature on fertilization and early development of 2 species of oyster (*S. glomerata* and *C. gigas*)
      1. S. glom from 4 major oyster-growing estuaries (T-induced spawning); Cg from farm (stripped)
      2. pCO2: 60, 750, 1000, 375
      3. T: 30C (elevated), 18, 22, 26 (all natural)
      4. Fertilization in treatment vs. ambient, larvae raised 48 h at treatment
      5. 4 d exposures of ambient-spawned larvae at stages umbonate, pediveliger, and spat
      6. fertilization success, number of D-veligers, % normal D-veligers, size of D-vel, shell growth (umbonate, pedivel, and spat) decreased with increasing pCO2
      7. lethal effects of pCO2xT at 1000 ppm
   4. Lannig et al. 2010. Impact of OA on energy metabolism of oyster (C. gigas) – change in metabolic pathway and thermal response
      1. Wild adult C. gigas
      2. pH: 8.07 (Ωar 2.31, Ωca 3.59), 7.68 (Ωar 0.87, Ωca 1.36) for 26-55 d
      3. samples: hemolymph, gill, mantle, muscle, hepatopancreas
      4. early response: delayed behavioral defense
      5. chronic: change in hemolymph (decrease ph; increase [HCO3-]e; changes in [Na], [K], [Ca]; decreased pO2; small decrease in body condition index)
      6. change in metabolites in mantle and gill (not muscle)
      7. higher increase in standard metabolic rate when temperature increased
   5. Findlay et al. 2009. Post-larval development of 2 intertidal barnacles at elevated CO2 and T.
      1. Collected 2 spp of wild barnacles on settlement panels (*Elminius modestus* and *Semibalanus balanoides*) and brought to mesocosms
      2. T: 14 & 18C; pCO2: 380 & 1000 ppm (8.07 & 7.7) for 30 days
      3. *Em* grew slower at high CO2 and T
      4. Mean Ca content in both spp decreased with increasing CO2 (ns), *Sb* Ca decreased with increasing T and significantly low in high T/normal CO2
      5. High T increased mortality in both spp – most important factor
      6. Co2 is sublethal effect and some differences between spp due to biogeographical preferences
   6. Walther et al. 2010. Effects of OA and warming on the larval development of spider crab from different latitudes.
      1. Female pregnant crabs taken from 2 extremes of range and acclimated to T of 3, 9, 15C and pCO2 of 380, 710, and 3000 ppm (ambient and gas mixture bubbling)
      2. Development was slower at lower T and higher CO2, but population-specific responses
      3. Zoea I: at 3 and 9C, North larvae developed slower under all CO2; at 15C north slower only at 3000 ppm; for both pops, development at 3 C significantly slower at 3000 ppm
      4. Patterns were different for ZoeaII
      5. Fewer moulted to crab at 3000 ppm
      6. T-dependent development has led to shift in phenology in wild southern population
      7. South larvae have higher C:N = higher protein:lipid = increased fitness
      8. North metamorphosis disturbed at T>9C which poses potential problems with global warming
      9. Narrowing of thermal tolerance at increased CO2 will mostly affect ovigerous females and megalopa
   7. Pörtner 2008. (review) Ecosystem effects of ocean acidification in times of ocean warming: a physiologist’s view
      1. Systemic to molecular hierarchy of tolerance limits
      2. Consideration for future CO2: at macro-ecological scale, distribution of marine fish and invertebrates is highly T-dependent
         1. Complex organisms specialized for specific bio-climate
      3. Importance of CO2 exposure scale: some affected mechanisms may come into play at low and high CO2 but only be detrimental at high; some only affected at high; length of exposure also important
         1. Minor disturbance to oxygen transport may make less energy available overall
      4. Short-term studies neglect acclimation
      5. Control of calcification usually integrated with other responses – need to look beyond
      6. Too simplistic to say that water Ω directly sets calcification rate since calcification usually occurs in specialized internal compartments
         1. Consider pHi and pHe buffering vi accumulation of HCO3
      7. Elevated sensitivity of marine invertebrates may be because low capacity to acid-base regulate pHe
         1. Hypometabolic, not enough resources
4. OA and disease
   1. Green & Barnes 2010. Reduced salinity but not estuarine acidification cause immune-suppression in rock oysters, *Saccostrea glomerata*
      1. Acid sulfate soils (ASS) release heavy metal ions (Al 3+)
      2. Exposure to conditions: salinity, pH (8, 6.5), Al -> replication of ASS leachate with sulfuric acid; added heat-killed vibrio injection to one exposure
      3. 2 exposures, sampled hemolymph
      4. 7 genes: SOD, Prx6, metallothionein, small Hsp, interferon-inhibiting cytokine (Ik), IkB, C1q-like protein
      5. changing in salinity affected Prx6: down-regulated in brackish conditions, no effect from acid or Al
      6. sHsP: insignificant upregulation in low salinity, downregulation with acid and Al, sig. downregulation at 35 ppt with ASS and Al
      7. vibrio injection: upregulation of IkB, down regulation of C1q
      8. interaction of salinity and pH affected SOD, Prx6, C1q
   2. Bibby et al.. 2008. Effects of ocean acidification on immune response of mussel.
      1. 32 day exposure to elevated CO2
      2. Phagocytosis decreases with pH
      3. Increase of superoxide production with time
      4. No effect of pH or time on # of circulating hemocytes
      5. Decreased number of hemocytes probably due to increased [Ca] in hemolymph
         1. Ca is important signaling in hemocyte function
   3. Anderson et al. 1994. Are outbreaks of *Martelia sydneyi* in Sydney rock oysters, *Saccostrea commercialis*, triggered by a drop in environmental pH?
      1. Oysters outplanted – environmental variables and infection monitored
      2. Minor fall in pH and salinity (rainfall + run-off) preceded heavy infection by 3 weeks
      3. Other incidence of heavy infection not preceded by pH drop
5. OA and metal
   1. Pascal et al. 2010. The toxicological interaction between ocean acidity and metals in coastal meiobenthic copepods.
      1. 96h (4d) exposures to HCl acidification or CO2 for *A. atopus* and *S. knabeni*; CO2 + metal (Cd or Cu) exposure for *A. atopus*
      2. both species more sensitive (mortality) to acidification by CO2
      3. mud-dwellers more resilient to increased pCO2 than organisms more porous sediment
      4. increased CO2 decreased toxicity of Cd and Cu
      5. pCO2 technique: mix low CO2 water with ambient (increases DIC at constant total alkalinity); HCl does not change DIC but alters TA (less realistic)
      6. pCO2 affects intracellular acidosis
      7. Problems: abrupt transfer to experimental conditions and acute exposure 🡪 no acclimatization
   2. Lopez et al. 2010. Influence of sediment acidification on bioaccumulation of metals in *Ruditapes philippinarum*
      1. Sediment from 3 estuarine areas with different metal contamination – exposed juvenile clams
         1. GR: mining spill in 1998 (high concentrations of heavy metals)
         2. H: heavily industrialized (concentrations of heavy metals, enriched in trace elements)
         3. Ca: low contamination (lowest metals)
      2. pH: 6.5, 7.5, 8.5, 1 month acclimation; 28 d exposure to sediments
      3. significant enrichment of metals in all clams, highest in mixed GR/Ca (only with significant mortality), lowest in Ca
      4. free ion activity of metals increases with acidity – bioavailability depends on pH
      5. highest sediment contamination not reflected in clam tissue – mobility of bound metals
      6. pH increases metal solubility/bioavailability: Cu, Zn, Pb, As, Ni, Cr, Hg
      7. elements like Cd with high affinity for CO3 and similar ions, may have decreased bioavailability with acidic pH
6. Ocean Acidification Background
   1. Feely et al. 2010. The combined effects of OA, mixing, and respiration on pH and Ω in an urbanized estuary
      1. Winter: water column of main basin well mixed, S sound more stratified, undersaturated wrt aragonite, MB & SS undersaturated wrt DO but > hypoxia cut-off (2 mg/L), HC stratified DO and more towards south HC
      2. Summer: HC more corrosive, hypoxia in SHC
      3. Estimated present-day decrease in Puget sound from OA is 0.05-0.15 units
      4. Surface Ω aragonite decrease 0.09-0.33 in surface waters since pre-industrial, more so in MB in summer
   2. Sabine et al. 2004. The oceanic sink for anthropogenic CO2.
      1. Anthropogenic CO2 is not evenly distributed throughout the oceans – highest [CO2] in near-surface waters
      2. If ocean surface pCO2 continues to increase proportionally to atm CO2, then at 2x atm CO2 (based on preindustrial levels) there will be a 30% decrease in [CO3 -2] and 60% increase in [H+]
      3. As [CO3 -2] decreases so does the ocean’s ability to absorb more CO2
   3. Byrne et al. 2010. Direct observations of basin-wide acidification of the North Pacific Ocean.
      1. pH minimum zone (<7.3) between 29-56 degrees N, generally coincident with O2 minimum zone (both because of decomposition of organic matter)
      2. 15 year pH different (1991-2006, Hawaii-Alaska) shows no change or declines
      3. evidence of human-mediated pH change at surfact-150 m along entire transect between 22-38 degrees N anthropogenic pH change extends to 500m
      4. significant upper ocean acidification proportional to anthropogenic increase in atmospheric CO2
      5. expect ocean pH to fall at accelerating rate if atm CO2 continues to rise
   4. O’Neill et al. 2010. Mitigation implications of midcentury targets that preserve long-term climate policy options.
      1. IPCC B2 & A2r scenarios
      2. Impose midecentury (2050) targets: <1/2 to 2x emissions from 2000-2050 gives outcomes in 2100 of <450-1000ppm
      3. More stringent end-of-century targets: costs for first half of century rise monotonically with increasing stringency but costs over second half decrease with increasing stringency
      4. Emissions at 2050 dictates feasibility of reaching end-of-century goals
      5. Limiting increases in global T to <2C has low feasibility given CO2 projections
         1. Reductions >50% by 2050 would achieve goal with 50% likelihood
      6. Trade-off: mid-century target that allows for lower end-of-century targets but also decreased costs
      7. Caveats: new cleaner technologies; social,political, institutional constraints
   5. Fangue et al. 2010. A laboratory-based, experimental system for the study of OA effects on marine invertebrate larvae
      1. pCO2: blend dry CO2-free atmospheric air with pure CO2
      2. continual flow-through of filtered seawater – never perfect equilibrium of gas pCO2 and experiment (head space in tanks)
      3. measure gas pCO2 at 3 points: inflowing seawater, gas-mixing reservoirs, larval culture vessels
      4. gases blend -> solenoid valve -> infrared CO2 analyzer
      5. 2 reference gas mixtures that bracket experimental
      6. paddle keeps water in larval chambers circulating
   6. Riebesell et al. 2010. Guide to best practices for ocean acidification and data reporting.
      1. The CO2 system in seawater – Dickson
         1. Seawater has dilute sodium bicarbonate: total DIC in N. Atlantic surface ~2 mmol/kg, 90% HCOc-, 10% CO3 2-, <1% CO2
         2. Co2 + H2O -> H2CO3 is a slow reaction
         3. At pH<5, [H2CO3] + [CO2 (aq)] is predominant but at higher pH ionizes to form HCO3- and CO3
         4. Aragonite and calcite = polymorphs of CaCO3
         5. Aragonite is 1.5x more soluble than calcite at 25C
         6. DIC = [CO2 (aq)] + [HCO3] + [CO3] -> measure by acidifying sample, extract and measure unionized CO2
         7. TA = [HCO3] + 2[CO3] + [B(OH)4] + [OH] – [H+] -> acidimetric titration
         8. pH = -log[H+] -> electrode or spectrophotometer with indicator dye
         9. pCO2 = x(CO2)p, x(CO2) = mol fraction of CO2 in air and p = total pressure -> direct measure of air, estimate fugacity
         10. as dissolved CO2 increases, additional CO2 reacts with CO3 to make more HCO3 -> more CO2 and HCO3 and less CO3
         11. info needed to know composition of CO2 in seawater: salinity, T, and 2 of: DIC, TA, pH, pCO2
         12. mid CO2 with and without larvae consistent, but differences at high and low CO2
         13. total [H+] with indicator dye
             1. dye is acid-base compound
             2. spec depends on acid and base forms having substantially different absorbance spectra
         14. seawater contains a number of acid-base species (aside from CO2, boric acid and water), mostly derived from minor nutrients
             1. mostly in deep water, can be upwelled
             2. components contribute to TA
             3. these nutrient species are of concern in lab – may not be able to reliably infer CO2 contribution to TA
      2. best choice of parameters: pH (spec) and total DIC (infrared) – allow description of CO2 systems alone without other coexisting acid-base systems
      3. Approaches and tools to manipulate carbonate chemistry – Gattuso et al.
         1. Changing DIC at constant TA
            1. Aeration at target pCO2

pH-stat: pH monitored continuously, controller opens/closes valves of gas delivery – 1) air + pure CO2, 2) CO2-free air + pure CO2, 3) air, CO2-free air, pure CO2

CO2-free air from molecular sieves for CO2 scrubbers; pH threshold measured by pCO2 and TA; can compensate for change in carbonate chemistry from photosynthesis or respiration; hard for system to reach equilibrium with high biological activity

bubbling with premixed gases

* + - * 1. Addition of high-CO2 seawater

DIC and TA are conservative – mixed water = sum amounts in 2 water sources

* + - * 1. Addition of strong acid and CO3 and/or HCO3

CO3 and HCO3 elevate DIC and acid balances change in TA – mimics changes from CO2

* + - 1. Addition of strong acids and bases
         1. Changes TA but not DIC; acid decreases TA, base increases TA
      2. Addition of CO3 and/or HCO3
         1. DIC and TA increased by adding N2CO3 (CO3) or NaHCO3
         2. Changes in DIC depend on changes in [ion]
         3. Changes in TA depend on changes in charge x [ion]
      3. Manipulation of [Ca]
         1. Omega = [Ca]\*[CO3]/Ksp
      4. Methods that mimic increases in DIC at constant TA (real-life changes from increased atmospheric pCO2): gas bubbling, high CO2 seawater, addition of acid and CO3 and/or HCO3
    1. Atmospheric CO2 targets for OA perturbation experiments – Barry et al.
       1. OA research should inform policy and regulations – needs to be done in consistent manner with realistic pCO2
       2. Select “key values” that overlap and span present and future OA research (following guidelines) to provide predictive capabilities for response of oceans to future pCO2
       3. IPCC estimates between 530-970 ppm by 2100
       4. Ideal experiment: response of system to pH range corresponding to 180 to >1000 ppm
       5. Baseline comparison = 280 ppm – millennial average concentration that has shaped animal performance and ecosystem function
       6. Table for which ppm to add for number of treatments
       7. 385 as control: natural systems have acclimated over decades, current change in response variables
       8. more treatments will help to find ecological tipping point
    2. Designing OA experiments to maximize inference – Havenhand et al.
       1. Independent replicates, consider variance during analysis
       2. Regression models for multiple time points
       3. ANOVA for end-point
       4. Multivariate for multiple response variables
    3. Studies of acid-bas status and regulation – Portner et al.
       1. Use free pH scale when determining effects of seawater pH on invertebrates – can be used to measure body fluid and environmental pH and gradients between
       2. Non-respiratory change in body compartment: influence of metabolic pathways, net exchange of acid/base across epithelia or membrane, change in protonation of protein
       3. pHi controlled mostly in cytosol – intracellular buffering and membrane transport
       4. to understand pHi, must know pHe
    4. Studies of metabolic rate and other characters across life stages – Portner et al.
       1. Environmental changes affect intrinsic factors (i.e. time to develop) – using larval age (post-fertilization) may lead to erroneous conclusions if organisms reach exact same stage later
          1. Use developmental signposts or follow whole process by making multiple observations over time
       2. Correct for age in sampling 1) use standardized time scales 2) virtual age based on development 3) include stages of development
       3. Sample to compare in 2 ways: at same time and at same developmental signpost
  1. Fourth assessment report of IPCC 2007
     1. Climate change = any change, natural or anthro, over time that can be identified by changes in mean or variability
     2. Warming of 0.74C 1906-2005, trend of past 50 years = 2x overall
        1. Widespread trend but greater at northern latitudes
     3. Corresponding sea level rise: 1961-2003 1.8 mm/year, 1993-2003 3.1 mm/year (average rate)
        1. Can attribute directly to melting glaciers and ice sheets
     4. Earlier timing of spring events (terrestrial)
     5. Marine and freshwater: shifts in ranges and changes in algal, plankton and fish abundances in high-latitude oceans: increased algal and zooplankton abundances in high latitude and altitude lakes; range changes and earlier fish migration in rivers
     6. Global greenhouse gas (GHG) emissions have increased since pre-industrial, up 70% 1970-2004
     7. CO2 is majority of GHG and amounts have been increasing even more rapidly in past couple decades
     8. Global atmospheric concentrations of CO2, CH4, N2O now much greater than ice core values from 1000s of years – mostly fossil fuel use then land-use change for CO2 increase
     9. Amount of radiative forcing caused by increasing GHG unprecedented in 10,000 years
     10. Most of the increase in global warming very likely due to increased anthropogenic [GHG]
     11. Very likely that sea level rise is due to anthro forcing
     12. IPCC special report on emissions scenarios (SRES 2000)
         1. Alternative developmental pathways: demographic, economic, and technological driving forces and their GHG emissions
         2. A1: very rapid economic growth, global population peaks mid-century, rapid introduction of new and efficient technologies
         3. A1FI: fossil intensive technological change
         4. A1T: non-fossil energy
         5. A1B: balance across all
         6. B1: same population as A1, more rapid changes in economic structures toward service and information economy
         7. B2: intermediate population and economic growth, local solutions to economic, social and environmental sustainability
         8. A2: high population growth, slow economic development, slow technological change
     13. Predict increase 0.2C/decade up to 2030 over most scenarios
     14. Even if GHGs and aerosols constant at 2000 levels, still increase 0.1C/decade
     15. Estimates for end of century
         1. T increase 1.8-4 C (B1-A1FI)
         2. Sea level rise 0.18-0.59 m (B1-A1FI)
     16. Past and future anthro CO2 emissions will contribute to warming and sea level rise for > millennium
     17. Resilience of many ecosystems likely to be exceeded in 21st century due to climate change, its associated disturbances, and global change drivers (land-use, pollution, habitat fragmentation, increased resources)
     18. Net C uptake by terrestrial ecosystems likely to peak by mid-century
     19. Likely 20-30% plants and animals risk of extinction
     20. Current decrease average 0.1 pH units in ocean due to uptake of anthro C
     21. Predicted based on SRES over 21st century 0.14-0.35 units
     22. To stabilize, emissions need to peak and decline – aim for low stabilization level and peak
  2. Hauri et al. 2009. Ocean acidification in the CA current system.
     1. Low pH in CCS (CA current system) direct result of upwellings – high in nutrients and CO2(aq), low O2, pH carbonate saturation state
     2. Regional ocean modeling system – flow and mixing of water, includes marine C cycle
     3. Strong seasonal change in omega aragonite
        1. Upwelling in spring makes omega <2 in entire water column within 20 km of coast
        2. Upwelling and high remineralization rates in summer make omega <1.3 within 10 km of coast and <2 within 30 km
        3. Low omega through fall
     4. First peak of low omega arg. in summer from plankton blooms
  3. Millero et al. 2009. Effect of OA on the speciation of metals in seawater.
     1. Trace metals complex with one of 5 inorganic ligands
        1. OH: Al, Fe, In, Th, U
        2. CO3: Cu, UO 2+, rare earths, Y
     2. Complexes with OH: no significant increases in free forms, but will be more complexes with fewer OH, i.e. Al(OH)4 + replaced with Al(OH)3
     3. Complex with CO3: strong increase in free forms, especially Cu 2+ (up 30%), free Cu is toxic
     4. Changes are projected for second half of century
     5. Pb: complexes with Cl- and CO3; will increase 10% in fee from, large shift to complexing more with Cl
     6. Yttrium: complexes with OH, CO3, Cl, sulfate; will increase 7% in free form and become more Cl dominant
     7. Significant acidification in estuaries because of low river pH + low ocean pH
        1. If ocean is the estuary’s source of CO3, Cu could become more toxic
     8. Organic material (as ligand for metals) can also be strongly affected by pH – charge will change and affect binding strength
     9. pH will also affect oxidation/reduction reactions, i.e. faster kinetic rates of Cu(II) reduced to Cu(I)
  4. Feely et al. 2004. Impact of anthropogenic CO2 on the CaCO3 system in the ocean.
     1. Ω depends on T, salinity, pressure
        1. Ω = [Ca][CO3]/Ksp where [Ca] is estimated from salinity and [CO3] is calculated from DIC ant TA
     2. [CO3] is the main driver for changes in Ω
     3. shoaling of aragonite and calcite saturation horizons in the Atlantic through Indian to the Pacific oceans due to higher DIC:TA in intermediate to deep water of the Indian and Pacific relative to the Atlantic (caused by enrichment of DIC)
     4. shallow depth undersaturation with respect to aragonite in all 3 oceans form anthropogenic CO2
     5. at least 60% of global CaCO3 dissolution occurs in upper water column
  5. Caldeira & Wickett 2003. Anthropogenic Carbon and ocean pH.
     1. Lawrence Livermore National Laboratory ocean general-circulation model – future projections
        1. Observations of pCO2 1975-2000
        2. IPCC predicted scenario IS92a form 2000-2100
        3. Exceeds 1900 ppm by ~2300
        4. Max pH reduction in ocean surface = 0.77 (little opportunity for buffering by other environmental factors)
     2. Four-box ocean/atmosphere model – past CO2 and pH
        1. When changes in CO2 occur on the timescale of less than 104 years, ocean pH is sensitive
        2. If it occurs over a period of 105 or more years, changes can be buffered through interactions with carbonate minerals
        3. No evidence that ocean pH was more than 0.6 units less than today’s over past 300 million years
  6. Schulz et al. 2009. CO2 perturbation experiments: similarities and differences between dissolved inorganic carbon and total alkalinity manipulations
     1. DIC = [CO2] + [HCO3] + [CO3]
     2. TA = [HCO3] + 2[CO3] + [B(OH)4-] + [OH0 + [HPO42-] + 2[PO4-3] + [H3SiO4-] + [NH3] + [HS-] – [H3PO4] – [H+]free – [HSO4-] – [HF]
     3. Anthropogenic CO2 in ocean increases [CO2] and DIC but not charge balance and TA
        1. Increase [HCO3] AND [H+]
        2. Decrease [CO3] and Ω calcite and aragonite
     4. Decreased TA causes increased seawater CO2
        1. Make comparisons with DIC
        2. At high CO2 less [HCO3] is more important, but at low CO2 more [CO3] is important
  7. Caldeira & Wickett 2005. Ocean model predictions of chemistry changes from CO2 emissions to the atmosphere and ocean.
     1. Model – 3 types of case 1) emission 2) stabilization 3) deep ocean injection
     2. Emissions from SRES
        1. All lead to Ω aragonite undersaturation in surface Southern Ocean by 2100
        2. B1: global surface pH decreases 0.3 units
        3. A2: pH decreases 0.5 units
     3. Emissions based on fossil fuels used derived from total available, ¼ - 4x over 500 years
        1. ¼: decrease pH by 0.3 units by 2300
        2. 4x: decrease pH 1.4
        3. current usage: pH decreases 0.8 units
        4. initially affects surface but mixed deeper, upper thermocline most impacted
        5. 2x and 4x are undersaturated with respect to calcite and aragonite at high latitudes
     4. stabilization of different ppm, business as usual over 500 years
        1. undersaturation in aragonite in Southern Ocean when stabilized at 650, 750, or 1000 ppm
        2. for 650 and 1000 ppm, decrease pH by 0.3-0.5 units
     5. business as usual and CO2 injection
        1. injection would not mitigate if 100% CO2 were injected because would re-enter atmosphere through degassing; significant pH drop of deep ocean
        2. if 10% were injected, could mitigate
        3. any injection plan needs to be coupled with curbing of emissions
  8. Dickson et al. 2007. Best Practices SOP5: Calculating pCO2 at different temperatures
  9. Ridgewell et al. 2009 (review) From laboratory manipulations to Earth system models: scaling calcification impacts of ocean acidification
     1. Biological calcification and feedbacks on climate change
        1. Negative feedback: process of calcification creates CO2(aq), so less calcification would mean less CO2
           1. Ca + 2HCO3 -> CaCO3 + CO2 + H2O
        2. Positive: less plankton calcification -> more buoyant and longer time in upper waters -> less efficient biological pump
     2. Posit that change in pCO2 or pH using acid/base are qualitatively the same (supported by some *E. huxleyi* work)
     3. Inter-strain differences within *E. hux* : less calcified are less sensitive to OA than heavily calcified