Environmental Stressors and Physiology

1. Molluscan physiology: food supply, temperature, salinity, and other stressors
   1. Zhang et al. 2011. Molecular cloning, characterization, and expression analysis of Hsp90 from Pacific abalone in response to selenium.
      1. 4 adult abalone: haemocytes, gonad, hepatopancreas, kidney, mantle, adductor, gill
         1. expression pattern: high in gonad > hemocytes > muscle > mantle > HP, gill & kidney
      2. fed juveniles different amounts of Se for 20 weeks and collected HP and hemocytes: deficiency (0 mg/kg), optimal (1), excessive (50)
      3. hsp90 is highly conserved across taxa, ab’s is closely related to aquatic invertebrates
      4. Se gene expression
         1. Optimal: max hsp90 expression overall in HP, intermediate in hemocytes (less than excess and more than deficient)
         2. Excessive: decreased expression in HP, max for hemocytes
      5. Tissue expression levels are consistent with other vertebreate and invertebrates
      6. Hsp90 is probably an important antioxidation response of abalone
   2. Lockwood & Somero 2011. Transcriptomic responses to salinity stress in invasive and native blue mussels (*Mytilus*)
      1. Collected adult *trossulus* (native) and *galloprovincialis*
      2. Abrupt decrease in salinity for 4h (simulate run-off and tidal flux)
      3. Forced gaping
      4. Collected gill tissue for microarray
      5. Similar between species transcriptional response to salinity stress
         1. Species-specific differences in 12 genes
            1. 5 up in *galloprovincialis*, only 2 ID’d: splicing factor 3B 14 kDa subunit, ornithine decarboxylase (ODC)
            2. 7 up in *trossulus*: 3 involved in translateion and/or mRNA splicing, exocyst complex component 1 (exocytosis), collagen, pi4k2 (cell signaling)
         2. both species response: genes important in osmoregulation and cell cycle control (may have undergone cell cycle arrest)
      6. Significant change in 117 genes (1.7%): 41 up, 76 down
      7. ODC (polyamine synthesis) shown to be important in osmotic response in others
      8. Mechanistic differences in tolerance probably downstream of transcription (post-translational modification of protein)
      9. Some similar genes are involved in heat stress, but opposite regulation pattern
   3. Buxton et al. 1981. Response-surface analysis of the combined effects of exposure and acclimation temperature on filtration, oxygen consumption and scope for growth in *O. edulis*
      1. Juvenile *O. edulis* – acclimated 30 d to T ranging 5-25C -> O2 consumption and filtration rates in T exposures 5-30C
      2. Filtration rate: cold-acclimated (5 & 10C) slow rates, 15 & 20C acclimated showed max rates at or above Tacclim.
      3. O2 consumption: 5C acclimated had faster increase in rate, 25C acclimated shows increase in metabolic cost
      4. Lower water filtration efficiency at Tacclim 5 and 25C
      5. Max scope for growth at Tacclim 17C and Texposure of 25C – increased growth during summer
   4. Warren & Davis 1967. Laboratory studies on the feeding, bioenergetics, and growth of fish.
      1. Scope for growth: difference between energy of food an animal consumes and all other energy uses for specific environmental conditions
   5. Navarro et al. 1992. Natural sediment as a food source for the cockle: effect of variable particle concentration on feeding, digestion and the scope for growth
      1. *Cerastoderma edule* – 3 different particles/mL (TPM), sediment taken from mud flat
      2. Filtration rate correlated to TPM, but at decreasing rate
      3. At threshold, increasingly more pseudofeces
      4. Food availability is main influence on SFG
      5. Limits to ingestion are determinant of maximum growth rate attainable
   6. Sanders et al. 2991. Relationships between accumulation of a 60kDa stress protein and scope for growth in *Mytilus edulis*
      1. Adult *Med* – 7 d exposure to range of Cu (1, 3.2, 10, 32, 100 ug/L)
      2. After a few days *Med* at 32 and 100 Cu stopped feeding, some mortality day 7 in 100
      3. 32 & 100 Cu: clearance rates, assimilation efficiencies, and SFG decreased
      4. no differences in respiration rates
      5. hsp60 western blot: band in treatments 3.2-100 (mantle)
      6. immunobinding in mantle: total Hsp60 increases with [Cu] (no sig diff between 1 Cu and control)
      7. linear relationship between Hsp60 expression and Cu
      8. increase in hsp60 detected at an order of magnitude lower than decrease in SFG
   7. Fisher & Newell 1986. Seasonal and environmental variation in protein and carbohydrate levels in hemolymph from *C. virginica*
      1. Oyster from 2 sites
         1. WP (Wachapreague, VA): 31-34 ppt, 9-15C
         2. TA (Tred Avon River, MD): 6-8ppt, 13-17C
      2. Experiments
         1. Salinity (1 month): 6, 12, 18, 24, 30, 36 ppt
         2. Temperature (17-20 d): 11, 15, 21, 26C
         3. Sal x T: 14 d at 6, 18, 30 ppt then 23-24 d at 30C
         4. Field: collected monthly over 1 year and hemolymph sampled
         5. T x starvation: 5 and 21C, starved 7d
      3. Results
         1. Protein and carbohydrate higher in TA for all salinity (no treatment effect)
         2. Increased protein at 26C for TA; TA carb and prot > WP for all T
         3. Sig diff prot due to location, sal and T (no interactive); less prot for TA 6 ppt and WP 6 ppt + 30C; carbs different based on location and T – less carb WP 30C
         4. Similar annual cycle for TA and WP, but TA > WP; protein increases in December to May (peaks) then sharp decline last half of may and low through summer; similar for carbs but minimum in aug/sept
         5. No effect of starvation
      4. Lab results confounded by collection date (environment and spawning)
      5. Probably less protein prior to spawning (gamete uptake)
      6. More food available at TA – probably cause of higher prot and carb
   8. Beiras et al. 1994. Comparison of the SFG with the growth performance of *O. edulis* seed reared at different food concentrations in an open-flow system
      1. Postmetamorphic *O.ed.*; [*Isochrysis*] 10, 30, 100, 200, 300 cells/uL
      2. Clearance rate (CR) decreased with food ration
      3. Ingestion rate (IR) increased with food ration, plateau at 100 cells/uL
      4. Absorption efficiency (AE) decreased with increasing food ration
      5. At lowest food, lowest O2 consumption and no growth; max growth at 200 cells/uL
      6. At saturated food, decreased filtering rates so do not overwhelm
      7. Constraints limiting energy gain
         1. Low food: amount available
         2. High food: ingestive and digestive capacity
   9. Grant & Cranford 1991. Carbon and Nitrogen scope for growth as a function of diet in sea scallop *Placopecten magellanicus*
      1. Post-spawn scallops, 6 diets for 52 d
      2. Poor diet caused reduced respiration
      3. Caloric or C budgets may overestimate SFG when not enough N
   10. Pinto et al. 2010. Gill transcriptome response to changes in environmental calcium in the green spotted puffer fish.
       1. Fish transferred to low and high Ca treatments, but within environmentally relevant range; sampled at 2 and 12 h
       2. Opposite physiological changes in 2 treatments: total blood plasma [Ca] down in low and up in high; gill epithelial Ca channel expressed higher in low and less in high
       3. Evidence of coordinated response of functionally related genes upregulated in low at 2h
   11. Pörtner & Peck 2010. (review) Climate change effects on fishes and fisheries: towards a cause-and-effect understanding.
       1. Species- and population-specific differences in thermal tolerance – acclimation or permanent population differences
       2. Ontogenetic change in width of thermal tolerance
       3. T max and min (not mean) are driving forces of ecosystem change of populations and communities
       4. Performance capacity within thermal window key to survival and competition
       5. O2 supply to tissue optimal within tolerance window – exceed tolerance and enter anaerobic metabolism, thermal damage to molecular structures
       6. Protective mechanisms: anaerobic metabolism, antioxidative defense, heat-shock response
   12. Xu et al. 2008. Starvation-induced changes of hemocyte parameters in the Zhikon scallop *Chlamys farreri*
       1. Adult scallop: satiation or starvation for 40 days
       2. Starved < satiated: condition index, total hemocyte count, % phagocytic hemocytes, acid phosphatase activity in hemocyte lysate
       3. % phagocytic higher in satiated than controls at time 0
       4. no effects on: ROS production, acid phosphatase activity in free hemolyph, or SOD activity
       5. possible reversal of normal hemocyte route during starvation – hemolymph goes to soft tissues to compensate for lack of nutrients
       6. hemocytes may need certain fatty acids for phagocytosis
   13. Hédouin et al. 2010. Influence of food on the assimilation of selected metals in tropical bivalves from a New Caldonia lagoon.
       1. Oyster *isognomon isognomon* and clam *Gafranum tumidum*
       2. Metals: Co, Zn, Mn
       3. Depuration kinetics of Co – most lost <1 day in oysters; clams assimilated more Co and took longer to lose
       4. Assimilation efficiency and relative retention time the same across [Co] in phytoplankton
       5. Assimilation efficiency different across differ phytoplankton diets
       6. Food quality and quantity important in metal assimilation efficiency
   14. Mafra et al. 2010. Feeding mechanic as the basis for differential uptake of the neurotoxin domoic acid by *C. virginica* and *M. edulis*
       1. Juveniles, exposed for 14 days
       2. Oysters accumulated 3-7.5x less DA than mussels
       3. High inter-individual variability in DA uptake
       4. Differences in toxin uptake highly dependent on inter-specific different clearance rates – oysters 7.5-8.5x lower than mussels
       5. With longer-celled *Pseudo-nitszchia* oysters decreased uptake (particle selecting) but mussels increased x400
       6. Oysters have heterohabdic gills and can size select with gill filaments; mussels have homohabdic gills and can only select with labial palps
   15. Hédouin et al. 2010. Metal and metalloid bioconcentration capacity of two tropical bivalves and impact of land-based mining
       1. Clam (*Gt*) and oyster (*Ii*) exposed to metals and fed – measured accumulation and depuration
       2. Whole body uptake kinetics of Cd, Cr and Mn not affected by increased concentrations – bioaccumulation proportional to dissolved concentration
       3. Oyster did not accumulate As proportionally to environmental concentrations
       4. At high [Co] oyster decreased uptake rate and clam increased depuration rate
          1. Similar with Zn in oyster but higher threshold
   16. Phillips & Hickey 2010. Genotype-dependent recovery from acute exposure to heavy metals in a freshwater clam
       1. New Zealand freshwater clam *Sphaertum novaezelandiae*; no larval phase, live young
       2. 96 h exposure to Zn 0-5 mg/L, fitness measured by reburial into sediment
       3. significant effects of treatment on mortality – no contribution from genotype (allozyme)
       4. significant difference of time to reburial across treatments; genotype influences recovery rate
   17. Blaise et al. 2002. Molluscan shellfish biomarker study of the Saguenay Fjord with the soft-shelled clam *Mya arenaria*
       1. Sources of contamination: industrial, municipal, boats, runoff – 6 sites in fjord
       2. Biomarker responses differentiated sites
          1. Maximum MT induction at site closest to industry and high population density
          2. Vn (vitellinlike proteins) highest at site adjacent to municipal effluenct
          3. Esterases increased at sites near municipal waste; proportional to phagocytosis, DNA damage, lipid peroxidation, and Vn – indicative of metabolic state of hemocytes
       3. Level of metallothionein-like proteins indirectly proportional to amount of vitellin-like proteins
       4. MT proportional to Zn in tissue
       5. MT proportional to lipid peroxidation in hepatopancreas – oxidative stress stimulates MT synthesis (hypothesis)
       6. Reproductive status and T influence biochemical responses
   18. Chavez-Villaiba 2010. Growth, condition, and survival of *C. gigas* cultivated within and outside a subtropical lagoon.
       1. Mexico aquaculture – massive die-offs began summer 1997
       2. 3 stocking densities (high 100%, intermediate 50%, low 25%); on grow-out trays inside (lagoon) and outside (gulf) lagoon
       3. greater growth at low density
       4. gulf oysters showed more variation in dry weight (decreased somatic growth in lagoon)
       5. condition index gulf > lagoon
       6. mortality was greater in lagoon than gulf and was affected more by season than density
       7. November-December mortality linked to decreased T by 10.5C – lagoon > gulf
       8. Mortalities in autumn/winter and spring (not summer) caused by T change of water masses
       9. Autumn/winter mortality also affected by increased discharge of seston from shrimp farms
   19. Lee et al. 2000. Influences of dietary uptake and reactive sulfides on metal bioavailability from aquatic sediments
       1. Acid-volatile sulfide can form thermodynamically stable metal sulfide precipitates in sediment and sequester toxic metals
       2. Metals: Cd, Ni, Zn at environmentally relevant concentrations in sediment column with vertical stratification of oxygen concentrations
       3. Filter-feeding clam *Potamocorbula amurensis*, facultative deposit-feeding clam *Macoma balthica*, surface deposit-feeding worm *Neanthes arenaceodentata*, head-down deep deposit-feeding polychaete *Heteromastus filiformis*
       4. Cd
          1. Both clams accumulated sig more than controls controlled by extractable Cd in sediment (same for Ni and Zn)
          2. *Nar* bioaccumulated from porewater (not for Ni and Zn , dependent on extractable metals)
          3. *Hfi* did not accumulate Cd, but did accumulate Ni and Zn according to levels in sediment
       5. Exposure for most organisms occurs through ingestiong of particles
   20. Soto et al. 1995. Bioavailable heavy metals in estuarine waters as assessed by metal/shell-weight indices in sentinel mussels *Mytilus galloprovincialis*
       1. Measured metal concentrations in soft tissue at differentially polluted natural sites
       2. Metal bioavailability winter > summer
          1. More metals when poor flesh condition, similar results in many other bivalves
          2. Also types of tissues dependent on season, i.e. gonad absorbs more metals
       3. Lurking variable of seasonal change in somatic growth – relate burden of metals to shell weight
       4. Metal bioavailability was higher in the more directly polluted area of the estuary
   21. Tran et al. 2001. How water oxygenation level influences cadmium accumulation pattern in the Asiatic clam *Corbicula fluminea*; A laboratory and field study
       1. Under low O2 for 15 days, ventilation rate in clam increases and Cd body burden increases concomitantly; even at low levels of Cd, in hypoxia significant amount bioaccumulated
          1. Specifically in gill and visceral mass, not other tissues
       2. Gills are main site of Cd uptake
   22. Geffard et al. 2003. Assessment of the bioavailability and toxicity of sediment-associated polycyclic aromatic hydrocarbons and heavy metals applied to *C. gigas* embryos and larvae
       1. Exposures accomplished within first 24 hpf
       2. PAH bioavailable during larval development – levels increased with sediment and elutriate concentrations
       3. Larvae have critical level of PAH exposure (0.3 ug/g) above which abnormalities are apparent
       4. Larvae enriched in low MW PAHs – different contaminant profile than sediment to which exposed
          1. Solubility = bioavailability
       5. Tissue and cytosol heavy metal concentration (Cd, Cu, Zn) increased with exposure level
          1. Critical levels: Cd 0.6, Cu 13, Zn 50 ug/g
       6. Metallothionein induction correlated with metal contamination levels – good biomarker, sensitive
          1. But abnormalities visible before MT induction
   23. Macey et al. 2010. Modelling interactions of acid-base balance and respiratory status in the toxicity of metal mixtures in the American oyster *C. virginica*
       1. Adult oysters, 1-27 days at environmentally relevant doses of Cd, Zn, Cu singly or together
       2. Concentration of all metals: hepatopancreas > gill
       3. Cd had linear accumulation, Cu and Zn did not
       4. Metal exposure correlated with thiobarbituric acid-reactive substances, a quantification of lipid degradation
       5. Gill Cd level correlated with increased hemolymph pH
       6. HP Cu level correlated with increased hemolymph pH and decreased total CO2
       7. Cu affects immune markers: gill levels positively correlated with total culturable bacteria; HP levels negatively correlated with total hemocyte counts
       8. Damage to cellular membrane sensitive to tissue content of all 3 metals and is dependent on change in hemolymph pH and pO2
   24. Jeon et al. 2010. Bioaccumulation of Perfluorochemicals in Pacific Oyster under different salinity gradients.
       1. Salinities: 10, 17.5, 25, 34 psu; T: 9-12C
       2. At each salinity, exposed to 10 ug/L of a PFC in presence or absence of food
          1. Bioconcentration = absence, 7d
          2. Bioaccumulation = presence, 56d
       3. [PFC] increased with time, reached max at day 28 and then decreased exponentially
          1. different degrees of bioaccumulation and depuration depended on compound
       4. PFC bioaccumulation increased 2.4x from salinity 10 to 34 – higher proportion of long-chain PFCs at high salinity
       5. PFC accumulation increased with salinity due to dietary uptake
       6. Faster depuration rates at higher salinity because oyster takes up more water at high salinity
   25. Saito & Marty 2010. High levels of Icosapentaenoic acid in the lipds of *C. gigas* ranging over both Japan and France
       1. Adult *Cg*
       2. TAGs and sterols major components of neutral lipids, low levels of wax esters, steryl esters, diacylglyceryl ethers, diacylglycerols and free fatty acids
       3. Higher tracylglycerols in Japan than France – food availability
       4. Wide range of fatty acids = varied diet
       5. Same 9 dominant FAs found in all samples
   26. Marie et al. 2009. Metallothionein gene expression and protein levels in triploid and diploid *C. gigas* after exposure to Cd and Zn
       1. Juvenile dips and trips: 14d exposure to 0.133 uM Cd and/or 15.3 uM Zn
       2. No difference in growths (soft tissue wrt shell length)
       3. Bioaccumulation of Cd and Zn in dips and trips but no difference between, although dips more efficient at accumulation of Zn
       4. MT protein
          1. No difference between dips and trips but more in Cd and Cd + Zn
       5. MT gene expression
          1. MT1 and 2 upregulated in dips and trips for Cd and Cd + Zn
          2. MT3 upregulated only in Zn alone
       6. Ploidy has no effect on MT gene expression – dosage regulation or silencing of additional MT allele
       7. Differential expression and upregulation of different MTs
   27. Strady et al. in press. Tracing Cd contamination kinetics and pathways in *C. gigas* by multiple stable Cd isotope spike experiments
       1. Juveniles from uncontaminated regions
       2. Spiked seawater and algae with Cd stable isotopes, 3 week exposure
       3. Cd concentrated more in gills and DG than rest of body
       4. At 21 d no different in MT protein amount between treatment and control (not measured in between)
       5. Different contaminant kinetics for direct (seawater) vs. trophic
       6. Direct exposure mostly responsible for contamination in gills and DG
   28. Bussell et al. 2008. Changes in the immune response and metabolic fingerprint of *M. edulis* in response to lowered salinity and mechanical stress
       1. Adult mussels
       2. Max hemocyte response to stress is 30 minutes after shaking
       3. Salinity at ½ normal = moderate flood
          1. Decreases in number of hemocytes, % eosinophils, % phagocytic activity
       4. Shaking : decreased respiratory burst activity; no effect of shaking x salinity for any parameter
       5. Metabolic/biochemical profile different between salinities, not shaking
   29. David et al. 2007. Peroxiredoxin 6 gene: a new physiological and genetic indicator of multiple environmental stress response in *C. gigas*
       1. 3 estuaries with different levels/kinds of anthropogenic pollution and one reference, moderately polluted estuary
       2. gene expression varied with site and season
          1. upregulated at 3 contaminated sites in winter, but all levels lower in summer
       3. Prx6 allows control of ROS to prevent cellular damage
2. Larval physiology and stressors
   1. Manahan 1990 (review). Adaptations by invertebrate larvae for nutrient acquisition from seawater.
      1. Bivalve transport systems for range of organic compounds activated soon after fertilization and continue through development
      2. Transport in bivalve larvae through velum
      3. Bivalves and gastropods have no digestive tract as larvae but are able to absorb DOM
      4. Bivalves and echinoderms (larvae and adults) transport different amino acids at different rates and relative amounts
      5. Metabolic rates increase in direct proportion to larval mass
      6. Patchiness in nutrient availability – probably exist microzones of high nutrients
      7. Urchin larvae transition from monophasic (prism larvae) to biphasic (pluteus) transport of alanine
      8. *Cg* larvae (and others) can de novo synthesize essential amino acids from glucose – biochemical plasticity in growth requirements
   2. Manahan et al. 1989. Ontogenic changes in rates of amino acid transport from seawater by marine invertebrate larvae.
      1. *S. purpuratus*, echiuran worms (*Urechis caupo*), *C. gigas*, gastropod (*Haliotis rufescens*)
      2. Rate of alanine transport saturated wrt [substrate] in *Spurp* prism but not pluteus
      3. *Hruf* increased capacity to transport from trocophore to veliger – nonfeeding larva throughout
      4. Rate of alanine transport in *Cg* veligers can be saturated
      5. All larvae showed more transport capacity of aa as development proceeded – probably because larval surface area increased (size and appendages)
      6. Max transport for *Cg* 9-fold greater from veliger size 80-300 um
      7. Larvae are able to meet increasing energy demands of growth
   3. Manahan & Crisp 1982 (review). The role of dissolved organic material in the nutrition of pelagic larvae: amino acid uptake by bivalve veligers
      1. Bivalve larvae show direct epidermal uptake and tissue incorporation of soluble nutrients – not necessarily via digestive tract
      2. Velum functions as transport for particulate food and uptake of dissolved nutrients
      3. Spat able to absorb nutrients through developing gill buds
      4. Most amino acids go to protein synthesis
   4. Kheder et al. 2010. Effect of nutrition on *C. gigas* larval development and the evolution of physiological indices
      1. 4 day periods of starvation at different days post fertilization: 2, 6, 10, 14, 18. 2 controls: constant feeding, constant fasting.
      2. Total fasting: good survival and competence
      3. D6 deprivation had worst survival
      4. Slowing/halt of growth whenever feeding stopped; the later the deprivation the slower the developmental catch-up
      5. Early deprivation – lipid consumption; late deprivation – lower but more stable
      6. All reserved triacylglycerols consumed during deprivation but not much impact on sterols
      7. Tolerance for deprivation and rapid physiological recuperation (plasticity); larvae use endogenous lipid reserves by 6 dpf
   5. Gallager et al. 1986. Lipid as an index of growth and viability in three species of bivalve larvae.
      1. *Cv, O. edulis, M. mercenaria* – stained larvae with lipid-specific stain Oil Red. O.
      2. Embryogenesis used 69-71% of parentally-derived lipid
      3. 8d feed increased total lipids: *Cv* x2.7, *Mm* x37.6, *Oe* x1.7
      4. elevated T (30C) for *Cv* decreased lipid accumulation and pediveliger survival
      5. TAGs are preferentially catabolized during starvation
   6. Gallager & Mann 1986. Individual variability in lipid content of bivalve larvae quantified histochemically by absorption photometry
      1. *Bankia gouldi* – nutritional stress
      2. loss of lipid during starvation
      3. inter-individual variation in lipid content increased significantly during starvation
      4. lipid store analysis = good assessment of stress response
   7. Phillips 2002. Effects of nutrition-mediated larval condition on juvenile performance in a marine mussel
      1. *M. galloprovincialis*
      2. 3 different food concentrations: 20,000, 2000, 500 cells/mL
      3. high food were larger and proportionally more lipid content when competent to settle
      4. grew at different rates after metamorphosis – high food grew faster (20 d)
      5. outplanted to field: more juveniles from better food conditions remained on settlement plates
3. Adaptation of marine invertebrates to environmental change
   1. Widdows et al. 1990. Measurement of physiological energetics (SFG) and chemical contaminants in mussels transplanted along a contamination gradient in Bermuda
      1. Bermuda mussels *Arca zebra* – outplanted to 7 sites (clean and contaminated in 2 harbors), 11-12 days, back to lab
      2. Clearance rates lower (but non sig) at contaminated vs. reference
      3. Castle Harbor (dump): only small increase of contaminants in mussel tissue
      4. Hamilton Harbor: significantly more contaminants and reduction in SFG caused mostly by petroleum hydrocarbons and TBT
4. Life history strategies of cupped vs. flat oysters from *Oyster culture*. Matthiessen 2001.
   1. *Crassostrea virginica*
      1. 10-50 million eggs per female, ~50 um diameter
      2. spawn when water T = 20C
      3. grow well in warm brackish water, waters from rivers and estuaries rich in nutrients
   2. *Ostrea edulis*
      1. Produce fewer eggs, ~1 million and larger 150 um diameter
      2. Spawn when water T at 15C
      3. Eggs extruded into mantle cavity and fertilized
      4. Larvae incubated ~1 week
      5. Grow best subtidally in cooler saline waters
      6. 4-5 years to reach marketable size
      7. *O. edulis* range: Mediterranean Sea to Norway
      8. Major declines in Europe from epizootics, much culture been replaced by *C. gigas*
         1. 1968 and late 1970s: protozoan parasites *Marteilia refringens* and *Bonamia ostreae*
      9. majority of flat oyster culture in France today along south coast of Brittany
      10. Spawning when summer T>15C, setting June-Sept (France)
          1. Natural sets because of hatchery cost and *Bonamia*
      11. Larvae eat small flagellates with thin cell walls
      12. *O. lurida* from AK to Baja Peninsula, Mexico
          1. Relatively small, 4 years to reach market size of 50 mm
          2. Sensitive to heat and cold
          3. Grown in diked areas
          4. Vulnerable to overexploitation because Puget Sound waters not warm enough for sets each year
   3. Commercial oyster landings sources, p. 22
   4. To avoid low O2 resulting from oyster aquaculture waste in Japan, drag entire operation further offshore in summer (all seed from natural sets)
5. Invertebrate Evolution
   1. Hadfield 2000. (review) Why and how marine-invertebrate larvae metamorphose so fast?
      1. Why are the basics of metamorphosis and settlement conserved across diverse marine invertebrate phyla but different from terrestrial?
      2. Marine invertebrate larvae: small, planktonic, ciliary feeding (most) constrains size by weight, dietary switch after metamorphosis (from phytoplankton)
      3. Post-metamorphosis competence for feeding few hours to days after settlement – “Need for speed” hypothesis, want to be less vulnerable
      4. Larvae have sensory structures to know when to settle
      5. 8 separate occurrences of larvae and metamorphosis: Porifera, Cnidaria, protostome non-arthropod (Lephotrochozoa), Crustacea, Insecta, deuterostome non-chordate, Urochordata, Vertebrata
         1. all except arthropods and vertebrates share small larval size, rapid neurogenic control, and rapid morphogenesis – convergent evolution in 6 lineages
   2. Newell 1952. Periodicity in invertebrate evolution.
      1. Anthozoa (corals)
         1. 4 peaks of genera differentiation in Silurian, Mississippian, Permian and Jurassic
      2. Brachiopods
         1. Ascent in rates of genera differentiation from Cambrian, plateauing Ordovician-Mississippian, high in Jurassic
      3. Crinoids (sea lilies/feather stars)
         1. fluctuations
      4. Echinoidea
         1. Low rates of differentiation during Paleozoic through Odorvician
         2. Adaptation and evolution in Triassic and Jurassic
         3. Flexible structures replaced by rigid in Triassic
      5. Evolutionary history seems determined by competition within and between groups
      6. Triassic fauna dominated by molluscs, others limited in variety – decimation of marine fauna at end of Permian, although molluscs and echinoids not affected
   3. Bretsky 1968. Evolution of Paleozoic benthic marine invertebrate communities.
      1. Details of communities: faunal associations, when occurred in geologic time
   4. Humphreys & Reinherz 1994. Invertebrate immune recognition, natural immunity and the evolution of positive selection
      1. Invertebrates have rapid allorecognition
      2. Burnet’s self-recognition hypothesis (1971) – foreign cells are not self
      3. Selection of immunocytes – positive selection on immune molecules that can recognize non-self
      4. Leptins probably effectors of this process
   5. Bachali et al. 2002. Phylogenetic analysis of invertebrate lysozymes and the evolution of lysozyme function
      1. lysozymes sequenced in: 2 hydrothermal *Bathymodiolus* spp. , 2 cold seep *Calyptogena*, and 2 *Mytilus*
      2. cDNA sequences 65-97% identical amino acids
      3. phylogeny of lysozymes = relationships between organisms
         1. genera grouped together and with other bivalve molluscs
      4. from sequences analyzed no evidence of deep sea adapatation to obligatory bacteria-mediated digestion
      5. lysozymes found across organisms – perform same function and similar 3D structure but high divergence of amino acid sequences
         1. not product of convergent evolution, conserved domains = common descent/true homology
   6. Ottaviani & Franceschi 1997. The invertebrate phagocytic immunocyte: clues to a common evolution of immune…
      1. Immune and neuroendocrine systems share similar structures and function, probably developed together from common origin
         1. Macrophages across taxa have adrenocorticotropic hormone, B-endorphin, a-melanocyte-stimulating hormone, and cytokine-like molecules
         2. Across taxa use NO synthase
         3. Above molecules have same function across taxa: cell migration and phagocytosis
         4. Stress response molecules the same but in invertebrates are concentrated in the macrophage
   7. Holde et al. 2001. Hemocyanins and invertebrate evolution
      1. Hemocyanin in molluscs and arthropods – O2-binding site has pair of Cu atoms
         1. Different molecular structures (very) but similar in Cu-binding regions
         2. Distantly related, origins are separate events
      2. Hemocyanin precursor probably cytoplasmic enzyme – different but related enzymes precursors for molluscs and arthropods
      3. Arthropods and molluscs evolved different methods of cooperative binding and decreasing osmotic pressure in hemolypmph
      4. Molluscan hemocyanin development is more ancient than arthropods
      5. Hemocyanin arose in both after Lophotrochozoa and Ecdysozoa split
         1. Once appeared evolution and diversification rapid