WERA meeting
Sunday 02/28/2010

Paul Rawson
Cross-Breeding and Field Trials for Disease Resistant Oysters (Station update UMO)

Disease is a serious impediment to aquaculture production of eastern oysters

- In Southern New England have: MSX, SSO, Dermo
- Dramatic increase in frequency and prevalence over past several decades—severe declines in oyster production
- Concern about a spread of MSX and dermo into the state
- High incidence of Roseovarious oyster disease (ROD)—so want lines resistant to ROD and multiple other diseases MSX/dermo

Goals:

1) Compare performance of interline hybrids constructed between currently available lines at sites impacted by all 3 diseases (UMFS & NEH crosses)
2) Examine whether oysters selected from local natural environments in Southern NE (SNE) that have survived heavy disease pressure perform as well or better than commercially available lines at sites throughout SNE

Mortality results

- Final mortality somewhat higher for NEH & NEH hybrid lines at all 3 Maine sites (no obvious signs of disease)
- Mortality near 100% for UMFS at New Jersey site
- As you add more NEH to genome of animals, almost stepwise increase in survival

Yield results

- Line to line variation primarily driven by differences in mortality
- Cape Shore has the worst yields but improve as you add more NEH to genome

Other results

- No line-specific differences in shell shape among the five lines tested at POC
- Condition index—F1 by NEWH hybrids had highest average CI but between line differences less than 8% (but only at one site)

Growth & mortality for two new locally selected lines (EGP & Clinton)

- EGP line took a pretty good hit at a number of sites (hatchery troubles…)
- Mean mortality (excluding EGP) is 11-34%
- Relatively steady increase in cumulative mortality for Clinton, NEH, and hybrid oysters at all sites
- Mortality for Clinton line slightly higher than NEH at 3 sites and substantially lower than NEH in Rhode Island site

Hybrids/ Clinton/EGP lines: Growth and Yield

- Local adaptation—one line at a disadvantage out of the hatchery catches up
- Not a lot of differences in mean yield for SNE sites
- By what degree are differences in results driven by differences in disease pressure (in bugs we focused on, opportunistic parasites, and others)?
- Clinton line looks like a reasonable line to continue working with

Other results

- Hybrid lines didn't have highest survival but they often had best growth and highest yield
- Results suggest additive effects on survival
- Clinton line has performance as good as the industry standard NEWH line at SNE
- Little variation in shell shape or meat condition among NEH, UMFS lines and hybrids
- Results argue for continued maintenance of the primary lines to preserve their unique characteristics while capitalizing on the increased growth performance and yield afforded by interline crossing

Questions:
Q: How will hatcheries make commercial spawns using hybrids? Strip spawn? Individually? A: If you are strip spawning you need a continuing source of parental taxa…., advocate strip spawning because you can maximize parental contribution

Q: Hybrids & hetrosis should be important but that doesn't seem to be working out for you. How do you think this impacts you moving forward?
A: We certainly don't have the set up that Dennis has (good, strongly inbred lines)…. Our goal wasn't to capitalize on heterosis and hybrid vigor….it was to see if we could get a line resistant to bacterial and protistan diseases….Two different diseases, one line does well under each disease pressure….

<u>Exploring mechanisms of disease resistance in oysters</u>
<u>Marta Gomez-Chiarri</u>

Testing disease resistant lines in RI

- Gome-Chiarri & Leavitt (RWU)
- Compare performance of one selected line (NEH), wild stock (GHP), hybrid cross (HYB) in 4 RI farms with 2 year classes

Results

- Disease at Naragansett bay farms but little disease at the coastal farms
- % to market size varies from 8% to 77%. Mortality at the end of year two varies from 21% to 85%
- NEH did the best at all four farms—good growth and pretty good survival. Does well under pressure from juvenile oyster disease and from MSX.

- As a side note—may be multiple bacteria causing the same signs & mortality Juvenile oyster disease and ROD the same? Rosovarious crassostrea

Testing disease resistant lines in RI farms (continued)

- Good performance of NEH driven by increased survival to JOD and MSX and/or good growth
- Results not confirmed for 2009 year class—results vary substantially by year (environmental conditions!)
- No hybrid vigor for NEH and GHP
- Sig relationships were found between growth and:
    - PH (Negative for GHP)
    - DO (Postive for hybrid and NEH lines)

SNP discovery within a matrix metalloproteinase gene in the Eastern Oyster (Proestou & Gomez Chiarri)

- Sequenced flowers, NEH & Greenfield pond
- Characterized SNPs
- Add more polymorphic SNPs to help find markers for disease resistance for JOD
- Several SNPs resulted in non-synonymous changes that could change protein function. For example, there is an indel in flowers line that could make protease more susceptible to being cleaved---really want to work more with this.

NRAC: Markers for resistance to JOD (with Guo (RU), Leavitt and Scott (RWU)

- JOD challenges: samples from families with differences in susceptibility before and after mortality for genotyping (90 microsatellite markers, want to add SNPs)
- 96 individuals pre and post challenge
- Ongoing challenge with three families, adding markers as they go

<u>Stan Allen and Anu</u>

<u>VIMS</u>

- ABC exists for the continuous improvement of oyster aquaculture through the manipulation and control of the genetics and culture of the oyster.
- We've been selecting for disease for so long, but if we grow our lines without disease pressure, we lose our advantage
- Super DBY, XB, LA lines.
- Shifting selection from disease to growth
- Deployed lines at three sites: low salinity, medium salinity high disease, high salinity medium disease
- 2 controls: now that we are selecting for growth, our super lines are doing better than industry controls and wild controls (growth)
- In low salinity area, we lose our advantage for disease
- In York, industry spawn control is doing much better than the wild control. But not much difference between our super lines and industry spawn.
- Same in Lynnhaven—industry spawn does just as well as ours with disease
- New selection strategy: projected out to 2014. Grow them for 18 mo, pick the fastest animals.

Selection process

- Growing 30K out in field, sample of 400 animals…
- Selection differential is 20g. Multiply that by heritability about 0.3 looking at about 6g difference between the two generations

Questions
Q: Do you have an estimate for the heritability for disease resistance?
A:yes.

Q: Do you have an estimate for the correlation between heritability for growth& disease resistance?
A: No. But heritability for disease resistance is less than H for size. Interesting given the progress we've seen with disease resistance.

Q: Why did you stop selecting for disease resistance and just deal with growth?
A: If you die in the first year you will not be selected. If you get to market size in 18 mos, you dispel the second season of exposure. So---fast growth in disease zones is also selecting for disease.

Q: You can increase growth as long as food is not limiting. Do you have any thoughts on food limitation?
A: We don't have data, but our hypothesis is that food is not limited. And when food IS limiting, genetics play a large role in which animals grow faster….faster growers are more efficient at using limited foods. It also matters how fussy the oysters are. If food is limiting, oysters that eat a larger variety of foods "get more calories down their throats."

Q: How do you select for a non fussy eating oyster?
A: Select for oysters that grow faster for whatever reason….without losing other traits

Q: Restrict intensity of selection and use a large number of breeders, 100 families (1 male, 1 female so N_e becomes 200 effectively)…are you afraid you'll go into inbreeding over time?
A: Everyone goes into inbreeding over time. We do a lot to restrict levels of inbreeding in the hatchery.

<u>Reece Laboratory Molluscan Genetics Update</u>
<u>VIMS</u>

Clam microsatellite markers

- 56 markers
- 8 working fairly well
- 7 monomorphic in test panel
- 9 loci with indels, stutter issues, and or null alleles in majority of individuals
- 14 sets of primers amplify 2 or more loci
- 18 microsatellite sequences identified but primers have not been developed

- Work initiated by M. Camara years ago

Test Panel
- Stocks and wild, variety of genetic backgrounds in order to test the markers
- From FL to a New England

Clam SNP markers
- SNPs abundant in all EST sequences examined
- Primers designed for 70 loci
- 25 SNP markers have been tested on panel
- 15 have been run on several different sets of samples

Family studies "50 families"
- Parents have been scored at 8 microsats
- Larvae from 6 families have been scored at 5 microsats and 3 SNPs
- Larvae from a different 6 families have been scored at 8 microsats
- Assignment power?

Population and hatchery studies
- Wild VA sample 96 individuals (8 micros, 8 SNps)
- Wild SC sample as above
- KK line as above (5 SnPs)
- QPX experiment (4 micros)
- Trying to fill it in so all have 8 micros, 10 SNPs)

Oyster (*C. virginica*) genetic marker application
What happens to the DEBY oysters deployed on reefs?
- Molecular markers to track them
- Do they reproduce? Genotype the spat fall
- Are progeny from the deployed or wild oysters? Or hybrids?
- Do deployed oysters survive? How long?
- What impact do they have on surrounding pops?

2005 a large scale deployment over 14 months (15 million animals deployed)
- 5% DEBY contribution in 05
- 3.4 % in 06, 0.7 % in 07
- Maybe a pulse in 05/06
- Microsatellite results
- 02-05 (non sig by msat markers) but 2.4 % in 06 and less than 1% in 07
- But they do survive. At least 40% of adult oysters on shell bar reef are DEBYs
- No hybrids found on any of the other sites.

Genetic evaluation of recruitment success in deployed domesticated *C. virginica* oysters in the great Winomico River
- Sampled juveniles in summer 2009 to determine DEBY contribution to recruitment in 2008
- Sampled spat in Fall 09 to estimate DEBY contribution for 2009

- Sampled largest geographic area to date searching for signs of DEBY influence
- mtDNA/nuclear msat data suggests small amount of DEBY spawning in 05-07 but no obvious increasing trend
- high spat falls in the GWR in 0507 largely a result of reproduction in wild pops
- Deployed DEBY oysters are surviving to market size in the system
- Lack of DEBY impacts in the GWR—too early to tell? Looking in wrong place?

Comments
Still important to have oysters on that reef. If they are surviving they might be helping improve larval retention, etc.

East Coast Shellfish Breeding Center Breeding and Conservation Genetics
Stan Allen?

Continuity is one of the most important things to have in the program---but $ runs out. What do we do?

One of the challenges on the East Coast:

- Cold water physiology (NA)
- Juvenile oyster disease (MA)
- MSC (mid Atlantic)
- Dermo (mid to south Atlantic)
- Huge salinity variation (mid Atlantic)
- A lot of genotype X environment interaction
- Lots of regional challenges to selective breeding

Solution: science at UMO, URI, Rutgers, U Maryland, VIMS, U NC Wilm. A distributed center (ultimate oxymoron)

- Our target is the USDA agricultural research service. An agency devoted to the domestication of animals for food purposes
- Now we have a northeast Hub and Mid Atlantic hub…but where do scientists go?
    - Northeast: Concentrate on molecular aspect of missions
        - Quantitative geneticist, molecular genetics, bioinformatics
        - Based in URI which has new capital facilities which are amenable to bringing in scientists without need for capital improvements. Infrastructure is available, we only need the scientists!
    - Mid-Atlantic
        - molecular physiology, agronomist

Hopefully, each of these institutions would get funds to provide solutions for regional problems. Cooperative agreements could also address specific regional issues. Overall this is to provide STABILITY and CONTINUITY!!

Positions

- A quantitative genetics position will bring state of the art animal models from agriculture to shell fish breeding with the expertise to incorporate recent genomics advances into existing genetic improvement programs to accelerate gains.
- A molecular geneticist will develop advanced genotypeing platforms and genomic maps, identify genomic veriations that correlate with important traits, and apply genome-wide marker assited selection
- A bioinformaticist because the data that modern genomics projects generate is huge. Specialized expertise is required to search, interpret, and understand these data using advanced computation tools. Bridge lab based approaches to….
- Molecular physiologist: a phenotype person to address the physiological base of disease resistance, growth, cold tolerance, which are critical in connecting genotypes to phenotypes
- Agronomist stationed in Cheseapeake Bay where large scale spat on shell restoration and commercial programs are located. 3 hatcheries in mid Atlantic produce large quantities of seed for field trials and industry plantings. Agronomist will leverage production and smaller numbers from smaller facilities to regionally test cultivars and newly developed lines on industrial scales. This position will also develop optimum planting strategies for restoration.

Incipient idea winding its way to flesh this out.
Total ask is 4.5 – 4.6 million

Q: One of the problems is not only the science but maintaining the hatcheries themselves. Any contingency in this plan to support hatchery maintenance?
A: it is difficult to direct the funding that way. We hope that the cooperative agreements will provide research $ that will help keep the hatcheries running effectively. Hatcheries support the work of the scientists so some of those funds could be moved into operations.

Q: Practical breeding programs are more industry supported. Is the industry thinking about their contribution to breeding effort through in-kind support or with actual $?
A: Categorically, not on a coast wide basis. Lots of inkind support happening at each regional level. Hard on a coast wide basis because of the huge differences across regions. Right now, we just want them to support the project at a political level.

Comment: Hard to channel some of these funds for operating costs for industry…
A: (from P. Rawson) In Maine, the University challenged the Maine growers to act like the potato and blueberry growers and put a significant portion of $ into research. But…. the industry operates at such a low profit margin that this is impossible for them.

Comment: On East coast, 1000 hatcheries, less than 10 of them have more than 10 employees. Dominated by mom and pops. No Taylor's. Herding cats.

Comment: To get support, the research must be on lines that are important to the industry. In catfish farms, some demonstration ponds on farms grow the animals….a lot of our shellfish growing IS happening on farms.

Comment: In Australia—they use seed as a way to raise funds. A levy (missed the details) as well as a royalty on the seed from selectively bred lines. That $ goes back to the breeding company. At this stage, not a self-funding process, so grants are still necessary, but some $ is coming from the industry.

Comment: In NZ—a lot of government $ for a while but then the government said, we have helped you enough, get industry to help you now. So—now it isn't much of a research program but they are producing gains. Producing about 25% of NZ seed, the rest is caught from the wild. That gives us a vehicle to implement the breeding program on site and deliver the product to industry but there is a really conflict….having that commercial activity happening in a research facility. The partnership has been critical.

Comment: If you provide the seed and take the revenues from seed to run research, that is a whole lot different than taking a tiny bit of revenues from all seed to fund research.

Comment: In AU….company is owned by the oyster growers and we have basically a commercial working relationship with the breeding companies. Have a good relationship with two major breeding companies but are still interested in the smaller ones. Breeding companies and professional broodstock manager.

Comment: In France: after many years, finally moving. Has been taken over by industry. Two companies now have breeding programs. One company is a single hatchery, the other is four hatcheries that joined together (the fifth was supposed to be in the group of four but did not agree, so two groups). We need the industry to understand that it is time to move forward. The industry got organized and got invsetmetns from sector for breeding. The production of triploids made hatcheries more profitable, so they can now move forward with breeding/ research. Done by industry with support of research.

Comment: There are 40 hatcheries on the east coast and none of them are making money. Unless they are vertically integrated, not doing very well.

Comment: In our area, driven by growers, not hatcheries. Grower says, "I want this." And hatchery will produce it. Hatcheries have not gone out of their way to market the selectively bred seed…. Small industry communication is intimate in terms of what is available/ useful discussions back and forth. Marketing campaign is not really warranted at that regional level. BUT says Mark, these growers need to see the oysters on their farm growing well---then they will pay extra for this seed.

Q: How evolved is the growers representation at a political level.
Comment: If something is going to happen (commercializing the science….getting growers to grow the lines), growers as a political organization have to own it and drive it.

Q: Research center does the genetic work so the lines are available. What about the step of impressing the growers on the ground? Might want to think about adding an economist to the breeding center…..to help us understand profits, tax on seed, offsets the cost of the

premium, etc. Requires an economic analysis. But all of our institutions have economists and we can work with them…?

Comment: Need an economist to put weights on traits—what are the costs/benefits? Need a bioeconomist. Easier to do in AU where industry is more uniform. Here people grow many different things in many different ways. Good challenge for an economist?

Comment: Stan can model value of improvements we are adding, comparing one stock to another on a model. Value you can add to profitability based on the improvements we can demonstrate in the field. This convinces people to use the stuff. This is what we need—getting people into the field, onto the farm, showing the growers how it will benefit them.

Comment: In Virginia, we're very "lucky" to have the diseases….growers are on board with the program because these oysters survive.

Steven Roberts
University of Washington

Looking at shellfish and environmental stressors—interested in fundamental organismal responses to different things (pathogens, growth, etc). Hopefully this is useful for people in the breeding world

Can look at stress response using transcriptomics, proteomics, epigenomics

Looking at interactions of pathogens and shellfish. Working with microbial pathologists

A few underlying projects with no new data:

- Disease resistance/ mechanisms behind disease resistance with Rick Carney
- Resistance in black abalone with Friedman lab. Examining the mechanisms contributing to disease resistance in populations with two different phenotypes.
- The mechanisms for resistance appear to vary in the two cases.

Past year: two main projects
Bioindicator: epigenetics

- monitoring the environment
- next generation sequencing doing libraries from different sites. Getting 40 million reads and comparing differential gene expression to see what is impacting animals
- Have farmed oysters in different locations to see how they are doing. Interested in mechanisms in performance.
- Focusing on DNA methylation: the genome can have a bunch of methyls sitting on it. Heritable and very important. Lots of implications in terms of aquaculture.
- Learning that epigenitics is prime candidate to explain phenomena including hybrid vigor, inbreeding depression, local adaptation, and disease susceptibility. Mammalian world has definitely shown this. Can have two pops that are genetically the same but epigenetically different that have different responses.

Environmental changes (ocean acidification and *Vibio tubaishii*)

- Looking at temp and pH to see how multiple stressors affect oysters and pathogen in lab
- Field sites in wild and hatchery sites
- Just started this. Tim Green in lab this summer doing some work.

Q: Epigenetics is very tissue specific. Are you controlling what tissues you take?
A: We're looking mostly at gill right now to be consistent across samples. Not sure what those differences are in oysters.

Q: Is methylation happening in a way that is transmitted across generations and could we select on it? Or is it happening in real time in animals exposed to various conditions?
A: In mammals it is heritable. We haven't examined that in oysters so we don't know. This is a KEY issue.

Q: At the protein level..post translational modification at the level of phosphorylation of proteins? What about epigenetics effects on the proteins themselves? Methylation can turn transcription off but phosphorylation can be turned off proteins themselves. Heritabale?

Freidman Lab UW
Kristi Straus

Geoducks
Abalone
Host Pathogen
Ocean Acidification

Geoducks

- Examining natural pathogens in wild & finding that wild geoducks are not disease free
- Making tetraploids- so to get triploids without using chemicals
- Looking at wild & cultured geoducks. Finding a high proportion of full sibs in cultured year classes. Five microsatellite markers.

Abalone and withering syndrome

- San Nicolas island show resistance to WS
- In experiment, higher mortalities in Carmel (naïve population) than WS, also differences in histology and in gene expression patterns.
- New pathogens observed in this experiment, other RLO: New and stippled.

Helen Gurney Smith
Vancouver Island University Center for Shellfish Research

Myt-OME project

- Development of genomic resources for cultured mussels (of a novel cDNA microarray for blue mussel species)(*M. edulis* & *M. galloprovincialis*)
- Mussels in BC new aquaculture industry with hatchery seed
- BC has major mortality events 95-100% mussels dying just prior to harvest
- Genetrating cDNA librarires for multiple stress responses (*Vibrio tubaiyshi*, temperature, etc). And also for aquaculture
- Gills, hemolymph, gonads, mantles, DG, mussel tissue
- Hard to get enough mussels per taxa. Although farmers are adamant about what species they have, they are often growing multiple species….hard to identify them in any way other than genetically.
- Other notes
    - Both normal hybridized libraries and subtracted libraries.
    - Collaborators who will give us libraries so when we develop the microarray looking at functional gene responses to different stresses, there will be a lot of information on that array.
    - We've had interest on EC of Canada in using the microarray in looking at stress responses to mussels to invasive tunicate species

Genetic analysis updates (concentrating on *M. edulis*)

- Blue / Mediterranean mussels not found naturally in BC. Originally brought from PEI and distributed between the farms that grew up different lines using very different hatchery protocol.
    - Blue frontier adventures breeding a color morph (inbreeding one initial pop for 13 subsequent individuals getting rid of dark mussels each year)—want some info on effects of inbreeding on populations
    - Island Sea Farms: individual *M. galloprovincialis* lines & *M. edulis* lines and producing hybrids. For broodstock, they see what does the best each year, discard everything else, and grow only from that line in future years. We're looking at different broodstock lines to see how they do with stress.
    - Taylor is doing usual husbandry methods (hatchery standard).
- Wont be comprehensive broodstock program because they don't have the resources to do it.

Q: Why was the EC mussel brought in when they have a local mussel?
A: They saw mass mortalities in *trossolus* so thought is wasn't an appropriate culture mussel. But now these sporadic events kill *edulis* & *galloprovincialis* also.

Q: Any indication of genetic interaction between farmed & wild mussels?
A: At one site in an enclosed bay, found hybrids between *trossolus* and *edulis/gallo* but by the second generation had a very limited impact.

Q: Do you have any hybridization between *gallo/edulis*?
A: Yes. This leads to massive problems with microsatellites.

Q: Do you face problems with genotyping?
A: We used four different nuclear markers to separate out pure *edulis* populations. From there went on to the microsatellite markers. Started with a big pool and are now down to 6 that seem to work quite well.

Q: Do mortality events happen at any particular time of year?
A: Yes, mid spring. There is some documented information that it might be due to a post-spawning event. However, it has occurred at other times of the year and *edulis/ gallo* don't generally spawn concurrently with *trossulus*, but mortality events still happening at the same time. We want to use microarrays to identify biomarker genes and do a proteomic approach (food availability? Type of food? Disease? Reproductive events?)

Mark Camara
USDA-ARS Shellfish Genetics Program

Overview

- Mission-oriented, stakeholder driven, applied research program
- Product = knowledge and technologies that address stakeholder problems
- No facilities or funds for practical breeding efforts
- Benefits to industry stakeholders through existing practical breeding programs
- Collaboration/cooperation critical

Short term

- Estimating quantitative genetic parameters (BLUP based "high dimension" estimates of genetic (co)variance structure in current west-coast Pacific oyster germplasm)
- Improving selection strategies

Near term

- Mapping QTL for economically important traits (survival & growth in outcrossed families)
- Gene expression in response to heat stress

Long term

- Profiling transcriptome responses to stressful conditions
    - Temp
    - Bacterial infection
    - OA

How to improve sterile (3N?) products

Target traits for selection are changing

- Hatchery problems & larval viability
- Pathogen susceptibility (*V. tubaiishii*?)
- pH problems
- other factors?

Market shifts & product quality/safety

- shifting to half-shell market so shell shape, meat/shell ratio, uniformity important
- human pathogens?
- Shelf life?
- Effective multi-trait selection requires rigorous estimates of genetic (co)variances

Genetic parameter estimation

- Partial (2x2) factorial mating scheme with 50-100 families
- Multiple, genetically linked cohorts (1 year)
- Multiple traits @ multiple stages (larval, spat, growout)
- Blue/BLUP individual based analysis (ASREML)
- Big Question:
    - Genetic trade-offs?
    - Additive vs. non-additive effects.

Current approach: among-family selection w/ families reared separately has issues:

- Full-sib families confound SCA & GCA
- Limited infrastructure → limited # of families → limited selection intensity
- Unreplicated larval/nursery cultures confound environmental (tank, position, density) effects, and genetic (family) effects in the hatchery and nursery
- Family-specific "associative effects" w/ in growing units at all stages (iea ll tank and bag mates are full sibs…so not just genetic but also environmental differences between families. Associative effects are just how you interact with your full sibs, you may interact differently with other animals.

Mixed Family Strategy

- More complex family structure possible
- Higher selection intensity by raising more families w/out expanding infrastructure
- Elimination of most family-specific effects
- How early can larvae be mixed? Larvae mixing experiment to determine how family rankings compare btw families reared separately vs. mixed.

What is the best selection/ genotyping strategy to obtain maximum response at minimum cost? In silico

Seed crisis & genetic screen experimental plan

- Wait for a call from the Whiskey Creek hatchery that larvae are dying like flies
- Spawn a # of families from promising MBP broodstock
- Mix families in commercial scale tanks
- Watch most of them die

- Genotype survivors and assign to parents
- The reality was good for the growers but bad for the science!
    - Bad water problems were sporadic in 2009
    - Two attempts → high survival both times.
    - Expensive! Not helpful!

QTL Mapping

- Growth, survival, and shell shape in outcrossed families (nearly complete)
- Regulatory regions for genes related to heat stress- in progress
    - Lab recreation of summer mortality conditions w/ 3 sampling points
    - Pre heat stress, after gradual temp increase, after acute heat shock
- Phenotype = expression levels of genes identified in
    - Previous microarray and cDNA-aFLP experiments, published literature
- Morphological and reproductive traits in Pacific X Kumo crosses (preliminary)
    - Make F2s and map genes that control reproductive traits in both species
- Larval susceptibility to pathogens & pH stress – largely imaginary
    - F2 females. Produce their progeny and stress them. Phenotype for that F2 female is the survival of the progeny under these stressors.

Transcriptomics

Competed

- Heat shock in adults (microarray w qPCR follow up)
- *Vibrio tubiashii* in adults (cDNA-AFLP w/ qPCR follow-up) to identify genes that seem to be responding to *V.t.* challenges.

On the Drawing board

- pH and *V.t.* responses in larvae
- next generation mRNA sequencing

Q: Curious about pH study. In Europe would be driven by acidification but here it is more driven by problems in hatcheries.
A: Well it is both. The larvae in the hatchery are sort of canaries in the coal mines. Conversation follows about ocean acidification, upwelling, DO, vibrios. Compounded by waters off of the PNW have very low buffering capacity…some of the lowest on the planet. Nearshore, biological activity can reduce pH even more. Highly dynamic, we don't totally understand it. So lots of work just starting to be done now.

Q: Industry just got some $ to deal with this (500K)---what is the plan?
A: The plan is to do more monitoring before implementing anything. We can implement hatchery systems that degasify the water, and put in systems to remove *tubaishii,* etc. These are correlated with mortality but there may be something else. We need more info.

Comment: Breeding animals that can tolerate these stresses is a more cost-effective way than having each hatchery have a zillion systems to control the problem.

Comment: In NZ, mussels sometimes will lose 10% of the batch, sometimes 50-100% always at a specific time. Did a lot of work to try to replicate the problem. In some females, lost every single family made from that female. Actually families, all the half sibs of those females are lost.

Comment: Big genetic variance experiment we want to do larval stress factors but the question is what do we stress them with? No doubt we will see genetic variation….but what is the right stressor to stress them with?

Comment: Female conditioning. Different genotypes wanting to be ripened at different times and spawned at different times…and the larvae do better when females are spawned at the right time of year.

Comment: Mussel problem in NZ. Small larvae can't eat isochrysis….maybe if eggs have enough DHA they survive or maybe if larvae are big enough to eat iso they make it. But the larvae without enough DHA and can't eat iso are screwed.

Comment: It is a question of cumulative stresses. Maybe animals can deal with 1 or 2 stressors but when hit with a third or fourth…they may not make it.

Chris Langdon
Coastal Oregon Marine Experimental Station and Department of FW, Hatfield OSU

After three selection cycles

- Some cohorts (C18) have 160% of the control but some cohorts have 70% of control
- MBP pedigrees—star performer cohorts after 3 selection cycles
- We will now focus on those two cohorts in a more systematic way

Heritabilities for growth, survival and yield averaged over 3 selection cycles in two sites (cohort 18)

- Subtidal: Growth 0.096, Survival: 0.488, Yield: 0.327
- Intertidal: Growth 0.071, Survival: 0.103, Yield: 0.148
- All values are different from control except for intertidal survival.

Strong correlation between survival and yield. Yield driven by survival, not growth.

Several of the initial studies for family selection…many of the selected families grow to marketable size within two years of planting (in Alaska). Growers are noticing

How do we get growers to amplify their top performing families?

- Commercial repository is up and running. Hatcheries run through 10K animals/yr. Goal is for hatcheries to produce their own broodstock.
- But gives hatcheries some independence of MBP. Commercial repository goes to commercial hatchery goes to grow out

Inbred broodstock parental lines for three top performing C14 families
After two selection cycles, three families in Cohort 14 are over 300% of average control.
Adam & Eve, Salt & Pepper, Yin & Yang
Industry have bought Eves/Adams from commercial repository, hatcheries can make inbred lines and cross them.

Production of spat from single paired crosses between inbred families in MBP hatchery (2009) Bad year, upwelling event, lots of mortality but Adam/Eve and Yin/Yang crosses did much better than controls or whiskey creek larvae. Salt/ Pepper did not do as well.

Growers report excellent performance of Adam X Eve crosses
"our most successful hatchery pacific oyster strain comes from MBP and has revolutionized our growout program with a fast growing, beautiful deep cup shell and widely tolerant environmental traints. This program is key…."

New Kumamoto oysters

- Quarantine protocol for introduction of new kumamoto broodstock was intense!
- Had to keep initial broodstock in quarantine, raise a whole generation in quarantine, from those produce G2s and we could use them as broodstock.
- Have done sepearte G2 families (49 families) and produced combined families of single paried crosses among all G1 families.
- One problem is that shell shape and morphology in new Kumos are more similar morphologically to west coast Pacifics. But after quarantine, the next generation looks more like Kumos (really deep cups).

Q: Some $ from industry, what is the future funding for your program?
A: Industry pays for maintenance of commercial repository. In discussion to try to get industry to put a premium on the MBP larvae. 1 hatchery into it, 1 not sure, 1 against.

<u>Genome Sequencing Update.</u>

- Huge effort from JGI to produce ESTs for Pacifics
- Gaffney headed it up and Dennis has been a main interface for the EST work
- Chinese group doing the sequencing. Ximing is working with them now—it was much harder to do than they predicted. They used illumina (short reads); with plasticity, assembly has been really challenging.

Together Pat & Pierre have applied for a sequencing project…since 2008, long process, still pending. If accepted it would be very helpful to assemble Chinese data. But genoscope wants Pierre to cover half of the costs……looking for 80K euros. Hoping that this will go through soon enough to be useful for the whole project. In order to get the Chinese stuff assembled, really need a scaffold.

BAC library was already supported by USA for funding. France is okay to cover half of the cost of the back end sequencing project….but needs to cover the other half (100K). If any American funding agencies can support part of it, this would push IFREMER to

cover more of it. Our hope both on French and US side, if we can bring this to the Chinese, it will be a more balanced situation. Not talking about ESTs, over and done now. For the whole genome, most of the work is done in China. Nice to have a contribution from Europe….it is a success but only half a success because Pierre needs to find more $ for it to go through.

Dennis mentioned looking into US Funding agencies that might help fund it.

Public Sigenae Contig Browser Oyster

- Helpful to get feedback from people using the database so that it continues to be maintained and updated.
- Hosted in Toulouse in the S of France—team of 15 people doing bioinformatics
- Paper published a few months ago in BMC? Genomics?

It would be good if all ESTs generated were posted on here. 30K unique genes, posted by genomics platform, cattle/chicken etc.. It has been free thus far.

Dennis was going to have a specific website for oyster ESTs. They are working on a crude internal thing now. The goal is to merge it into Sigenae. Everything will be public eventually on genome database….but good to have a more sophisticated dedicated tool.

Q: Basically there are 30K genes that are quasi annotated. Will there be ability to add BACs, genomic, short read data?
A: I think they are open to everything if this is something already developed for other species, than it is fairly routine. If we have specific needs than there need to be more staff to do the work… harder to deal with.

Pierre comment: If we think this tool is good enough and will be the main portal for oyster genomics, then hopefully it will be stronger in France and it will continue to be maintained and improved.

Q: If we get a lot of stuff on the Eastern Oyster, can we add that?
A: Don't know…adding one more species and the Eastern oyster is not grown in France. We need to discuss. Worth asking but not a lot of comparative tools in this portal.

Helen Comment: Mussel stuff from Center for Shellfish Research is just about to go live. We will start uploading onto that and others are welcome to include their information. They will also include sequences on little neck clams. A bioinformatics person in Halifax will run the site & post the annotations. It will be pre-annotated data available for download. Free to access but specific user names.

Comment: At some point FR wanted a linkage map hosted in Scotland…but to have a portal which would host all the mapping information would also be important. It could be linked to this EST & database but right now we don't have it. As we get 50K SNPs that are known…we need to get them into a product that you can buy and use.
Nick King

Shellfish aquaculture update from New Zealand

CAWTHRON

- Independent non profit research institute set up at request of Thomas Cawthron
- 180 staff
- Focus on marine & FW science: aquaculture, coastal/freshwater ecosystems, aquatic biotechnology, sustainable business, analytical services.
- Produce commercial spat (half research and half commercial…but non profit so that $ goes back into research)

Systems

- Pond based systems where they grow food for their commercial spat
- Little larval rearing tanks for experimental work
- Continuous rearing systems

Domestication Programs

- Program has grown with each new iteration
- Pacific Oyster—CAW based on MBP system, but smaller & less glorious than it once was. CAW provides about 25% of hatchery seed.
- Greenshell mussel ™ couldn't take oyster technologies and apply them straight to mussels because they didn't work. Don't have hatchery capacity for mussels—most mussel spat is collected wild. Industry is now beginning to build hatcheries to take advantage of the breeding program. A breeding program not being used by the industry is like a sailboat without the wind.
- New high value shellfish species. What new species can we grow using our infrastructure that would be good for export?
- Really good at developing relationships with industry; industry applies research findings early on.

Oyster culture in NZ

- 75% of farmers use stick culture—wild caught spat gown on sticks. Trend is to go to purchased seed into oyster bags. No bottom culture in NZ. Nearly all intertidal on sticks or in bags. Worth about 30M$ US per year, half is exported
- Breeding
    - cohorts1999, 2001, 2003, 2006, 2008
    - Approximately 200 families
    - Objectives
        - Grow-out cycle under 12 months
        - Half-shell presentation (shell shape, meat coverage)
        - Out of season condition (triploids)
        - Now are selecting for these traits within fast growing. Not a quantitative program any more. Already had quantitative gain with growth rate but it is working and industry likes what is getting.

Greenshell mussel

- where most of the funding is at the moment

- Grown on a long line system
- Goals:
    - Eliminate dependence on wild spat
    - Improved yield, efficiency, and product quality

Greenshell mussels

- 350 families. Cohorts in 02, 03, 04, 06, 08, 09
- Current breeding objectives
    - Growth rate (SL)
    - Process yield (half shell weight—good because traits around shell are quite heritable)
    - Consistent condition

Tagging vs. engraving

- Started out with oysters, so started with mussels trying to use the same design as used with oysters
- Glued on about 50K tags. After a couple cohorts and really disappointing results, now we just use a little engraver and engrave a # on them when they are 25-30mm in size. This has worked out really well. Faster, cheaper, better data.

Response to selection

- Once mussels could be labeled well, they could be measured well & we can do accurate selections. Seen a 5% gain in shell length and a 15% gain in weight compared to wild. This is in one generation
- Single family droppers: still see good response if all mussels in one family are grown in one dropper

Future breeding objectives

- Conversion efficiency (modeling growth rates and figuring out physiological differences btw fast & slow growing mussels)
- Resilience (breeding mussels that handle the process. Survival, harvest, stay closed instead of gaping when harvested, shell strength, etc)
- Consumer attributes (what do people like,, can we make it?)

Hatchery development

- Currently almost all spat caught from wild
- Working with industry to develop hatchery capacity

Research themes

- Larval rearing including probiotics
- Transfer technology
- Cryopreservation
- Hatchery automation

New species:

- Goal: identify high value shellfish species that can be grown using existing infrastructure where possible

- Identify potential candidates
- Identify drivers for species selection
- Select species
- Solve the technical problems
- Current species we are thinking about
    - Flat oysters (if we can deal with Bonamia)
    - Scallop (if there is a market for our species)
    - Geoduck (if we can find a grow-out system that fits with NZs resource management environment)

Implementation

- Work with industry
- Transfer hatchery technology to industry
- Bridge the gap between hatchery and farm
- Commercial trials
- Industry partnership to commercialize breeding program
- Deliver benefits to industry

Q: one bullet referred to working with industry to help develop hatcheries. What did you mean by that?
A: Industry doesn't like to take risks. We've put money into developing reliable techniques so that when they have $ to invest they are confident it is going to work (larval rearing, etc). Another limit is space to do it. So Cawthron has opened up space on their site so that industry can work right with them. More technical assistance.

Q: Do you have summer mortality in NZ? Is survival an issue?
A: Harvest stock over a year so almost don't have time to have summer mortality. Apart from Bonamia, don't have much disease

Comment: Growth rates are 2x what we see on the West Coast.

Comment: Pacific Oysters have only been in NZ since the 1950s

Q: Nursery system. We are looking at size and the hatchery manager said they can't really afford to buy larger seed so they trade off…..Q is if the govt supports the industry phase you are at, will industry be able to support growing the seed to the size they are getting them now.
A; Actually the whole spat production is run as a commercial program. They have looked at cost benefit and figured out it is better for them to pay more to get large seed than to buy cheaper, smaller seed….more die, have to buy finer mesh, have to clean the bags more often, etc.

Q: New species, you mentioned flat oyster (*Ostrea chillensis*). Is there any market potential for that species?

A: We think it is better than scallops. But we need to look for exports to satisfy the industry. Whole lot of people grew up on flat oysters who now can't get it…so should be lots of local demand.

Conversation about industry support/ industry perspective

Very different beasts, industry on the east coast and industry on the west coast
On east coast prefer to buy the small seed because it is "cheaper" but don't value their own time in cleaning etc. Not a rational economic perspective
On EC, early stage of development. We need people who think like businessmen instead of those who just want to go out and work on the water alone.
Very difficult to get growers to agree on anything…they would much rather just work on their own and not have anybody bother them.

PCSGA has been around for quite a long time. The ECSGA has been around for six years, P has 10X the budget, staff, etc. E is a nascent organization. ECSGA grew 46% last year and will need to do that 4 more times to be a real viable organization. Easily less than 10% of growers are members and some of those just spend time telling me I am doing things wrong. Can't say much else about the WC except that I am envious.

Engendering industry support. Trying to get a shellfish breeding center started. Should get an industry advisory committee going…industry needs to be part of the process. They can chose the traits that should be selected for first rather than scientists. Let them figure out what fees should be and how things should be distributed, who sets up structure and pays for it, etc. On EC, they seem to be totally against the concept of paying for anything. If there were federal $ used to develop strains, they feel they have already paid for it. Very frustrating, price structure for seed does not support the cost of operations at the hatchery. If not vertically integrated as a hatchery, the seed does not cover the cost of operations.

The nursery phase is clearly economic. You can make $ buying 1 mm and selling 10mm, but not selling seed or eyed larvae.

We are probably 10 years away from having industry buy into the shellfish breeding program. We have to show them that our oysters grow better, survive better & make them more money. Then they need to grow it themselves so they believe it….At least in NNE, hasn't been a recent epizootic that wiped everything out…so they don't remember there is a need for this. In Mid Atlantic, they know they need it.

Lots of guys don't know they are using the improved stuff. Genetically improved seed need to be properly labeled. Need to start charging for improved seed. The fee can be tiny to start with, to identify this seed as being special…then growers will pay attention to it, pay attention to how it performs and see if it is worth it. Until then, they will mix it up and not handle it properly to notice any differences.

Does it need to come with documentation/ paper trail? To have a charge on the seed, we need to have a good paper trail. Hatcheries are now going to give certificates with the MBP crosses.

In Australia, didn't you have better performance before charging a premium? Yes. Don't always have a success story but no feedback/ interaction happened until a fee was charged. Didn't do them any favors. Now someone who is interested in it can get it because it is valuable to them and they will pay a premium. Mom & dad growers are going to prick their ears up as soon as they have to pay anything more for the seed.

How much improvement do you need to see before you can say, "this is improved." It has to be observable. Significantly different does not equal financially significant. 15% better AT LEAST! Visibly different. They aren't going to measure them and say we got an 8% gain. Has to be obvious.

I like the idea of the breeding center bringing together the whole East Coast…so many things to bring together, so many different lines. Instead of having to phone Paul up and ask him, should be able to look on a website and find the details about their seed.

Key thing that they did in NZ was come up with a growth form ranking (GF). Level of improvement on growth and form….everyone in the industry understood this! Someone might want GF 17 for 500$ per kg and someone else might want GF 22 for 5K$ per kg. That was very helpful. Who made the GF index? Forest Research Institute, a government funded growing program. This made it very accessible. That works as long as environment is not too heavy on the traits. Can never predict all of the environments you are going to face. Can split it up to 6 traits—indicates where it sits relative to other lines. Similar to a breeding value for dairy farms. If you want the top bull you pay the top dollar. Can set up your breeding program so you are looking for generalists…works for WC but doesn't work in the EC where there is SO MUCH environmental variation.

Really difficult question. How do you prevent one of the 40 hatcheries from breeding matured seed and creating their own improved seed without getting a kick back? Hopefully the center will always be 2 or 3 years ahead of what they are breeding. Some of it will have to be volition and support from the industry themselves. If you can show them that you are going to take care of all of this for them….maintain broodstock, keep things separate, labeled, etc they don't have to do that….and their bottom line should reflect that. We are talking about professionals though. I view our industry as a thousand hobbyists who are not behaving as professionals.

We've had really good buy in from the farmers in Maine. But the things that get in the way are things we can't address. *Polydora* infestation of shells, etc. So much affects them because we don't have a defined environment. You'll be up against if you as a scientist will go tell the farmers that this is an improved seed and they should buy it. You need someone IN industry who is involved and on board who can get the message out to the growers. Better penetration and more believable.

A really good example is the whole triploid thing. It was immediately demonstrative. Obvious differences. There were royalties attached but across the world people are adopting this. It is growing heavily everywhere….so if we can find something that is obviously better, the industry will be on board. Breeding will get you there but it takes 15 years instead of the next year. Already been demonstrated that people are ready to adopt better things…but breeding is so gradual, we have to take it slow.

Triploids are a single bullet shotgun. Tiara on the princess, not the princess. Not resistant to everything. If you can do it this easy way, why bother with breeding? Well then you have a problem where triploids are not the answer to the problem. Now trying to combine both but this is a big jump. Moving from triploids to selectively bred triploids is a very big jump. Clear that triploidy was a good way to show that genetics is good for oyster industry. On the other hand, in FR people waited too long to build up on that.

Whiskey Creek hatchery manager hates triploids. Harder to deal with in hatchery. Growers like them but…when times are good they do really well. When times are bad they die first. We need to incorporate breeding and triploidy to make them better.

We need to show the grower improvement for them to be convinced. We did that with triploids. But we need to show them with breeding program.

Q: How to get industry support? At the beginning, gave our stocks to try them out. At the industry meeting, the growers are going to the hatchery and asking for DBY, LOLA. The growers are asking for the stuff. If you want industry support, maybe going to the hatchery is not the way to go.
A: But you need a commercial hatchery to produce millions of seed to get them to a bunch of different growers and try them out. Otherwise, you won't make a dent.

In the good old days we gave them lots of groups of families with a dozen suggested crosses or more. Too complicated. Now we are producing large numbers of limited #s of parental groups to cross. This magnification process is an important step in getting industry to see the effects of breeding on a large scale. We're giving away broodstock at the moment (10$ per individual) but that just whets peoples appetite to get this seed out.

Stan is terrified of distributing broodstock rather than getting the fee for larvae coming out of the hatchery….then we are held responsible for how good the year was, sex ratio, if they fattened up, etc. Effectively they only want the females, what are you selling them? Maybe *gigas* is a completely different animal than *virginica*.

They would have to accept the fact that sex is random and they have to be responsible for conditioning.

One of the things you have to do though is involve industry. They have to agree with a plan and help them develop it with you. Say to them…what sort of thing is going to work here. If they help develop the solution, they will be on board. Then if things don't work,

they are interested in helping to improve it, make it happen, make it change….otherwise this will be a long, hard road.

NZ—mussel and oyster programs run in totally different ways. Oysters they are producing the whole thing. Mussels they are doing their bit and handing it over.

Decision to go that direction with oysters had to do with their major buyer. They didn't have a hatchery. They had to build a hatchery, produce hatchery spat and then add the breeding program on top of it. In AU, the industry took over the breeding program and does it cooperatively. But these growers work well together (more like WC than EC).

Hallmark of a good breeding program is that you make it successful enough and add enough value that some commercial company wants to buy it and capitalize on the value. If so, the royalties on that seed are going to go up up up. When commercially vs. institutionally exploited, it will be MUCH more expensive, even though we get creamed for doing it. Unless the commercializing body is cooperatively industry owned. But most of these guys hate each other and would never work together.

Right now there is no buy in because they don't see a perceived value. Buyers asking why do we want these seed.

In AU the breeding program is commercialized. But CSIRO provides genetics info through a research and development project with both industry and government $. Design research & development projects involving a lot of selection and breeding.

Q: How did you deal with industry individuals who didn't want to be part of it?
A: Democracy…elected representatives to represent you. And then respected whatever they came up with. Industry reps in the room made decisions on behalf of industry.

In NZ they just talked to the four most enthusiastic big companies and saw what they came up with. When those four came up with something, and were willing to take it forward, lots of the little guys joined up too. In AU, they still don't have all the hatcheries on board with the breeding program…but they only NEED the two major hatcheries….so the others are welcome to join in or not.

You need someone to go out and promote and market our product to industry. And you can't beat having a grower do that marketing for you. If you aren't in their psyche and don't understand their world, you are going to have trouble hitting their buttons.

Stan's model

Effects of traits on economics of oyster culture budget as part of a bigger program on nutrient credit trading.

- Used this model as a template and changed just two things: survival rate and avg. time to market (proxy for growth rate)
- Cost/oyster, 10 year IRR
- Model uses cage culture as this is the most pop way to grow oysters in CB
- Breakdown of approx. annual operations cost for 4M seed (labor, packing, seed, shipping, admin, permitting, triploid licensing, utilites, office, etc, ABC licensing)
- Value: genotype column, environment column, grow time col, survival col. # s are fairly well anchored in real data
- With 4 million seed: Starting with a wild oyster in a disease environment, you get a growout time of 20 months and a survival of 40%. Revenues are 15K. In a non-disease environment, you can make 22K.
- With 4 million seed: Take a select oyster in the disease env, 18mo growout time and 60% survival, can make 50K. Take a triploid in a disease environment, gat 16 mo grow time, 60% survival can make 73K. Etc.
- At the end, according to this model, improvements we've made over the other options seem to have some net worth, including the upfront cost on the seed
- Bare bones economic model is a large part of the sell for this whole thing. Helps us to make our point and sell the need for improvements. Whatever improvements we make to oysters can make a huge impact on profit margins—if we improve survival or growth by 3%, farmers can be profitable.
- Without disease, breeders need to add value. Growth, meat to shell ratio, etc.

Comment: The other way to sell disease resistance is to sell it as insurance. Not say, you'll make this $ every year but every fourth year you are going to lose this much $.

Q: Did this win any hearts and minds of growers after the meeting?
A: They didn't argue, so probably yes.

Comment: Can show growers this to perk their interest and then have a field day to visit a grower who is doing field trials…talk to a farmer who is on board and doing this in reality and lots of people might be on board

Comment: Selecting for growth is better than making small incremental changes in survival. Because if you grow really fast, you aren't in the water long enough to get sick.

Oysters at Smoky Bay South Australia

Gary Zippel

- Very clear waters, food limited environment but still get reasonable growth
- Bags off bottom—no on-bottom culture
- Mostly intertidal

- Another system of growing by growers in SA, extremely rough conditions and baskets swing on hooks. Hooks at different heights so bags can be lifted right up. Helps reduce handling, have more management control.
- Haven't seen any fouling on any of these…these sites are good for that, fairly clean environment.
- Still bag manually so they can look at all the oysters & make sure no gaping, etc.
- Woops, a commercial release of a disaster line. Very fast growing but super long and skinny. Nearly ruined the breeding program.
- Color very uniform across lines. Nice uniform shape and colors
- In control oysters (a commercial line), less uniformity in color, shape, size
- Now they don't bother with a control…one less spot for a breeding line
- Mass selection line has been dropped too…wasn't meeting the aims.
- We want faster, fatter, better shaped oysters with good quality shells that don't die.
    - The mortality part we don't have a good handle on and our oysters are a little softer than commercial lines
    - Condition vs. shell meat/whole weight by shell thickness. Essentially if you select for fat oysters you inadvertently select for thin shells. This is a problem because thick shells are good. If I compensate for the shell (SW/(whole weight- shell weight). You have to take into account the shell weight or you breed for thinner oysters. Quite good genetic heritability for condition.
    - Meat to shell ratio is not that well related to weight.
    - What if you select for a thicker shell?

What is a family line? A line we've generated by single pair mating. Break them into different reps and different sites with different reps. Consistency within a family in different environments and a lot of variation between families. Broad sense heritability.

Five different sites across two states that we use.

Oysters in SA can't be shipped back to Tasmania. They won't allow oysters back into the state. But oysters in SA are maintained as a back up or emergency…or if there is an outbreak in Tasmania then nice to have things in two places.

Comment: It would be great if this community could find a single measure of condition index. Why do we need to have eight of them? We should figure out which of the eight has the best genetic correlation and simply use that.

Monday WERA Meeting
030110

Jeff Silverstein: USDA Agricultural research service
It would be great to have NOAA, the other federal agency doing shellfish aquaculture here today as well.

ARS team

- Animal production and protection
    - Aquaculture (65 scientists)
        - Met with PCSGA & ECSGA last month.
        - Program in entomology that deals with arthropod control all the time….nice cross fertilization for working with ghost shrimp. Some methods have very little worry about affecting anything other than target organism.
    - Animal health (Cyril Gay & Eileen Thacker)
    - Animal Production (Mark Boggess)
    - Veterinary, Medical and Urban Entomology (Dan Strickman)
- Food safety-vibrios (Jim Lindsay, Mary Torrence)
- Nutrition (John Finley)
- Natural Resources habitat (Charlie Walthall, Mark Walbridge)
- Bioenergy-algal experience (Bob Fireovid, Kevin Hackett)
    - Could be nice synergy for shellfish growers, who know how to grow algae! Help produce algae for biofuel or for DHA/ (fish oil replacement)

Federal USDA priorities

- Global food security
- Climate change
- Food safety
- Human nutrition/ childhood obesity
- Biofuels

Commitment to industry priorities

- Responsive to current industry concerns also do research that will pay off yrs
- Currently ARS aquaculture center is on WC (Hatfield) but trying to extend to EC
- Improvements in animal performance on farms—fund evaluation on farms
    - Disease resistance
    - Shape
    - Larval performance
- Agronomic performance
    - Density, depth, interactions among animals
- Research to drive future innovation

Aquaculture in US

- 4300 aquaculture farmers
- 1Billion$ in goods
- 3 B in value generated through multipliers
- Need to reach out to the broader community
    - Hatchery, growout, processors, distributors, outlets (supermarkets), investors, insurers, consumers

Communities need to use the strength across aquatic systems

- Broad stakeholder base Ag, Conservation, Sport, Fishing, USFWS, NOAA, USDA (NRCS), Fundamental research (NSF)
- When Pierre talked about sequencing needs, this would be a great outlet for our $
- Interests vary but the tools are similar
- Joint subcommittee on aquaculture (science and technology strategic plan)
    - Need to see a unified vision on aquaculture research to give them a way to push for further support
- International partners

Science and technology

- In animal domestication, we're about 8000 years behind terrestrial animals
- Sequencing technologies
    - Sequencing and resequencing
    - Expression profiling
    - Counting experiments: ChiP seq., RNA seq, epigenetic changes with Methyl seq.
- SNP Chips
    - Chickens, Pigs, sheep, dairy
    - Dairy industry pushes this technology and it is getting cheaper all the time
    - Bovine 60K SNP chip is available
    - Underway for catfish, trout, salmon

USDA Animal Genomics Bluepirnt

- Science to practice (StoP) priorities
- Discovery science
- Infrastructure
- Has a lot of impact around the world for agricultureal genomics

StoP priorities

- Move to whole genome selection
- Prediction of genetic merit of individual animals from genome based data combined with phenotypes (need reliable methods to measure phenotypes)
- Integrate genomic data into large scale genetic evaluation programs and use genomic info to design precision mating systems. Need to save some of our research $ for scientists to take measurements at farms…don't have farms do it.

Q: We need long term funding. Can't run breeding programs on 2 year grants. Any thing from ARS?
A: The history of aquaculture funding within ARS is unique. 3 million dollars in 89 to 37 million today and ALL came through congress. Not USDA priority driven. Right now with the tight budgets the chance of aquaculture programs growing low. But there are programs like MBP…we need to work together to leverage those resources as much as we can. If industry sees a need, they can push for support. But what we (gov) can do is limited. Beef industry kind of swamps the aquaculture industry.

Q: Can you talk about your relationship/interactions with NOAA?

A: Even within 2 years as I've gotten to know my NOAA counterparts…we have a good relationship. On finfish side there is a USDA NOAA alternative feeds program. Less interaction over shellfish but we do have active conversations. Trying to develop a workshop for ARS shellfish program on west coast with MBP program. Talking about how to get NOAA involved. Working together from the ground up.

Q: How does something like the alternative feeds thing get off the ground?
A; Ask NOAA folks we work with the same questions and push for this. Administrators are glad to follow up on good ideas. Two years ago when we were worried about *tubaiiashi*, LOTS of mobilization, we sat in a room together with NOAA and ARS and tried to respond. NOAA has a mandate for environmental monitoring and research which is a great complement to the agricultural side of things with animal husbandry etc. At the physiology side of things there is a lot of overlap

Q: A lot of us are with universities. Universities interact with ARS through cooperative research agreements. Could you talk about that?
A: A cooperative agreement is a contract. ARS is built around 5 year research projects. Base funding with the same funding every year to accomplish a set of goals (basically a research project plan). Goals may be best achieved by working with U collaborators and funding is available to do that. It could be to fund a graduate student to work in the ARS lab or U lab on a project that is important to ARS. It could be for 300K/ year to do a much more significant piece of work but needs to be built around the research project plan that has been externally reviewed and approved for ARS work.

Q: So ARS sets agenda and Universities work with ARS to implement agenda?
A: Yes but ideally Universities and ARS work together to figure out what needs to be done and think about research priorities and how to solve them. Together we figure out what the goals are. Your job is to figure out the best way to solve those problems, achieve those goals. Very collaborative, cooperative process.

Q: In previous years, collaboration with Ifremer & NOAA. But little matching funds, things weren't funded…hard to have real international collaboration other than this kind of meeting. How do we build exchange of researchers, can you push so that there are more facilities and framework to have real international collaborations with at least a bit of $. *Gigas* is everywhere in the world…we should work together. Ifremer/Noaa agreement is empty because it lacks $. A lot of potential, we have similar problems we can complement each other on research.
A: Two great examples. Rainbow trout the ARS lab has a very strong collaboration with ENRA (?) and they jointly applied for NFAA (?) competitive funds. Scientists taught back-end sequencing and there has been great exchange. NFAA has also funded international collaborations on cattle/chickens too. I agree, we need that kind of framework. I don't know the solution but I hear this all the time. Constituent governments need to show relevance at home…need to fund their researchers. Not that many programs where $ can go towards international collaborators. Framework in EU is more restrictive. Pierre says in France there is a program with specific $ for bilateral collaborations. He will look to see if there is something that can be opened for our field.

Q: I've noticed a decline in the number of courses, programs, and professors in aquaculture. This is a serious national problem because we are not training the next generation to work on these issues. We absorb engineers and pathologists who are interested but we aren't training our own. Any discussions on a national level about addressing this problem?
A: Perfect segue for Gary Jensen.

Gary Jensen USDA
New Name and Direction

A: We are currently collaborating with NSA, WAS, and fish culture section of AFS to create a database with all the "aquaculture" degree programs (1 year to PhD) and administer a questionnaire. Hope to complete this work in 2010 and have hard data that show trends. Anecdotally, lots of programs are shifting and changing and US students are not as interested in aquaculture. We fund programs in curricular development, funds for pre&post doctoral students, etc.

New agency and the direction we are going.

- Key changes
- Societal priorities
- AFRI 2010
- Strategies
    - How do you build strategies that take full advantage of federal programs?

Aquaculture team

- Gary's full time job but lots of folks who manage programs with elements of aquaculture. (animal nutrition, animal genome program, organic agriculture, etc)

Challenge is that aquaculture is so diverse. Few resources but salmon, catfish, shellfish….lots of different things pulling on resources.

Back in 2005, there was the second national survey of aquaculture. There is currently no budget for 2010 survey.

New agency: NIFA: National institute of food and agriculture

- First time, have a presidentially appointed director (Roger Beachy) who is now the chief scientist for USDA
- Strategic move to elevate the importance of agricultural within federal research
- Created October 1, 2009

Recommended reading A new biology for the 21st century by the National Academies

- Rising to societal challenges is big center part.
- food challenge, environmental challenge, energy challenge.

Priority Science (Societal challenges) / NIFA Priorities

- Global food security & hunger
- Climate change
- Sustainable energy
- Childhood obesity
- Food safety
- Note: These line up very well with the challenges by the National Academies

New Science Enterprise
NIFA will expand collaborations:

- Other federal science agencies (clear, expected, has to happen!)
- Foundations
- Private sector
- Integrated interdisciplinary research community (more $ to support education integration into research)

Purpose

- Leverage resources (each program has its strengths, has its area as to how to aquire funds. We are really pushing hard as to how we can best achieve that. Very relevant to shellfish aquaculture)
- Achieve maximum impact (impact, outcomes, measurements. Show us what you are achieving…beyond publications!)

Transformational Changes

- Implementation of program & organization changes (we will have four institutes within the agencies)
- Global perspective: New NIFA will absolutely fund international collaborators. This will be a very important part of RFAs coming out later this month
- Focus on scale (more $ per award), scope (longer periods of time), and impact
- Budget driven by societal priorities and performance. If we are effective at doing this, congress should recognize this and budget should increase. We are trying to recast the agency to show how it affects broader societal problems.

AFRI Funding trends

- 08, 09, 10---190M, 201M, 262M, in 2011, 430M!!
- Some NIFA programs have moved into AFRI budget, that is part of increased $

New structure: four institutes

- Food production and sustainability
- Bioenergy, climate and environment
- Food safety and nutrition
- Youth and community development

USDA is interested in becoming more involved in global programs. The international program is right under director's office.

Areas of emphasis

- Refocus & attract brightest scientists. We want scientists to come to USDA! More $ per award, longer duration
- Integrate across functions
- Collaborate across federal agencies
- Move agendas forward efficiently: performance and possibilities of more fund renewal
- Accelerate pace of science discovery, speed of application, support new generation of scientists
    - NIFA fellows, pre & post doctoral students 5M$

Grant Changes

- More focused areas
- Larger dollar amounts (millions of dollars)
- Bold outcomes (real results) beyond peer review and publications. This is really going to be a test. We want folks to become more effective at reporting benefits.
- Application science
- Measurable outcomes for continuous awards

AFRI 2010

- Flagship competitive grant program for research, education, and extension
- Anticipate RFAs about Marc h 19
- New societal challenge priorities (five)
- Foundationsl awards
- New NIFA fellows awards (pre & post doc)

2010 AFRI challenge areas

- Keep American agriculture competitive while ending world hunger
- Improve nutrition and end child obesity
- Improve food safety for all Americans
- Secure America's energy future through renewable biofuels
- Mitigate and adapt agriculture to climate variations

AFRI awards:

- Integrated (research, education, extension)
- Interdisciplinary & larger awards
- Larger duration (5 years, and some renewable)

2010 AFRI foundational areas

- Animal health, production & animal products
- Plant health etc
- Food safety, nutrition, and health
- Renewable energy, natural resources, environment
- Agricultural systems and technology
- Agriculture economics & rural communities

Ongoing follow-up to USDA national aquaculture stakeholder workshop (2008)

- Catfish
- Trout
- Shellfish (opportunities and need)
- Leverage all resources (intramural and extramural)
- Integrated approaches with industry participation for focus
- Interagency partnerships for science to practice

Lot of common areas between USDA & NOAA. How do we develop our framework of collaboration? Need a roadmap of research…X is doing this, Y is doing that but here is a gap area that I can address. Know what the priorities are

Emerging issues session: integration of extension with federal research labs

We all have different expertise, our facilities vary…but what collectively do we want to accomplish and what roll can each of us individually plan to accomplish these things?

PCSGA has a research plan…how does it get implemented? How might that connect with the east coast? Can we get a research road map for all of shellfish aquaculture? But how do we pull that together for a US strategy for shellfish aquaculture. That is the real challenge. We play a vital role in achieving that….we can figure out what are the key federal programs and what role can they play?

Q: Did you talk about the fellowship program? That is a nice initiative but one of the things we're seeing is that aquaculture faculty are being lost and not replaced. Any interest in building at the faculty level aquaculture positions to retain expertise?
A: This is a really disturbing trend. These are decisions that institutions make. The agency is concerned about the next generation of scientists and educators, this is why the study is important. This can be a common link among the three professional organizations and maybe they can work together…. But how do you change a state? They identify their priorities and determine what faculty they are going to hire. The land grant mission of some of these universities has really taken quite a hit.
Q: Maine aquaculture is concerned and they helped a lot with building brick and mortar buildings…so now we have great facilities but not the scientists in to work in them. Can we move forward with federal stimulus of aquaculture (or agriculture in general).
A: Honest reality…Americans consume more turkey than seafood on a per capita basis. I'm really hoping that this project can really elevate the problem

Q: you mentioned the land grant program. There is sea grant but it is run through NOAA and it is different. Is there any inclination to do a similar model for aquaculture?
A: Really good model but the $ to the Universities have been flat. And Congress is putting the new $ into competitive programs. Those $ are being used to maintain the core of the agriculture experiment station…cover costs which (not salaries) to keep the programs working and viable. Over the years the % of federal dollars that support those programs is becoming smaller. Same with RAC. Since 1986 it has been flat funded…. look at the inflation adjusted dollars. That program is not headed in the right direction.

Comment: In NZ most of the companies got together a few years ago so that they can lobby government effectively.

Q: I would really encourage the survey makers to compare what is going on in this country with what is going on in other countries. South America, Europe, Asia.
A: Believe me, I know. Some of these other countries are putting lots of money into aquaculture/ agriculture and developing a real great workforce.

Q: Will international students be able to apply for fellowships?
A: can't answer that question.

The five key areas from the National SF recognize that we are missing agricultural information. We as scientists need to show that agriculture is relevant and is going to make a difference. NSF gets 50X our funding. NIH gets 100X our funding…. We need to emphasize the connection with life sciences

This group is coming up on the end of a 5 year strategic plan. We should put some thought into our goals for the upcoming years. How are the coasts different? What are our needs? We should pull a small group together to develop a strategy of how we might go forward and think about the outcomes that come from our collaborations (multi U, interdisciplinary). That might really guide the research roadmap for aquaculture.

We also really want to get NOAA people here and industry people here, as well as other federal agencies that could help. You might also contact local sea grant director and see if something could be done with those programs as well.

<u>Gary Zippel</u>
<u>Australia industry</u>
<u>Do daughter studs have a place in the oyster industry?</u>

What if hatcheries want to put their own resources into selective breeding?
Should this be independent and haphazard or should it be part of a centralized system?
Is it possible to link all selective breeding?

Sub breeding program of a centralized breeding program (CBP)
Individual hatcheries May want only 5-10 crosses
Will need to continue to get fresh genetic material from the central breeding program to minimize inbreeding

Hatcheries may

- Want to concentrate on certain characteristics
- Want lots of broodstock available
- Want some level of exclusivity to the lines they produce

Daughter stud concept is a supplement and not instead of lines produced by the CBP
Therefore the central breeding program must produce ~50 lines a year

Rules:

- One CBP person is present when new lines are created to ensure correct procedure and record keeping
- The CBP may make available genetic material and specialized equipment
- Resulting crosses jointly owned by hatchery and CBP
- CBP has rights to at least 2000 animals from each cross for testing and breeding
- Only hatchery has the right to use the crosses for commercial lines but CBP can use them in breeding program
- No hatchery rights exist over any line subsequently bred by CBP

How is funding for the CBP to cocur?
What services are to be paid for?
At what rate?
What royalties should be paid to help meet costs of CBP?
If there is a need for royalty on all daughter stud commercial releases, hopefully at a min

Overall goal: To make selective oyster breeding ubiquitous and coordinated

The concept wasn't picked up in AU but seems like a good source of discussion

Comment: I think it is neat. We're trying to convince people that what we do is valuable. Some make their own seed, grow em out, make more. I like this as a way to bring people in without overwhelming them. I think it could be entirely proprietary. I'd like to be able to test our stuff against anyone's commercial stuff just to see where we stand. But they might not want us to compare their stuff against other stuff and release those results.
A: Can you turn this around? Can you get them to test your stuff at their sites? You can help them do the data collection and keep it all confidential for their and your use only.

Q: At an international scale, this is a huge problem for disease. Key issue for all of this is to improve disease monitoring. Otherwise a legal and practical constraint. Coming from the plant genetic sector…do we know anybody in FAO who has any interest in our species. Maybe one funding door.
A: So we have 3,4,5 separate *gigas* programs running around the world. This may be fine in the early days but what if you want to introduce more genetics? If you go back to the wild, you lose a lot of genetic gain. But you could trade with other international breeding programs. But barriers are going up, not coming down. Maybe we can trade genetic material with frozen preserved sperm but no current protocols to allow us to do that.

Comment: USDA has repositories for frozen genetic material. Once it is in there it has been cleared and can move around more freely. Can promote decentralization by doing more of a mixed family approach….Make 50 families, pool them together, and the grower grows them and somehow determines best animals by whatever criteria they want. Then genotype the good ones (good by farmer determination) and design a mating scheme for the farmer. Lines kept separate at the breeding program but mixed by the farmers. Mass selection.
Response: Problem with mixing them together and picking the favorite ones is that you don't know what their brothers and sisters are like.
Response: But if you genotype a lot you can reconstruct the pedigree, do a BLUP analysis, etc. Relying on natural selection for mortality. But for growth, shape, etc this could work. Centralized thing---centralized BP can breed for disease resistance while the individual farmers can breed for whatever they want, stripey, garlic flavored, etc.

Comment: One of the other important things to think about with this idea. It isn't the # of lines that can be produced, it is the # of lines that can be managed on the farm. By combining everything that problem goes away. Industry is managed to handle large numbers. If you give them small groups they want to mix them for efficiency.

Comment: In NZ, the farmers are asking for more individual families…they want to compare them themselves. They are so enthusiastic that they are prepared to do 50 individual families.
Response: That is great, our guys aren't there yet.

Stan: In some sense it is show me the $. How much does it cost now to do what you suggest and how might this change.

Pierre: this is routine in finfish now and it costs 10 euro a fish to do parentage assignment. In AU it is about 4-5$ a prawn.

Pierre Boudry: French update
pboudry@ifremer.fr
Summer mortalities

- Last two summers were very bad for seed survival
- 2008 was bad & 2009 was even worse
- Small seed only, doesn't affect larger animals much
- Smaller the animal, the more mortality
- Mortality happened at lower temperatures than it has in the past
    - Usually it was a 19C threshold and now it is 17C
    - More sites are affected
    - Last summer, all farming sites are affected from Corsica to northern France, with a clear temperature relationship (as T increased with time, over time mortlity goes from the South to the North)

    - More evidence that this is infectious—if you mix dying and non dying you can transmit the phenomenon. But it is also highly environment dependent---in the nursery, the seed survive but in the field they die.
- Have looked for pathogens, the # 1 candiate is the herpes virus
    - Very high prevalence in 2008
    - Detected a new genotype for the virus by sequencing
    - No direct indication that the new genotype is more virulent but it is clear that it is more present ---change in the population of this virus over time. Now are looking at archived samples to see if this variant was present in the past

Good news

- Morest (?) program divergent selection experiment started in 2001, 1 generation of selection and repeated lines by within line crossing….
- Show that what was selected 10 years ago is still effective
- Monitoring via qPCR. Over time, viral load goes up in S line while load goes up then down in the R line. So the R line deals with herpes virus while S line cannot.
- In press in animal genetics

QTL mapping (paper in press marine biotechnology)

- Recorded viral load and now have two QTLs for viral load in *gigas*. The map is not good enough (100 microsat/ snp markers) so error bars are huge
- 5 QTLs some are family dependant and others are not
- This line of research is very promising

Journal of virology paper

- More about the qPCR approaches, etc
- C. Sauvage first author

Fleury (paper in press marine biotech)

- Microarray study in R & S lines over time
- Examined during 3 months before mortality. Last sampling before mortality, 60% of differentially expressed genes are related to immune function. (before that the differentially expressed genes were all over the map)

Very different reproductive allocation in R and S lines, the R line has smaller gonads
Don't think it is a causal factor of mortality…but may be a cofactor
We see a clear genetic correlation between survival and allocation to reproduction

Montpelier (Evan Bachier)

- Working on SAGE
- Data comparing libraries from oyster hemocytes from infected with virulent and non virulent vibrios
- Successful, has a list of genes that are up/ down regulated in different challenges
- But SAGE uses small tags so you lose a lot of information, annotation is limited
- 10% of tags are linked to a given EST. To do this used the SIGENAE EST database mentioned yesterday. The new ESTs should help a lot!

- Do qPCR of differential expression of the genes—progress of response of gigas to virulent vibrios. Not specifically connected to *tubaiashii* but…?

Brest

- Work a lot on reproduction
- Functional genomics of reproduction: learning hormones related to gametogenesis
- Different approaches: gene proteins, reverse endocrinology, AMP dependent kinase which is an enzyme that controls balance between somatic & gonadal growth, matching ESTs which allows us to model the shape of the proteins which look very well conserved in oysters. Moving into proteomics to determine whether AMP kinase is known in oysters in the balance between energy allocation towards reproduction vs. maintenance.
- Paper in FEBS reporting RNA interference in Vasa (related to germline). Now we've tried to use this approach in other genes…gap between annotation and real annotation of function of a gene. RNA interference is one way to fill that gap. Doing it now in TGF beta which is related to gonad development. In vivo injection of knock down approach…gonad development is recorded via histology. A way to reduce gonad development. Pure research at the moment. Not sure about application unless there is heritability of the phenotype
- In a given gene, RNA interference is one way to assess its function directly

Reproduction allocation

PhD student finished last year worked on reproductive allocation in triploids to see if there is genetic basis for gonad development in triploids. Yes there is some evidence for genetic component for gonad development in triploids. For big or small gonads in diploids, response to selection in dips and trips. Difficult trait to work with. Highly influenced by environment but genetics behind it….to select for trait is tricky. Why do triploids sometimes produce gonads?

Development of the selective breeding program in France

- Hatchery industry is ready to start selective breeding led by industry, more like the Australian model
- New group called SYSAAF. Organization dedicated to assistance to selective breeding. Started for poultry (first A for aviculture) then added an A for aquaculture. Role is to help private companies implement selective breeding. Union of hatcheries. You pay to get in and they help you implement your breeding program. Two companies have now instituted breeding programs. SFC (French shellfish collection) and another family based company. Main trait right now is seed survival. Plan is to run three to four hundred families per generation using a flow through system like Nick showed yesterday.

- The companies are shy at the moment and they have nothing to show…but maybe in 2-3 years Pierre can show data from those two projects. Two projects is a bit of a shame, one would have been better..but it is France, people don't agree.

Q: Would someone from SYSAAF be interested in coming to Baltimore next year? Yes,
A: He'd like to come but not talk…listen a lot. Please invite him.

Q: The larval survival problem seems to be associated with upwelling & acidification. Have you looked at interactions where sea water chemistry may enable pathogens?
A: Don't think it is a major factor in France because it happened everywhere, from Med to channel….VERY different environment. It looks like Temp is the easy factor connected to the spread of mortality. May be other factors but likely not ranked as high.

Q: have temps been higher than normal?
A: No. And in 2008 didn't see the S-N trend but temperatures were not normal…Atlantic was warmer than med. In 2009 really obvious trend and relationship with threshold temp.

Q: When it started did it start in Mediterranean or the Atlantic? Can you tell if there was a pathogen transfer?
A: No. So much transfer of seed in industry. In the Mediterranean there is no spat collection and no hatchery so all the seed are imported. It is difficult to trace. There was no difference in mortality observed in hatchery vs. wild seed, and no differences between triploid and diploid.

Q: what size limit do they start?
A: above 3-4 cm is okay. But mortality can be very high at very small sizes.

Q: Has anyone looked at relationships with algal blooms?
A: Yes. The problem is you have mortality everywhere. It is hard to imagine toxic algae everywhere. HABs impact oysters but nothing blooms from Med to Northern France.

Comment: In NZ, an algal bloom in a pond wiped out 90% of small seed and 50% of larger seed. Now they can see it coming….oysters stop feeding. If they move them to aclean pond, in 3 or 4 days, they will recover.

In Fr. This dying phenomenon happens when seed are growing very fast. No sign of growth slowing before death. Experiment in a lagoon. Seed in an open didn't die at all but when they were moved to the bay, they die. So it is clear that environment is a key factor to mortality but may not be the cause…maybe environment starting a disease which is already present.

Q: Have any farms tried to manage around the problem by bringing the seed past the safe size in the hatchery and then planting?
A: Yes, some have tried but now affects the whole industry…

Q: Now hatcheries are trying to spawn earlier so seeds are bigger by the time the temperature gets too high.
A: Hatcheries now produce about 20-30% of seed for the market. Industry is very worried because they've had two successive bad years of seed so they will lack oysters at commercial size in the coming years.

Q: An article I just read had a figure that the French oyster industry was worth 845million $ does that sound right?
A: I don't know….Gary worked it out to be 420million…at least half so in the ball park. And Pierre says price may be higher than Gary thought so.

Many farmers think that they would like to import another species or another strain and that will solve all the problems. We say, no, there is no escape from it. This is why it is so useful to show that in other countries breeding already started and showing results.

Comment: Miyagi has endemic herpes so these might be an interesting strain that should show some natural resistance to herpes. That could be an interesting collaborative project.

Q: Can the R line be transferred over as a breeding line?
A: No because that line is based on 3 males and 4 females. And got these lines when Moress (?) was finishing and nobody cared. Now everyone is asking for them.

Q: You talked about looking for hormones for gonadagenesis. From industry, we think it is great that they put less energy into spawning but what do the hatcheries do. Is it feasible to develop a hormone drug that allows the breeding proteins…
A: problem is that our knowledge of endocrinology is very limited in bivalves. So the basic science is where we are now. Getting the whole genome sequenced will help a lot!

Triploidy conversation: implications and strategies for selective breeding

Geoduck aquaculture: concerns for culture and wild interaction
How do we break the connection? Triploidy or other ways to sterilize animals are going to be important and in some cases mandated. How do we break that genetic connection between wild & cultured animals?

We want to do selective breeding with geoducks but don't want to do so because of concerns with CWI.

Triploids are hard to raise in hatcheries on West Coast so…..that might not be the best bet.

Logical way is through GMOs…but that isn't going to work.
Some sort of progeny testing where progeny are sterile. Make combinations, evaluate them and then return to parents. Straight cross breeding program where you create a lot of

diploid and tetraploid inbred lines and cross them. Run a tetraploid breeding program and a diploid breeding program….you are starting a 30 or 40 year breeding program.

Pierre:
16 male by 6 female cross using either diploid or tetraploid males
Looked at progeny
# of individuals that we phenotyped was not big enough to reach significant conclusions
Phenotyping was heavy
Microsatellites to assess parentage, and hard when crossing tetraploid and diploid
Parentage assignment is hard with no parentage program that assigns parents with different ploidy levels.

Marta;
Thinking about mammalian work and sterility. Has anyone done family work and breeding. Any of them have problems with sterility? Can we identify the genes involved in sterility? If they have a dominant effect…RNAI knocks down so that is just an example of an approach.

Pierre:
We are trying to find gene expression levels that are correlated with gonad development in triploids. It works in diploids but not triploids. It would be good to find a few genes for which expression level is correlated with gonad development. We have a few candidate genes. We do biopsies and do qPCR on the biopsies but gonad is heterogenous so tricky.

Marta: a lot of the genes in sterility in cattle seem to be regulated by splicing….
Pierre: we know so much more about endocrine regulation in cattle than in bivalves

Gary: Could you breed animals in a hatchery but whose larvae aren't viable in the wild?
Brent: But they could outcross in the wild and then what?
Anu: On the EC the triploid is a preferred animal. Why is this triploid so good? A breeding program that improves a triploid…over generations you can inherit additive gain from diploid line and you continually improve your triploid product.

Move the additive gain forward by the diploid breeding program.
Problem we face now is that there is a lot of triploid production but we have very little information about how the additive variance is expressed in triploids

- So many factors interfere. They don't make much gonad and this affects the whole animal. Transfer of energy is different and additive value is expressed differently. It's a black box
- Do you think there is a design that could address that?

We should spend some time with the plant literature

- Potatoes are all tetraploid. Sugar beets have diploid and tetraploid lines and market sugar beets are triploid. Many breeders 15 years go said that genetic progress would be faster if they could just mate diploids and improve them….in sugar beets breeders chose not to improve the tetraploids as it was too slow. So just improved the diploids. Very complex and long term breeding strategy.
- Reminder that we are far from the first to address these issues.

Rather than splitting the # of lines into two different breeding program, may be better to do all genetic gain with diploids and then just cross those with tetraploids.

- Assumes the transfer of gain is additive.
- But you can still trial triploid crosses. Part of the assessment process is to do trial crosses with several improved diploid lines with tetraploids.

Where the whole heterosis scheme intersects with selective breeding.
Combine a traditional selection of diploids with crossing program of tetraploids to give growers a choice of diploid and triploid product.

Thought that shellfish growers in Tasmania had a selective breeding program for triploids. But…as far as I know it went nowhere. Got stuck on the "getting the tetraploid house in order" piece. Not sure if they've gone on to the next step.

Question: Regarding tetraploid breeding program and a diploid breeding program. Why would you want to improve a tetraploid when the triploid will give you so much more gain in one move.
A: Because triploid larvae die in the hatchery. This is one trait we want to improve. How do we do that?

In NZ, they started testing their triploids and are using DMAP that is giving them close to 100% trips. They assumed diploid gain and triploid gain are additivie…and just did a commercial scale batch. I think they got better performance from triploid larvae than diploid larvae.
Comment: The trick is to try to make that consistent.

Comment: Given high genetic loading in oysters, three chromosomes gives you one more chance to get a positive, dominant allele.

Q: What is the affect of inbreeding in polyploids?
A: Don't think there is a single paper. Data gap.

Comment: What is it in the stressful environment that causes the triploid larvae to not do as well? Is there something they could do? More food, less food, higher t, lower t?
Response: Our job is not just to id the genes but to id the environment that maximizes genetic potential.
Response: Whiskey Creek and Taylors have the same issue with triploids. It doesn't mean they die, it means, if there is death around, they are worse.

Response: If you extend this logically, they won't have trouble with all triploids but will have problems with some crosses. Is there a systematic approach to figuring this out?

Comment: What about epigenetic issues in tetraploids? Some tetraploid lines might be more stable and faithful in their segregation of chromosomes than others. Over generations they seem to stabilize. Another issue is that individuals lose chromosomes. We have to use tetraploids before they are four years old because by the time they are two they are a patchwork of diploid, triploid, and tetraploid at the somatic level but the germ level seems to be fine. Different results in France.

Comment: I favor the idea of reciprocal breeding rather than transfer back additive variance from diploid.

Comment: Identification of genes along gonad development might help solve this kind of question. Another way to investigate this problem is to look at alternative methods to produce tetraploids—how you generate your tetraploids might influence the outcome.

Comment: Regarding succeeding generations, larval culture of tetraploids gets better as does the general health of the population. Bad ones are culled out.

Comment: Would be very interesting to compare a tetraploid diploid cross and a chemical triploid cross with the same genetics. International proposal that could address a fundamental question is a good idea.

Comment: Characterization of polyploidy in crops. Even in plants, where polyploids are widely used, there is not much. This is part of quantitative genetics that needs to be explored. Like when you start to calculate H when you use tetra and diploid crosses….you need to do it from scratch.

Comment: Triploidy is an emerging topic for native species. How to handle tetraploids as well. In France, there are people concerned about interaction among cultured and wild conspecifics and there are people who want to to seed selected lines in the wild and see where they settle and thrive. In France because the industry relies on wild caught seed, they want to go that way. What if I take 1 million improved seed and dump them, do I improve the wild caught seed as well? Everything is related to effective population size. Compromise between conservation and aquaculture. Nice way to progress rather than fighting.