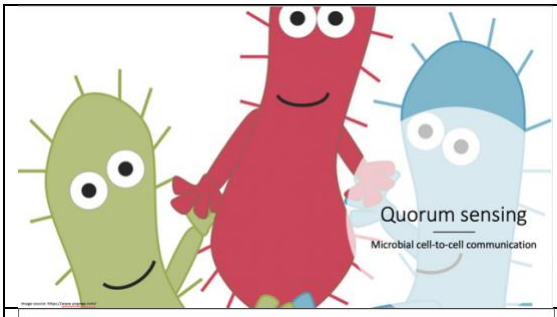
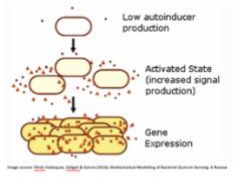
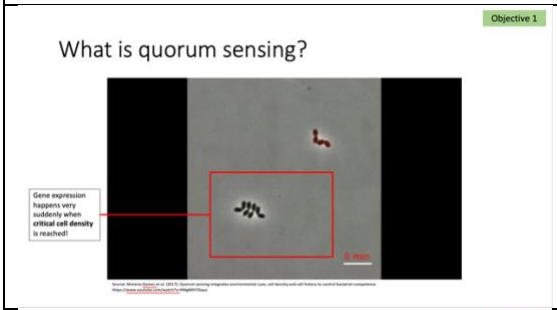
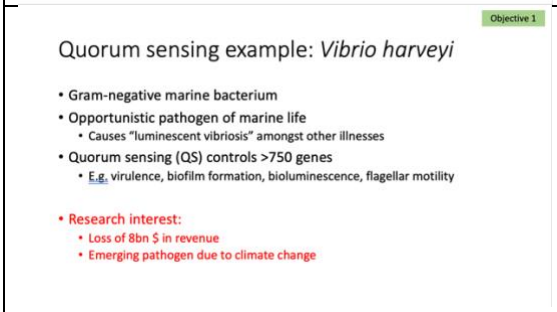
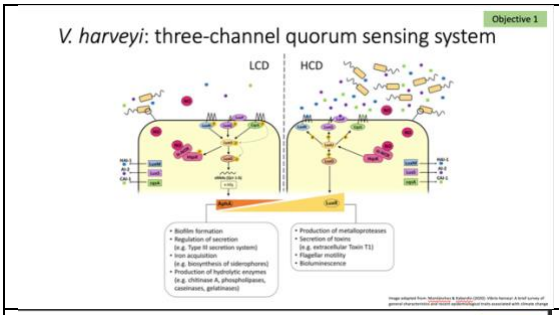
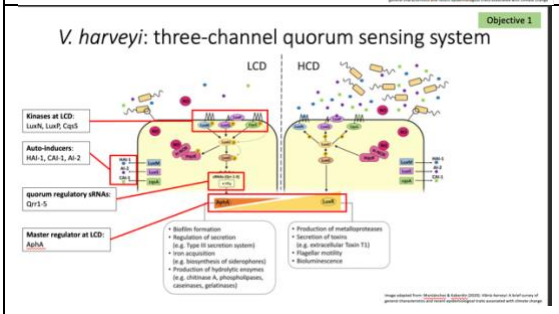
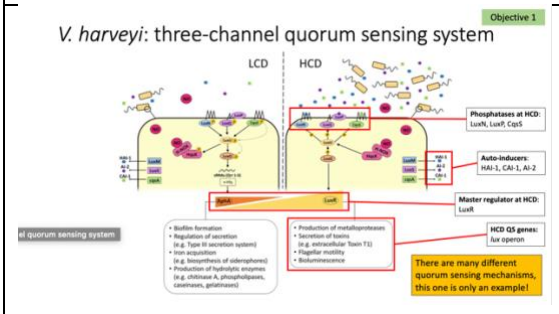
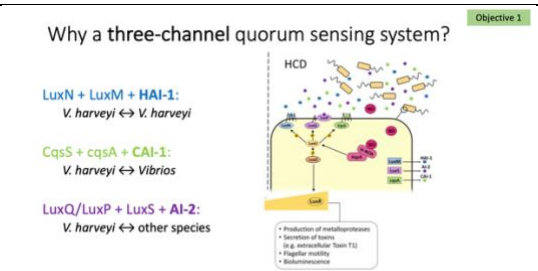
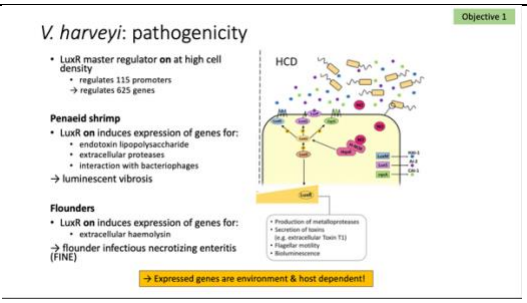
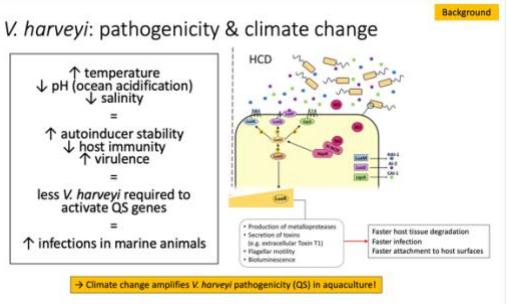
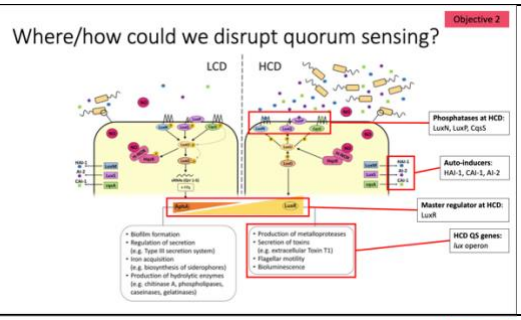
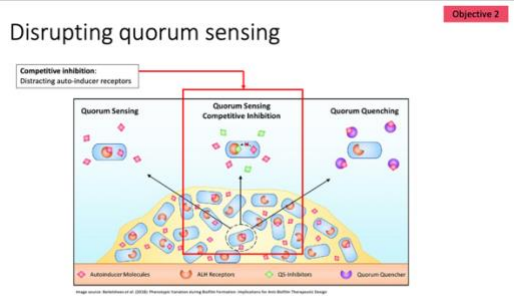
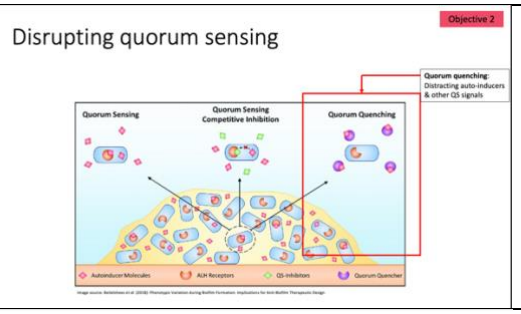
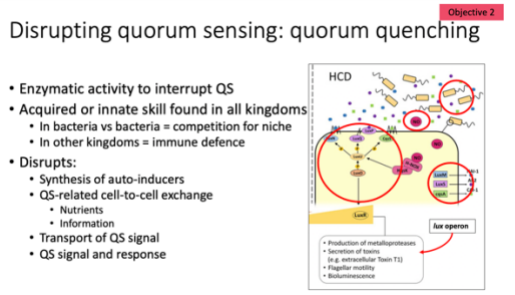
	- Lecture in microbial cell-to-cell communication - Quorum sensing - For Questions pop them in chat and raise hand
Learning objectives By the end of this lecture, you should be able to: • Describe the process of quorum sensing by bacteria — Objective 1 • Explain, using detailed example(s), how quorum sensing can be manipulated to control microbial populations — Objective 2 • Describe applications for manipulation of quorum sensing — Objective 3	- We have some learning objectives for this lecture - *Read the slide out loud* - Boxes on right side will be on each slide to indicate which objective we are discussing
Hint! Previous exam questions: • 2019: Describe the process of bacterial quorum sensing, and explain applications for the manipulation of quorum sensing. • 2018: Describe the process of bacterial quorum sensing, and explain how it can be manipulated to control microbial populations. • 2017: Describe the process of quorum sensing and explain how it can be manipulated to control microbial populations. • Worth 20% of your overall grade!	- A quick hint about the exam questions from the past - Answer lecture objectives = answer exam question
What do you know about quorum sensing? 	- *Do not move on until someone has answered*
Objective 1 What is quorum sensing?  • Bacterial communication → Within & between species • Division of labour for difficult tasks • Regulation of gene expression as a response to changes in population density → more cells = more **autoinducers** = gene expression	- Great answers! - So quorum sensing allows bacteria of the same and different species to communicate with each other - Generally, one bacteria secretes autoinducers. They are degraded in the environment. - **Unless** there is enough bacteria in the environment that secrete the same autoinducer. Then, the autoinducers bind to receptors on the cell membrane of the bacteria and induce a signalling cascade that results in gene expression.

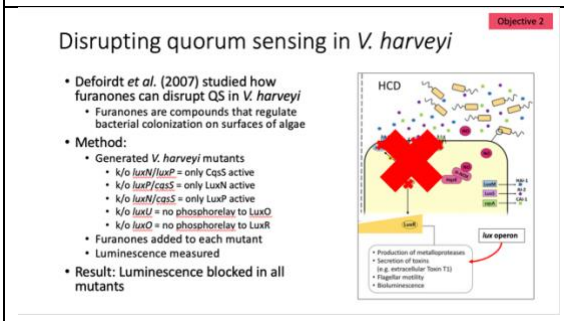
	- Often this happens so they can divide the labour of otherwise very taxing tasks - Examples of these are the formation of biofilms, secretion of virulence factors, swarming motility, bioluminescence or the production of public goods, like nutrients - So overall, this means that quorum sensing is the regulation of gene expression as a response to changes in population density
	- Here is a video that shows quorum sensing and as you can see, the gene expression starts very suddenly, so a critical cell density in the environment needs to be met for the signalling cascade to start
	- To dive a little deeper into quorum sensing I want to introduce you guys to Vibrio harveyi - Gram-negative marine bacterium that is an opportunistic pathogen of fish and crustaceans, so it causes disease when it gets the opportunity to do so, and one example of this is luminescent vibriosis, where crustaceans glow when they are infected with vibrio harveyi - The disease is due to the quorum sensing, which in vibrio harveyi controls over 750 genes, and some examples of this is of course, bioluminescence, but also virulence and biofilm formation - vibrio harveyi is of interest to the aqua culture and fishing industry because it causes a loss of around 8 billion dollars in revenue, and due to climate change this is become an emerging pathogen. And I will go into this a little later - first, lets look at the quorum sensing system in vibrio harveyi

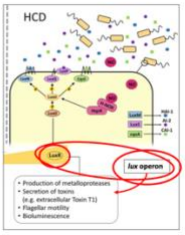
	- vibrio harveyi has a three-channel quorum sensing system where the autoinducer receptors have different functions at low cell density and high cell density
	- the autoinducers for vibrio harveyi are called HAI-1, CAI-1 and AI-2 - at low cell density, their receptors on the bacterial cell membrane are kinases. So they are unbound from the auto-inducers and autophosphorylate. This initiates a phosphorylation cascade where the enzyme LuxU phosphorylates LuxO, and this triggers the transcription of several small regulatory RNAs called Qrr-1 to 5. These RNAs trigger the up-regulation of the low-cell-density master-regulator AphA, which you can see here, and this expresses various genes, and some of those are listed in the box below. Importantly, the small regulatory RNAs also **inhibit** LuxR, the high-cell-density master regulator - *kinase = enzyme that catalyses the transfer of a phosphate group to a substrate/protein* - *phosphatase (next slide) = enzyme that removes a phosphate group from a protein*
	- this is my little Segway into high cell density. And that's what's really of interest to us, because the point of quorum sensing is that bacteria can "sense" when other bacteria are around. - When there is more bacteria in the environment, there are more autoinducers available. These then bind to the receptors, which turns them into phosphatases. This then stops the phosphorylation cascade. LuxU becomes de-phosphorylated, so it becomes inactive and stops

	transcribing the small regulatory RNAs. The absence of these RNAs then stops LuxR inhibition, so it "activates" LuxR and therefore, allows the transcription of genes regulated by LuxR. And one examples here is the secretion of toxins. - Pause.. so this is just one example of a quorum sensing mechanism, but there is many more. - I will just wait a moment here for questions because I know this is a bit complicated.
	- So why would we have a three-channel quorum sensing system? Does anybody have any ideas? - *Wait for a moment* - Yes, so different auto-inducers and their receptors have the ability to communicate with different types of bacteria. - You can see here, HAI-1 and its receptor allows vibrio harveyi to communicate with its own kind, CAI-1 is a shared auto-inducer between different kinds of vibrios, and AI-2 is a common autoinducer amongst other bacteria, including gram-positive ones.
	- To bring these things together, lets take a quick look at vibrio harveyis pathogenicity. - The High cell density master regulator regulates 115 promoters, and 625 genes altogether - Many of these genes are for pathogenic purposes - One example is penaeid shrimp - In penaeid shrimp, luxR induces the expression of genes that produce endotoxin lipopolysaccharides, extracellular proteases and it enables interaction with bacteriophages, which can enhance expression of these genes as well - This then results in luminescence vibriosis - Another example are flounders

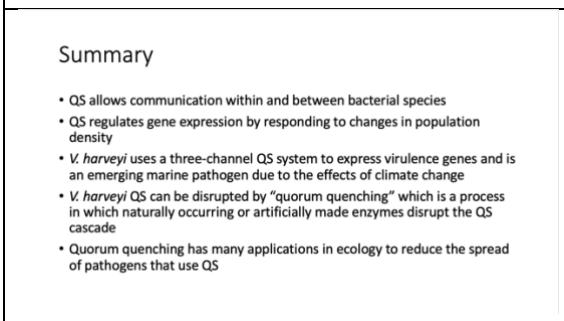
	- Here, luxR induces the expression of genes for extracellular haemolysin, and as you can probably guess from the word, it means that it produces flesh-eating enzymes. This results in the flounder suffering from flounder infectious necrotizing enteritis - What you can see here it that the expression of LuxR genes depends on the environment and the host the bacteria finds itself in.
	- How are we doing for time? - Okidoke so I will briefly go over this slide - I have mentioned before that vibrio harveyi is an emerging pathogen due to climate change - the increase in temperature, drop in pH and decrease in ocean salinity has different effects on the host and the bacteria. These conditions for instance increase the stability of autoinducers so they can remain in their environment for longer and this increase the likelihood of them binding to their receptors. This means that there are less bacteria needed to activate luxR genes. (*click to make box appear*). - The sooner activation of LuxR of course results in faster degradation of host tissue, infection and attachment to host surfaces. - So this means that vibrio harveyi is more virulent under climate change conditions and causes more infections in marine animals. - So probably one thing to prevent this from happening is to stop the virulence genes from being expressed.. so that would mean we want to disrupt quorum sensing. - which brings me to our next part - [for questions: Decrease salinity: larvae weaker. Due to glacier melting into wetlands and estuaries. Also

	affects virulence, Temperature-induced up-regulation of virulence]
	- I have left this image here of the cascade to make this question a little easier to answer. - Does anybody have suggestions on how or where we could disrupt quorum sensing? - *Wait for a bit for people to answer*
	- So quorum sensing can be disrupted in two different ways - One of them is competitive inhibition, where molecules similar to the autoinducer molecules can block the binding of the actual autoinducers
	- But what we are looking at is quorum quenching, where enzymes inactivate the autoinducers
	- Quorum quenching is a skill found in all kingdoms, and this can be an acquired skill, for instance when genes that carry this ability integrate into genomes of some organisms - Or an innate skill, where this ability is already in an organism - Usually, if this is a skill in bacteria, it allows competition for a particular niche - But quorum quenching in other kingdoms often is a type of immune defence, or even a way to regulate quorum sensing in a way that give the organism an advantage - On the side here you can see what it disrupts, some of these things were already suggested - So quorum quenching can stop the synthesis of auto-inducers

	- Or it can stop or degrade materials used for cell-to-cell exchange - It can prevent the transport of the quorum sensing signal or prevent the production of the quorum sensing signal or its response - It takes a lot of effort to identify what quorum quenching does in the quorum sensing system...
	- So I wanted to show you one specific case study here to that tried to work this out for furanone compounds - Furanone compounds regulate bacterial colonization, for instance of vibrio harveyi on algal surfaces and when defoirdt started their experiments it had already been established that furanones disrupt quorum sensing - So they set up an experiment where they looked at each element of the quorum sensing cascade in vibrio harveyi to determine where in the quorum sensing cascade furanones disrupt the cascade - The experiment was done in three steps and the first one you can see here. they knocked out the genes for each receptor, so they knocked out enzyme luxn and luxp so that only cqsS is active, then they did the same for each of the other enzymes as well – and then knocked out luxU and LuxO to disrupt the signaling cascade - They then added furanones to each mutant to see if they would still produce bioluminescence - None of the mutants luminescent, which means that furanones must interrupt the quorum sensing cascade further down..

Objective 2
Disrupting quorum sensing in *V. harveyi*
• Method 2:
  • Wild-type *V. harveyi* + furanone
  • RT real-time PCR measured mRNA of *luxR*
• Result 2: *luxR* was normally expressed
• Method 3:
  • Radiolabeling LuxR protein & *lux* operon promoters
  • Autoradiograph and gel electrophoresis to visualize binding
• Result 3: LuxR protein not bound to *lux* operon promoters
• Conclusion: Furanones disrupt QS in *V. harveyi* by blocking LuxR promoter protein binding to *lux* operon
 
 	
	- They then undertook their second experiment, where they looked at the mRNA of luxR to see if luxR is transcribed. They added furanones to wild-type vibrio harveyi and then us RT real-time PCR to measure the mRNA expression. This showed that the mRNA was normally transcribed. So that meant that they neeed to dive even deeper into the signalling cascade.
	- For their third experiment they radiolabelled the luxR protein and the lux operon promoters to check if they bind to each other
	- And they did that by using an autoradiograph and gel electrophoresis to visually exaine that
	- And that's when they finally found where it went wrong, the luxR protein was not binding to the promoters of luxR
	- So that showed that furanones disrupt the quorum sensing cascade by preventing the luxR protein binding to the lux operon
	- *Autoradiograph = x-ray -> determines if luxR mRNA and lux promoter are close together*

Objective 3
Applications for QS manipulation in ecology
• Aquaculture
  • Phytoplankton (e.g. *Chroococcus turgidus*) compound DTBMP reduces expression of *V. harveyi* master-regulator LuxR
  • Prevents biofilm formation and expression of virulence factors
  • Now commercial produced and commonly used to clean surfaces in shrimp farms
• Agriculture
  • *Bacillus* enzyme AiiA inactivates *Erwinia carotovora* auto-inducer AH2
  • *E. carotovora* = common vegetable pathogen causing soft rot
  • Enzyme disrupts QS cascade for virulent activity
  • Genes for this enzyme have been transformed into Chinese cabbage
• Antibacterial therapy, Wastewater treatment systems, etc.!
	- Now finally, and I promise we are nearly done. I wanted to give a couple of examples on how manipulating of quorum sensing can be applied in ecology
	- If you remember, vibrio harveyi cost the aqua farming industry 8 billion dollars in revenue. So it's obviously in their interest to stop vibrio harveyi from causing havoc in fish and crustacean farms.
	- What researchers found was that phytoplankton produces a compound called DTBMP which reduces the expression of LuxR, that then stops vibrio harveyi from forming biofilms and producing virulence factors

	- This compound is now commercially produces and used in shrimp farms to clean the surfaces, so that the bacteria cannot attach - I have another example that is a little more recent and from agriculture. In agriculture, erwinia carotovora is a pathogen that causes soft rot in vegetables. Researchers then found that the common bacillus enzyme AiiA inactivates an auto-inducer in this pathogen and stops virulent activity in the bug. - In 2019 the researchers then managed to genetically modify Chinese cabbage to carry the genes for the enzyme, so the vegetable can protect itself from soft rot - There's lots of other examples for applications of quorum sensing manipulations in ecology, ranging from their use in wastewater treatment systems to antibacterial therapy..
	- so.. I think we've gone through quite a lot of content here. - In summary, we know now that quorum sensing allows bacterial species to communicate with each other, and that quorum sensing regulates genes expression by responding to changes in population density - We learnt that vibrio harveyi uses a three-channel quorum sensing system that is used to express virulence genes, and that this skill makes it an emerging pathogen in the face of climate change - And finally, we know that quorum quenching, which is a form of manipulating quorum sensing, has many applications in ecology which allows us to reduce or control the spread of bacteria that use quorum sensing to express virulence genes

