



# UV-desinfeksjon - effekt på amøbens evne til å framkalle sykdom



Mark Powell  
Universitetet i Bergen  
NIVA



UNIVERSITETET I BERGEN





# Takk til

- Anders Lepperød (UiB) MSc thesis
- Herman Kvinnsland (UiB) MSc thesis
- Aina Charlotte Wennberg (NIVA)
- Marc Angeles D'Auriac (NIVA)
- Joachim Johansen (NIVA)
- Henriette Glosvik (MH Labrus)
- Gordon Ritchie (MH)
- RFF Vest – AGD control and disinfection in cleanerfish (ACDC) project.



# Mål for studiet

- Se om UV-behandlet amøber kan indusere sykdom hos berggylt
- Beskrive sykdomforløp hos berggylt med AGD
  - Brukte gjellescore, histologi, qPCR og i-STAT



# In vitro study objectives and design

- To test the effects of UV irradiation on the survival and growth of gill amoebae (*Paramoeba* sp.)
- To test realistic contact and exposure times relative to marine hatcheries
- Collimated beam UV lamp

Table 1. Exposure times [sec] to UV irradiance of *P. perurans* in four separate experiments each using a low and a medium pressure UV lamp in a collimated beam set-up.

Sample no	Exposure time low pressure [sec]				Exposure time medium pressure [sec]			
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 1	Exp. 2	Exp. 3	Exp. 4
1	60	10	5	5	60	15	5	5
2	120	20	15	10	240	30	15	15
3	210	40	20	20	480	60	30	30
4	300	80	80	80	720	90	90	90
5		150	150	150			150	150
6			210	210			240	240



# Effects of UV irradiation on amoeba morphology

Floating stages  
(non-exposed)

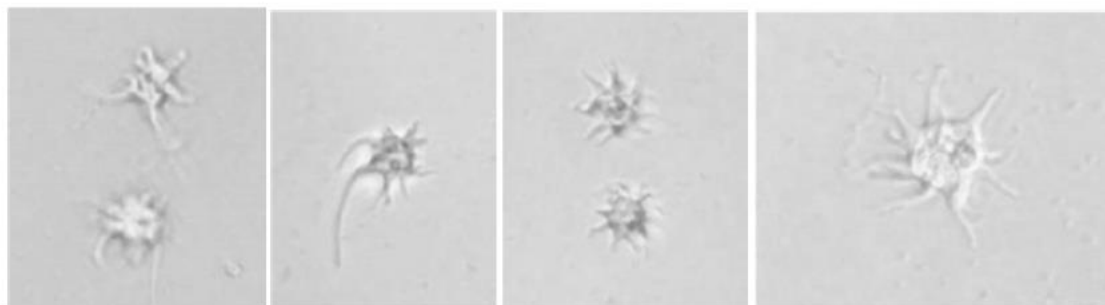


Figure 1. Floating stage of non-exposed amoebae (Photo: A. C. Wennberg)

Attached stages  
(non-exposed)



Figure 2. Attached form of non-exposed amoebae with active pseudopods and multiple vesicles. (Photo: A. C. Wennberg)

Attached «stressed»  
Stages (presumptive  
pseudocysts)

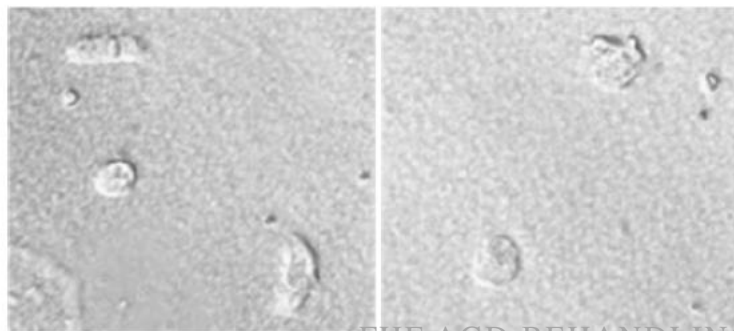


Figure 3. Stressed or damaged amoebae that has balled up several days after UV exposure (Photo: A. C. Wennberg)



# Evaluation of UV irradiation exposures

Low pressure

Table 4. Acute and long term effects on *P. perurans* of exposure to low pressure UV doses.

UV-doses [mJ/cm <sup>2</sup> ]	Acute effects (same day as exposure)	Long term effects (4-7 days after exposure)
0 (control)	None. Cells are polymorphic with active pseudopods and vesicle transport.	Exponential growth with approx. 20 hours doubling time.
4	None. No difference from control	No growth, or maximum one doubling. Morphology same as day 0.
4-64	None. No difference from control	Small and round or partly rounded up without active pseudopods or vesicles.
66-220	Round cells with short or no pseudopods	Small and round or partly rounded up without active pseudopods or vesicles.

Med pressure

Table 6. Acute and long term effects on *P. perurans* of exposure to medium pressure UV doses.

UV-doses [mJ/cm <sup>2</sup> ]	Acute effects (same day as exposure)	Long term effects (4-7 days after exposure)
0 (control)	None. Cells are polymorphic with active pseudopods and vesicle transport.	Exponential growth with approx. 20 hours doubling time.
2-5	None. No difference from control	No growth, or maximum one doubling. Morphology same as day 0.
5-10	None. No difference from control	Small and round or partly rounded up without active pseudopods or vesicles.
11-50	Round cells with short or no pseudopods	Small and round or partly rounded up without active pseudopods or vesicles.
48-215	Small round and possible damaged	Most cells are disintegrated



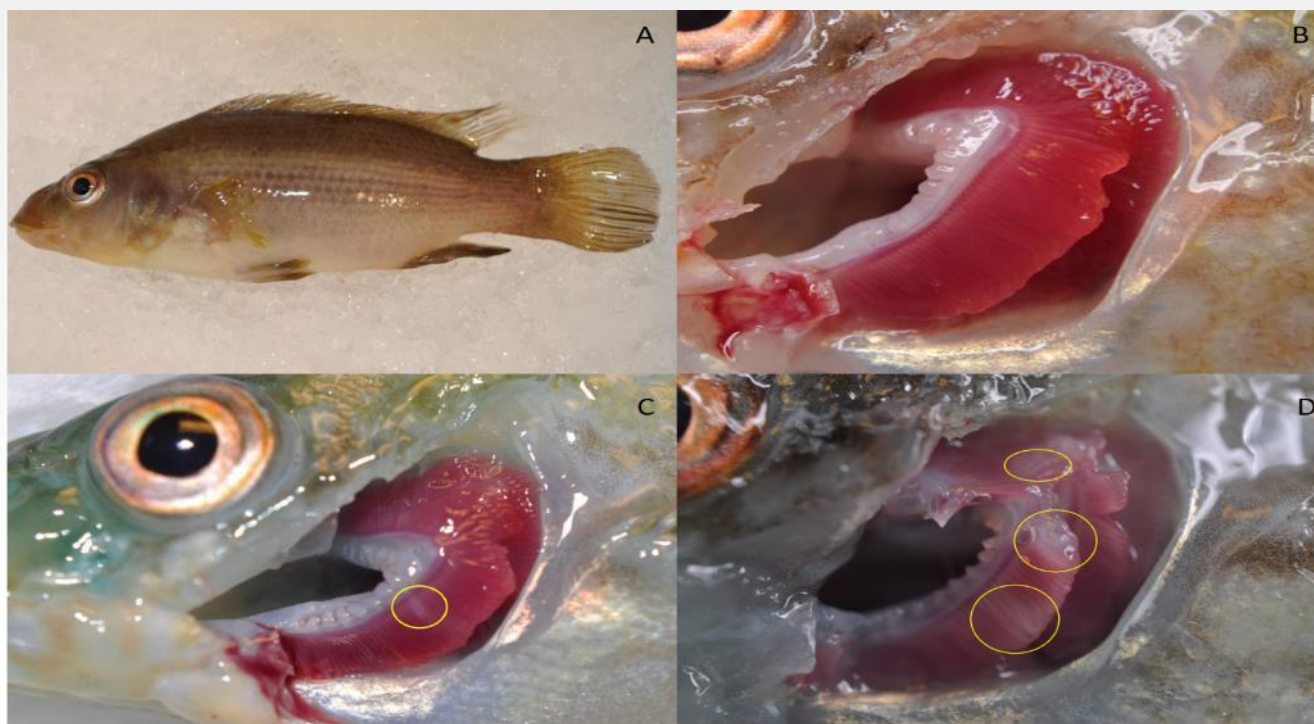


- 8 separate tanker (ca. 50 fisk per. tank)
- (700 L) med 400 L sjøvann – 13.6°C, 34,68 ‰, pH 7,98 - 8,02
- Medium pressure UV-lamper ble brukt til UV-bestråling av amøbekulturen
  - 10 sekunder for lav dose – 2 mJ/cm<sup>2</sup>
  - 90 sekunder for høy dose – 20 mJ/cm<sup>2</sup>

## **Karoppsett:**

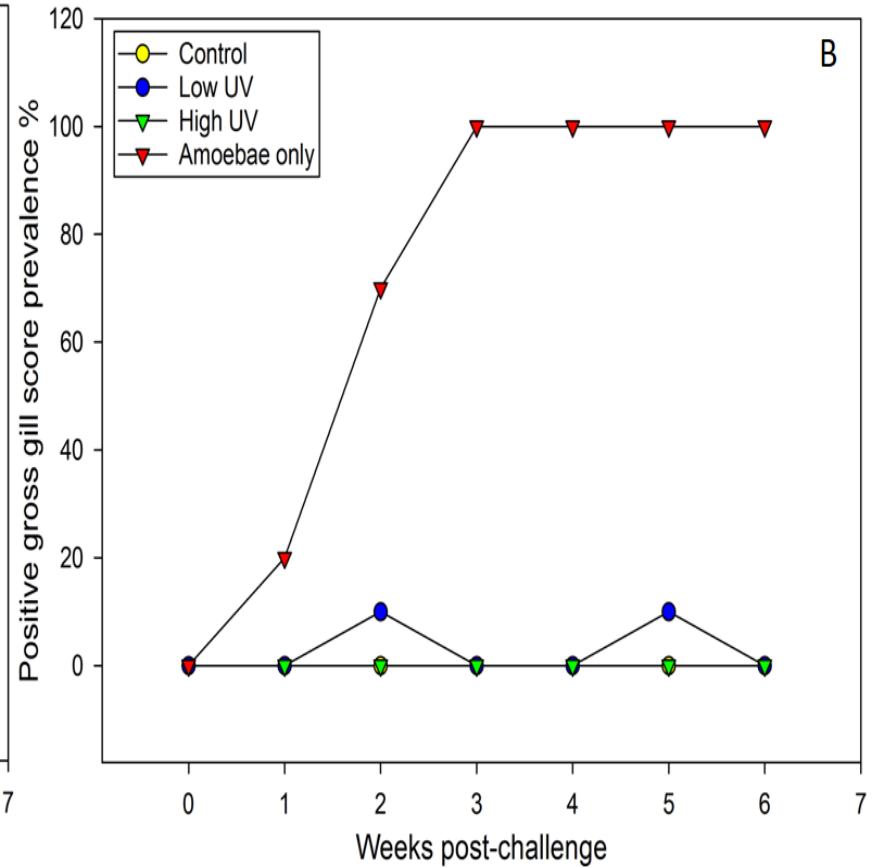
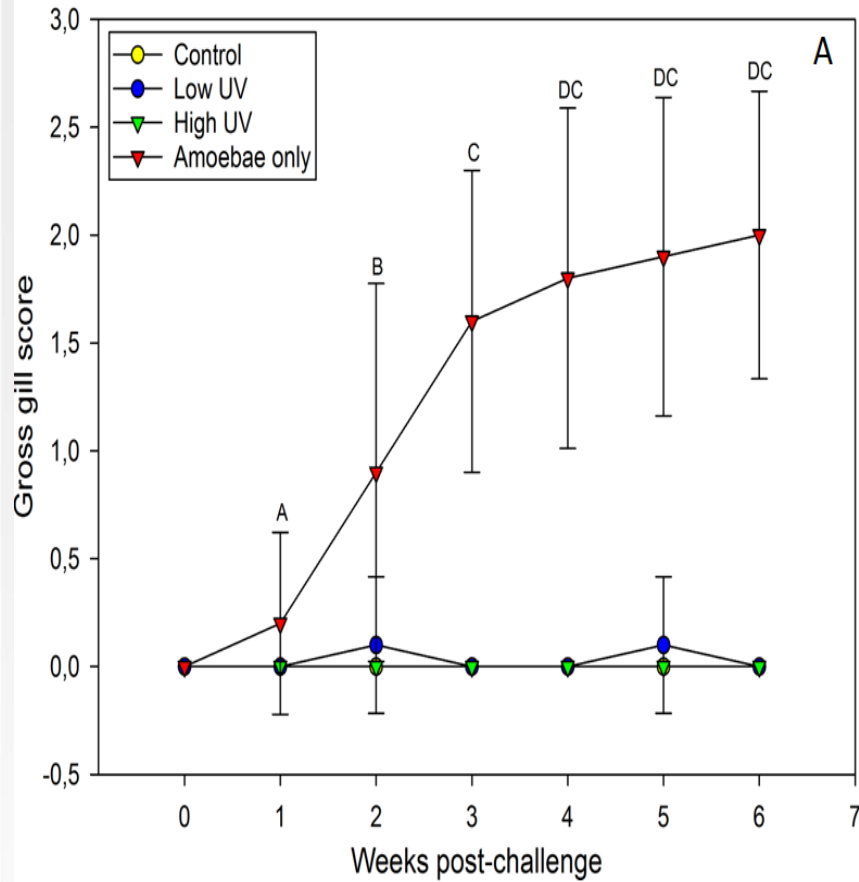
- ❖ Negative kontroller – 2 kar med 0 celler/L
- ❖ Høy dose UV-bestråling (20 mJ/cm<sup>2</sup>) – 2 kar med 1000 celler/L
- ❖ Lav dose UV-bestråling (2 mJ/cm<sup>2</sup>) – 2 kar med 1000 celler/L
- ❖ Positive kontroller (amøber som ikke var utsatt for noe UV-bestråling) – 1000 celler/L
- Ukjentlig prøver fra 5 fisk per kar – Gjellescore, gjellevev til histologi og qPCR og blod fra halevene for i-STAT-analyserer

Infection level	Gill score	Gross description
Clear	0	No signs of infection and healthy red color
Very light	1	1 white spot, light scarring or undefined necrotic streaking
Light	2	2-3 spots/small mucus patch
Moderate	3	Established thickened mucus patch or spot Groupings up to 20% of gill area
Advanced	4	Established lesions covering up to 50% of gill area
Heavy	5	Extensive lesions covering most of the gill surface



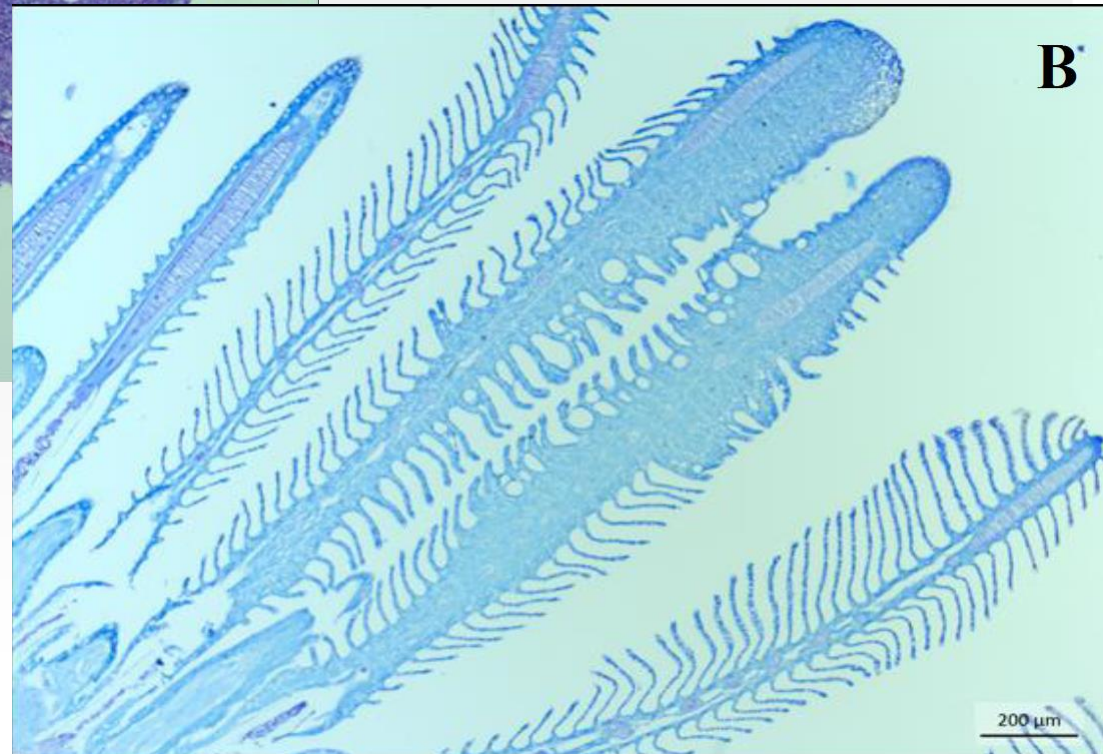
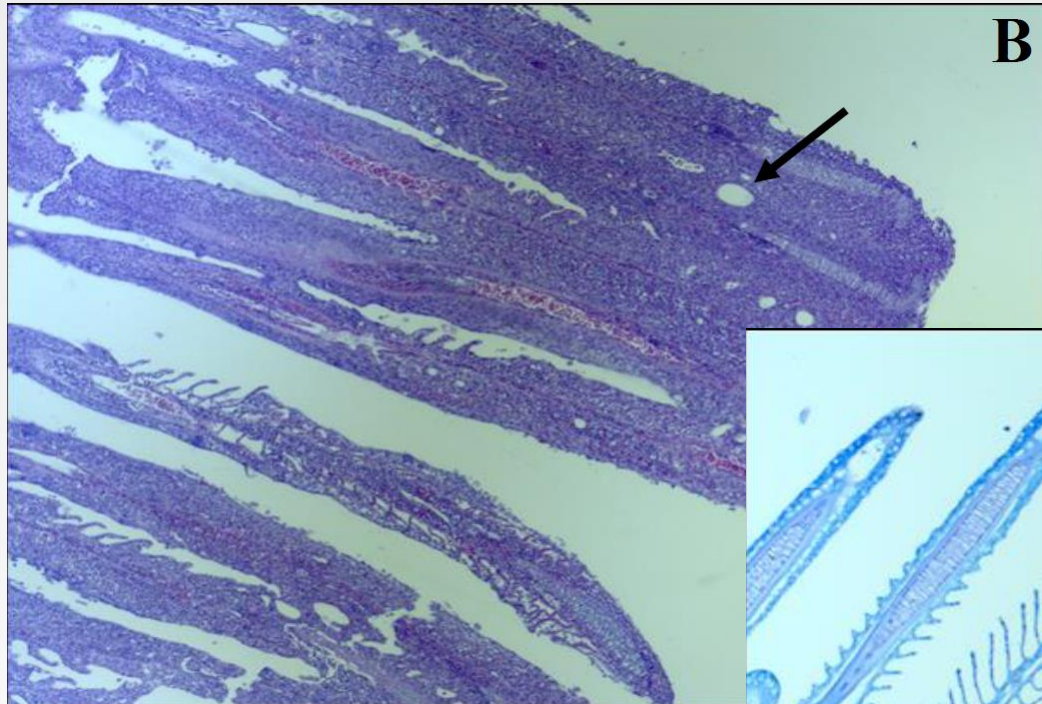


# Gjellescore



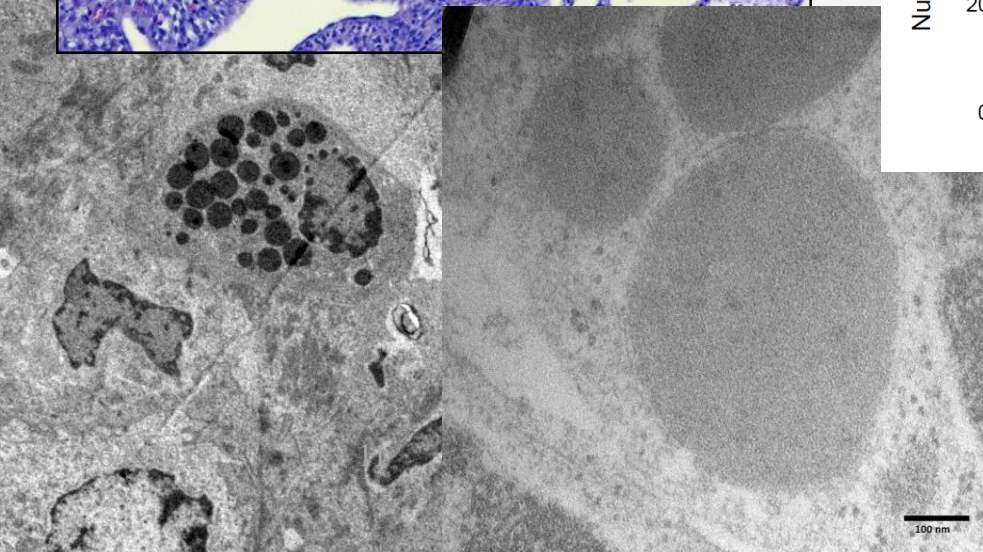
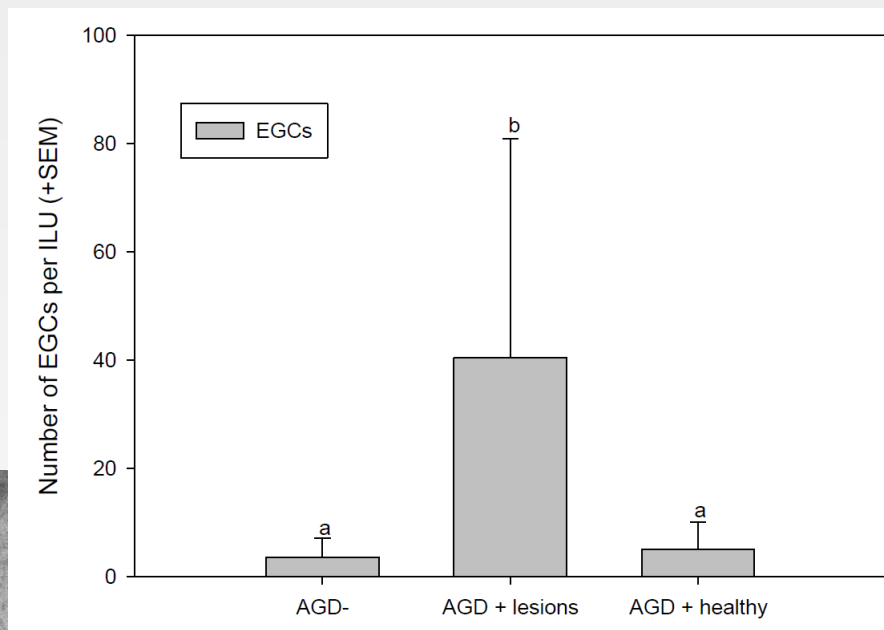
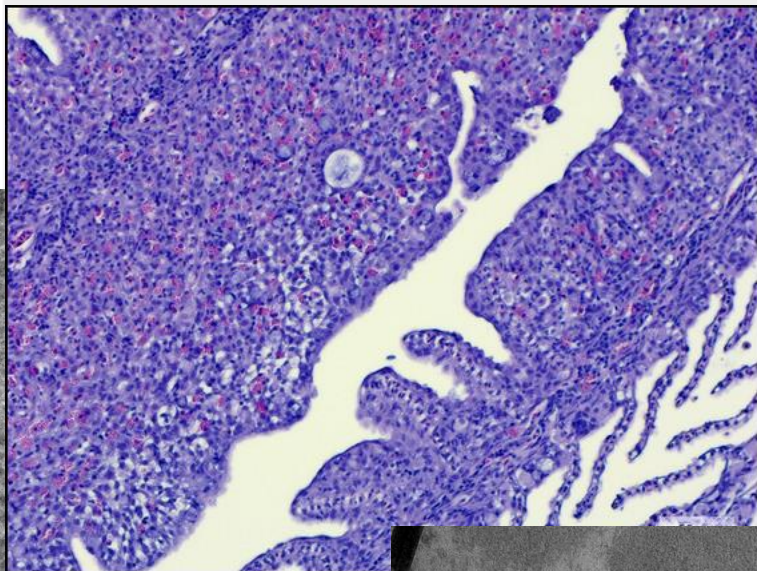


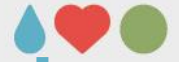
# AGD i berglyt



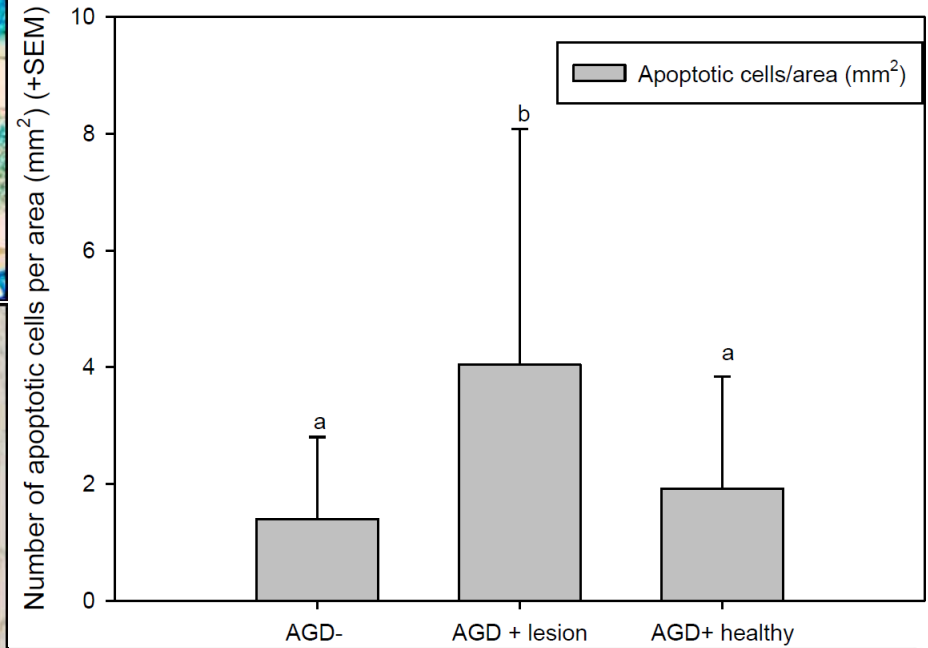
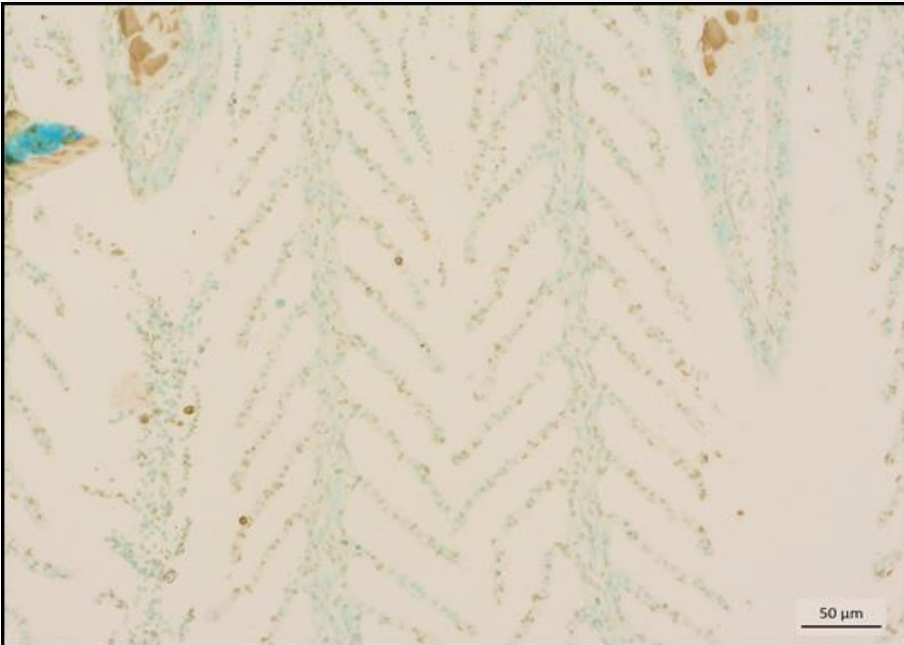
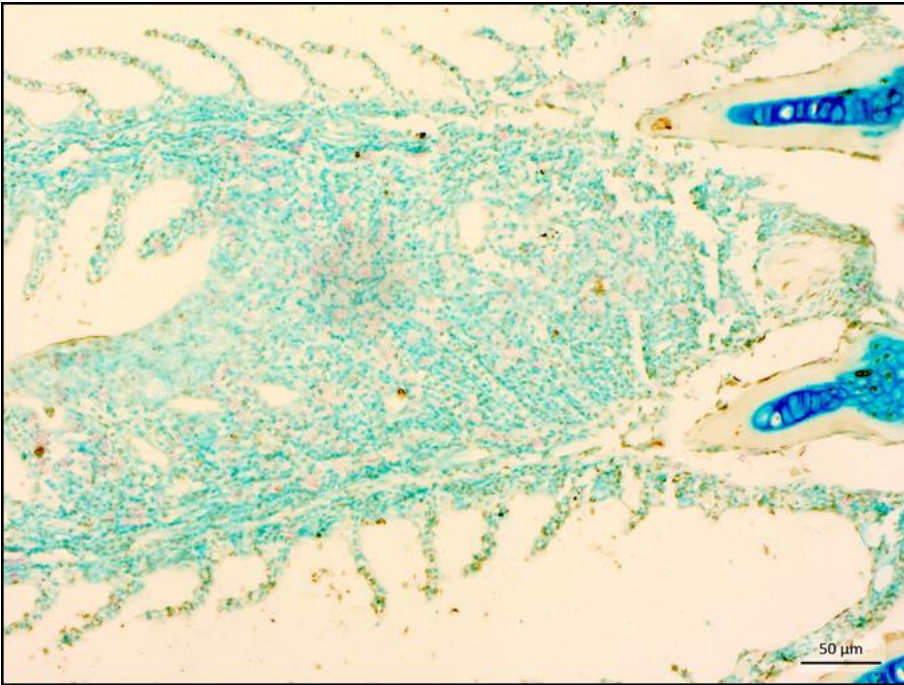


# EGCs i lesioner som bergyldt

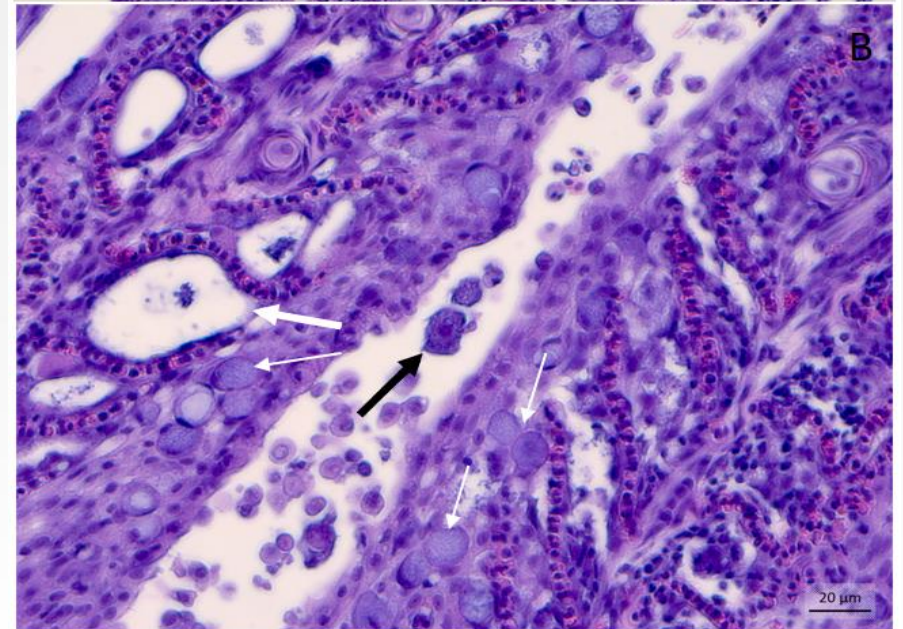
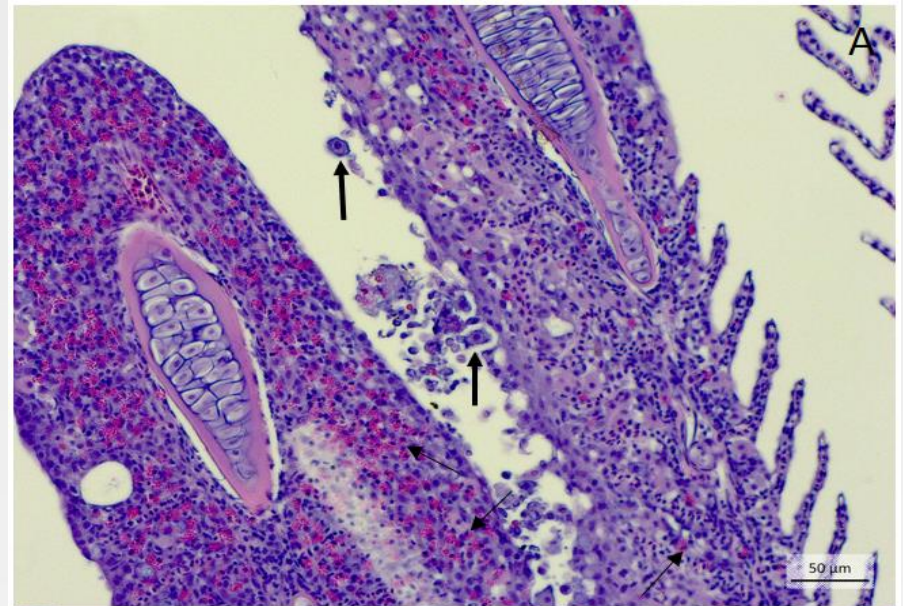
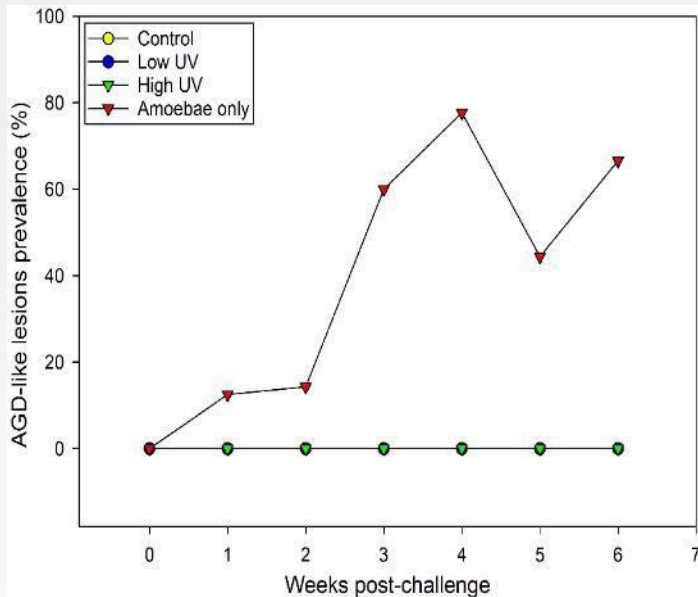




# Apoptosis i AGD lesioner



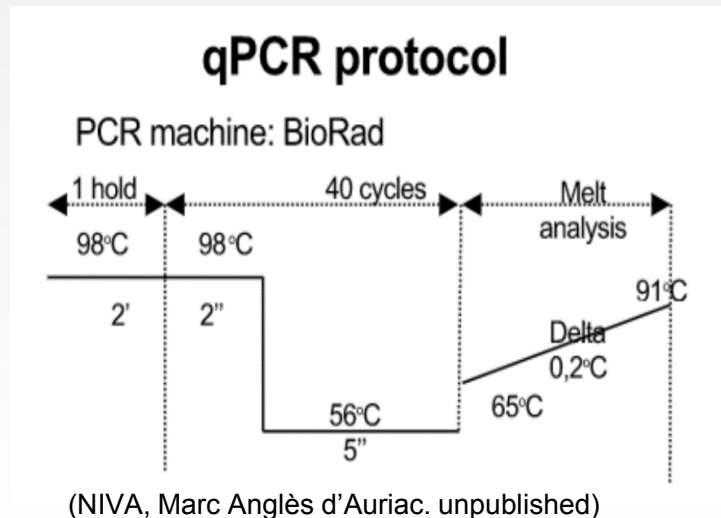
# Histologi: AGD- lignende lesjoner

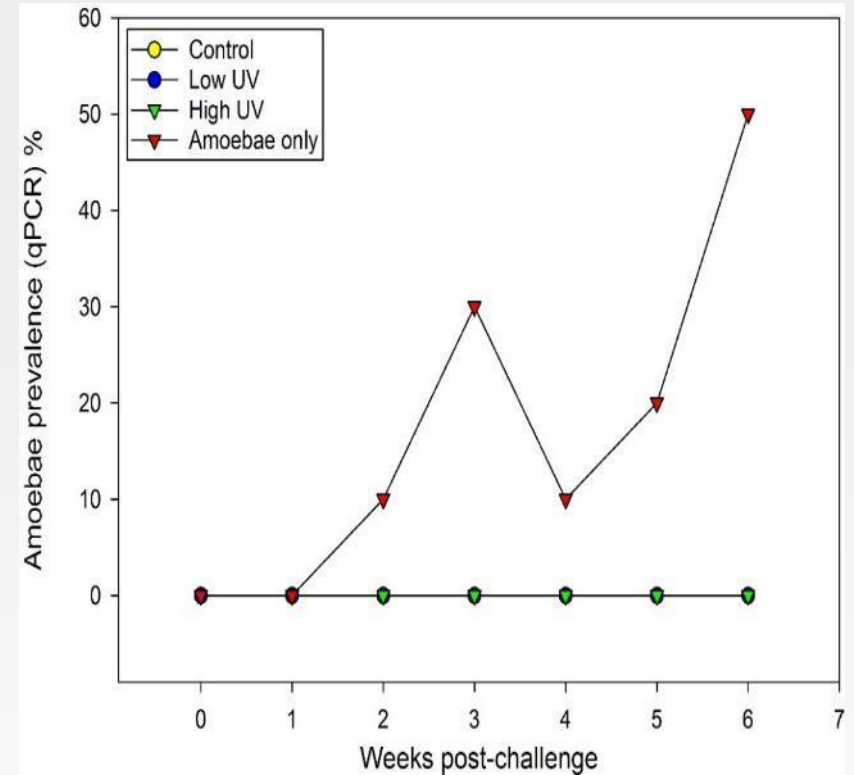
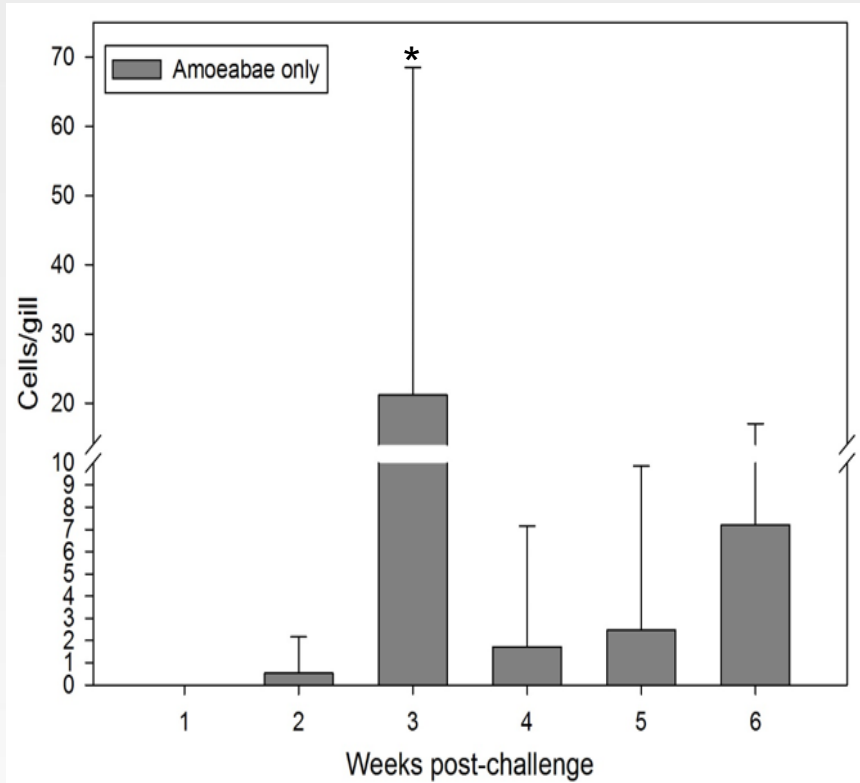




# DNA-basert qPCR

- Gjelleprøver sendt til NIVA, Oslo
- DNA basert qPCR for å kalkulere antall amøber per gjelleprøve
  - Kalkulering basert på kjent referensekultur
- Gene target *Neoparamoeba perurans*/18SrDNA
- Oligonucleotide primers (Bridle et al., 2010)







# Oppsummering og diskusjon

- UV-radiering med enten lav eller høy dose ser ut til å inhibere amøben til å forårsake sykdom hos berggyllt
  - Positiv kontroll med signifikant økning av gjellescore
  - AGD-lignende lesjoner og positive qPCR prøver eksklusivt i positiv kontroll
- Minimumsdosen for UV-bestråling av inntaksvann i landbaserte anlegg er  $25 \text{ mJ/cm}^2$
- Kan UV-bestrålte amøber begynne å vokse igjen og infisere fisk?
- Kan pseudocyster være mer resistent mot UV?





# NIVA reports



REPORT SNO 6909-2015

Disinfection of *Paramoebae perurans*  
with UV and ozone  
In situ dose-response testing



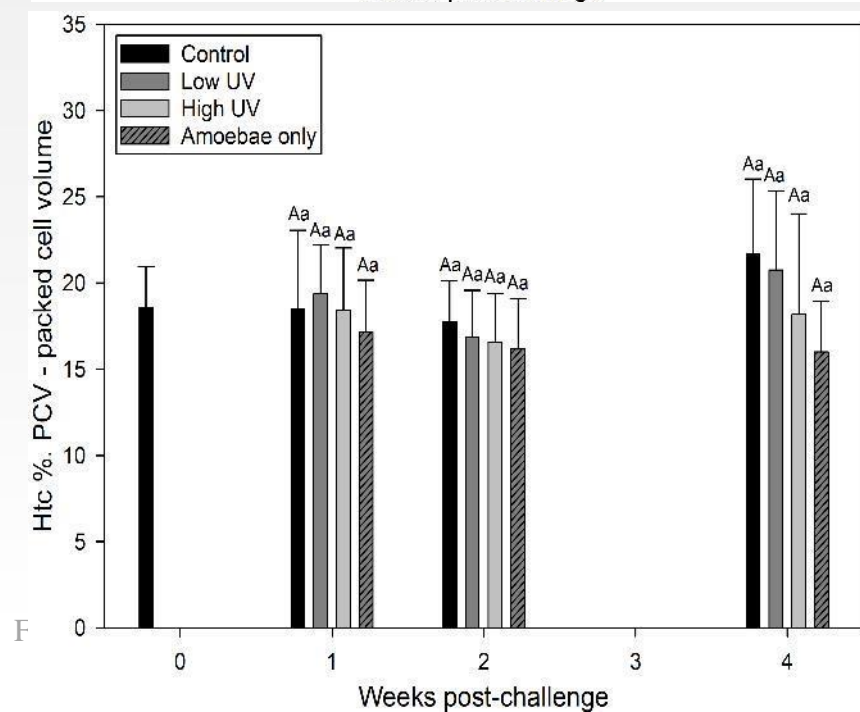
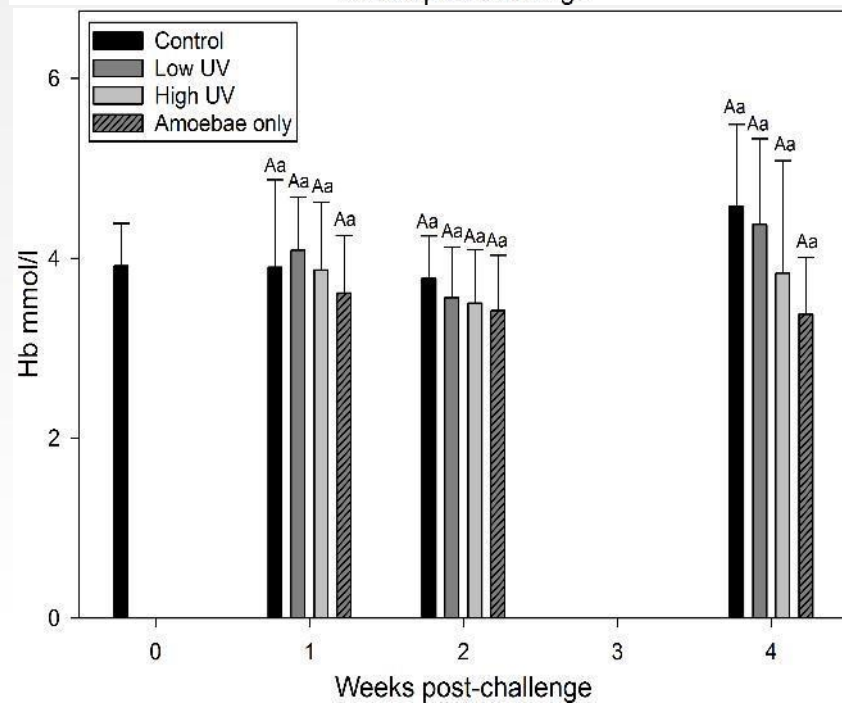
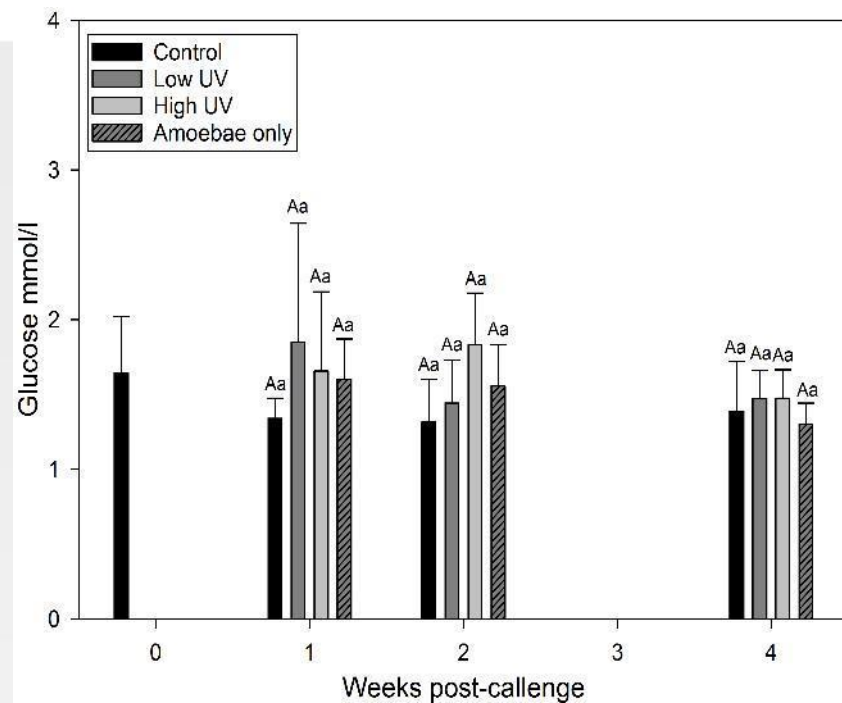
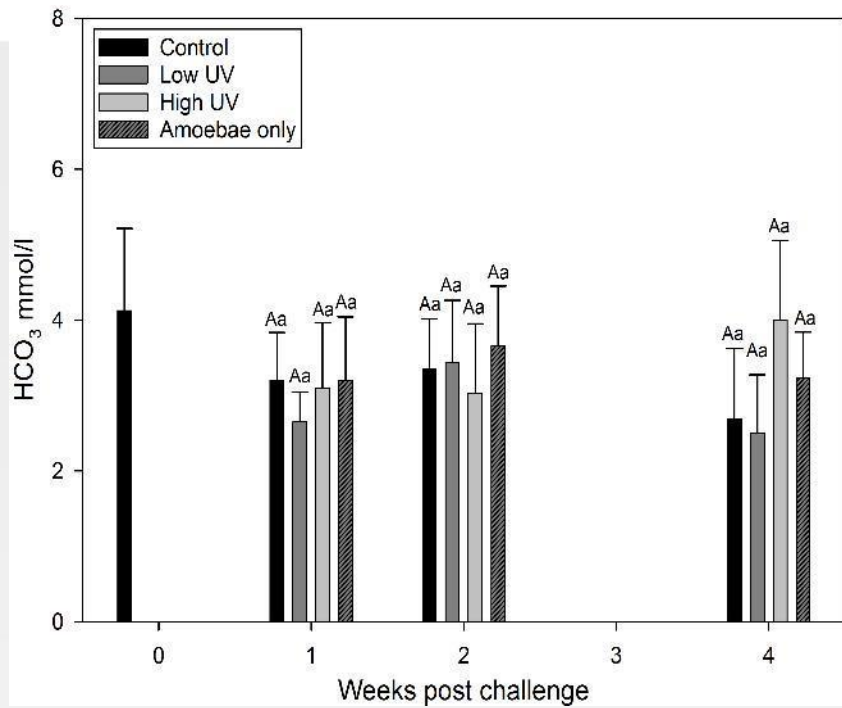
REPORT L. 7075-2016

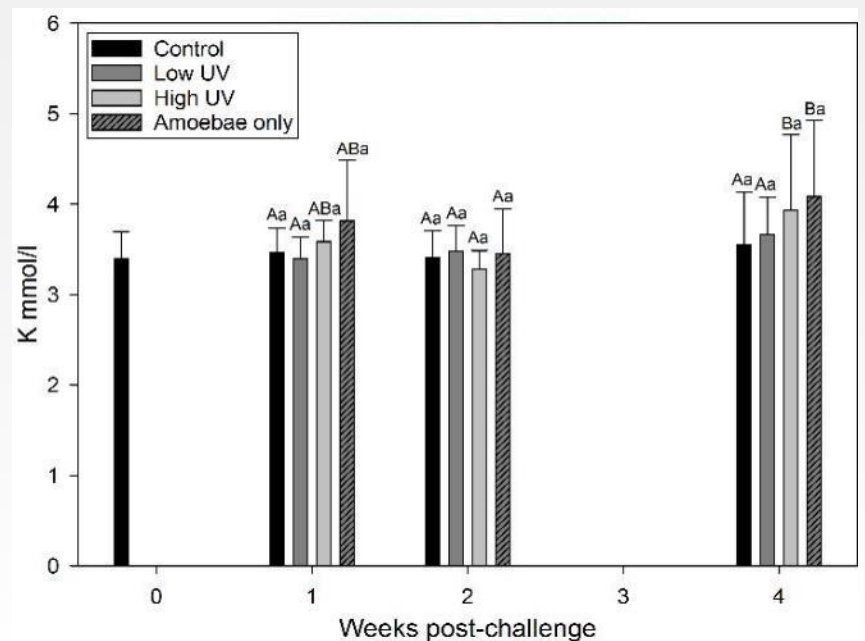
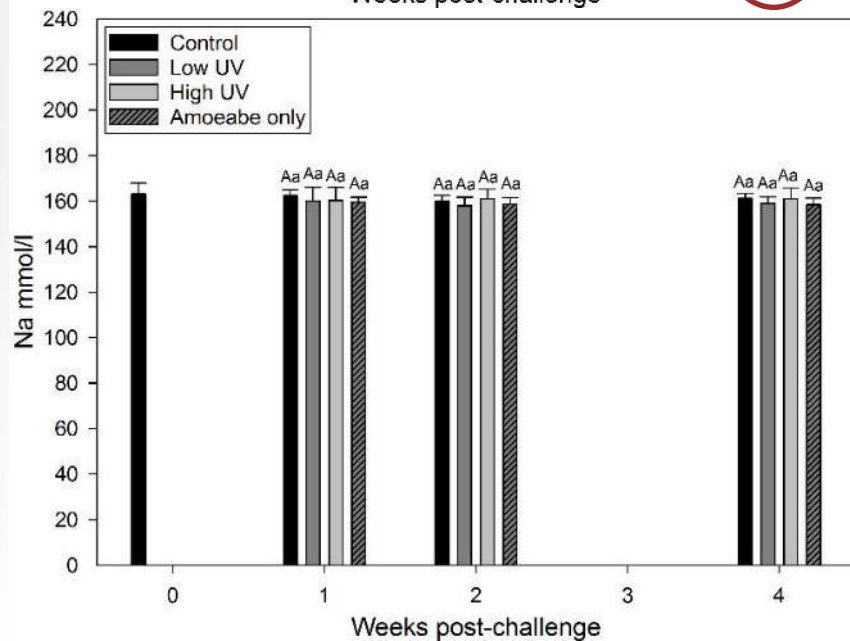
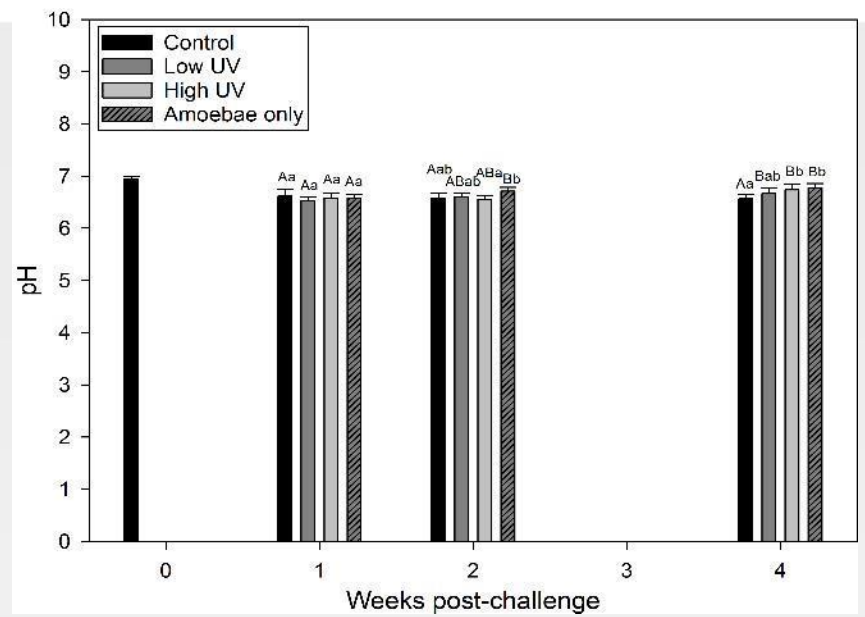
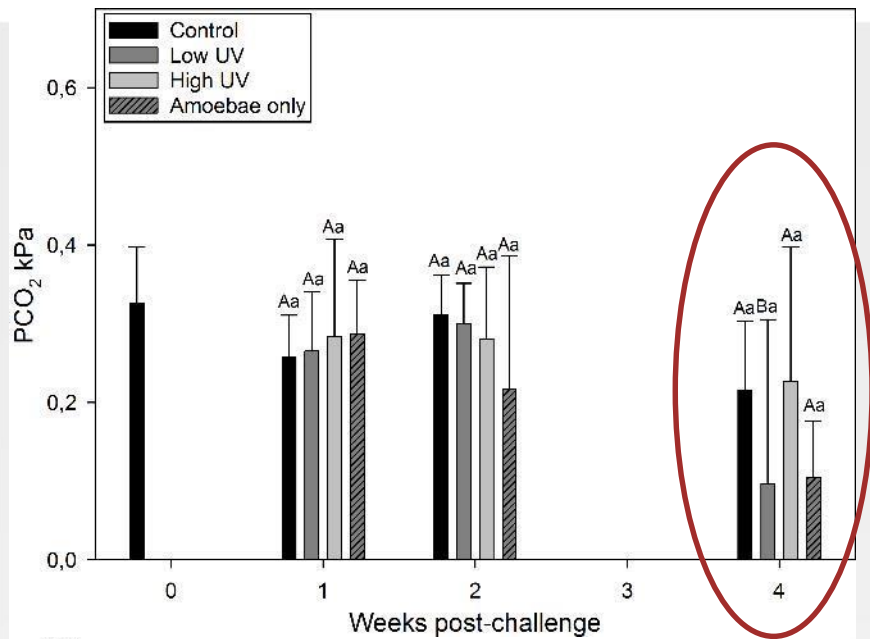
AGD control and disinfection  
in cleanerfish  
Part 2: Challenge trial

# Forbyggende tiltak i landbaserte anlegg



- **UV-bestråling**
- Denaturerer DNA og RNA til mikroorganismer
- Effekt påvirkes av turbiditet, UV-gjennomtrenging (Uvt), partikler, og gjennomstrømning
- Minimumsdose for inntaksvann i Norge – 25 mJ/cm<sup>2</sup>
  - Innaktivering av mikroorganismer skjer fra 2 mJ/cm<sup>2</sup> - >230 mJ/cm<sup>2</sup>
- Mest resistente: Virus og bakteriesporer







Cell contents: ❖ Correlation Coefficient ❖ P Value ❖ Number of samples	Hemorrhagic lesions	Clubbing lesions	Hyperplastic lesions	Gross gill score	PCR values
<b>AGD-like lesions</b>	0.0855 0.233 196	0.0196 0.785 196	<b>0.215</b> <b>0.00254</b> 195	<b>0.551</b> <b>&lt;0.001</b> 196	<b>0.703</b> <b>&lt;0.001</b> 196
<b>Hemorrhagic lesions</b>		0.125 0.0813 196	<b>0.148</b> <b>0.0395</b> 195	<b>0.172</b> <b>0.0157</b> 196	-0.0529 0.462 196
<b>Clubbing-lesions</b>			<b>0.142</b> <b>0.0479</b> 195	0.123 0.0871 196	-0.0912 0.203 196
<b>Hyperplastic lesions</b>				<b>0.437</b> <b>&lt;0.001</b> 195	0.0361 0.616 195
<b>Gross gill score</b>					<b>0.154</b> <b>0.0168</b> 240

Cell contents: ❖ Correlation Coefficient ❖ P Value ❖ Number of samples	Haemorrhagic lesions	Clubbing-lesions	Hyperplastic lesions	AGD-like lesions	Gross gill score
<b>Na/K mmol/l</b>	0.123 0.277 80	-0.183 0.103 80	<b>-0.246</b> <b>0.0292</b> <b>79</b>	<b>-0.221</b> <b>0.0491</b> <b>79</b>	<b>-0.206</b> <b>0.0399</b> <b>100</b>
<b>HB mmol/l</b>	0.133 287 66	0.182 0.144 66	<b>0.276</b> <b>0.0262</b> <b>65</b>	-0.0416 0.740 66	-0.155 0.168 81
<b>HCO<sub>3</sub> mmol/l</b>	-0.205 0.122 58	0.183 0.168 58	-0.207 0.122 57	-0.0815 0.543 58	0.0947 0.429 72
<b>PCO<sub>2</sub> kPa</b>	-0.0949 0.429 58	-0.0122 0.927 58	<b>-0.454</b> <b>&lt;0.001</b> <b>57</b>	<b>-0.397</b> <b>0.00204</b> <b>58</b>	-0.179 0.131 72
<b>pH</b>	-0.0923 0.491 58	0.227 0.0869 58	<b>0.305</b> <b>0.0202</b> <b>57</b>	<b>0.380</b> <b>0.00322</b> <b>58</b>	<b>0.321</b> <b>&lt;0.001</b> <b>72</b>
<b>Htc %</b>	0.135 0.279 66	0.182 0.144 66	<b>0.279</b> <b>0.0245</b> <b>65</b>	-0.00261 0.983 67	-0.153 0.172 81
<b>Glucose mmol/l</b>	0.0266 0.831 67	0.146 0.238 67	-0.322 0.798 66	-0.00261 0.983 67	-0.0776 0.491 81
<b>K mmol/l</b>	-0.169 0.134 80	0.109 0.336 80	<b>0.244</b> <b>0.0303</b> <b>79</b>	0.270 0.0535 80	<b>0.208</b> <b>0.0376</b> <b>100</b>
<b>Na mmol/l</b>	-0.177 0.116 80	-0.164 0.145 80	-0.179 0.115 79	-0.0635 0.576 80	-0.0947 0.346 101

6/8/2017

# Oppsummering og diskusjon



- Gjellescore
  - Kun signifikant økning i positiv kontroll
  - Patologi varierer mellom arter
- Histologi
  - Noe bakgrunnspatologi i alle grupper
  - AGD-lignende lesjoner observert ligner det som er beskrevet hos andre arter med sykdommen
  - Amøben ikke til stede ved alle AGD-lignende lesjoner
- qPCR
  - Kun i positiv kontroll en kunne se positive gjelleprøver for *Neoparamoeba perurans*
  - Under utvikling, noe indikasjon på lav sensitivitet
- Bloddata
  - Reduksjon av  $PCO_2$  over tid og en økning i pH
  - Korrelerte med økning av hyperplasilesjoner og AGD-lignende lesjoner
  - Blod fra halevene

# Konkluderende bemerkninger



- UV-dosen bør overstige  $2 \text{ mJ/cm}^2$  for å inhibere amøben til å forårsake sykdom hos berggytt



# Histologisk score

- Prosentandel filamenter med en eller flere gitte lesjoner
  - Clubbing
  - Blødninger, trombose og telangiectasis
  - Hyperplasi
  - AGD-lignende lesjoner
- 75 % velorienterte filamenter for evaluering

