



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



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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)

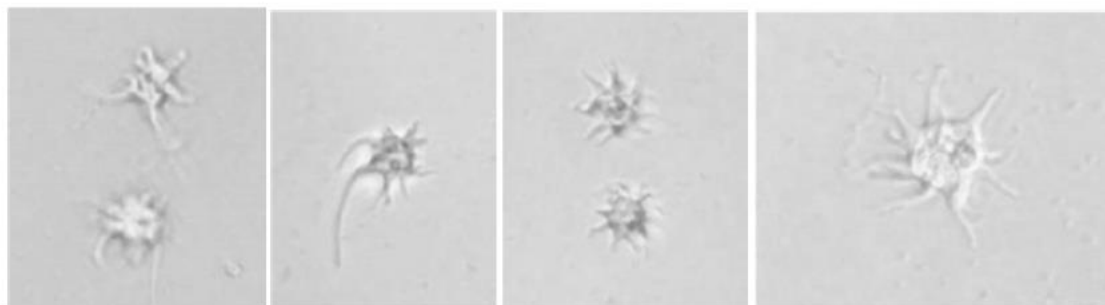


Figure 1. Floating stage of non-exposed amoebae (Photo: A. C. Wennberg)

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)

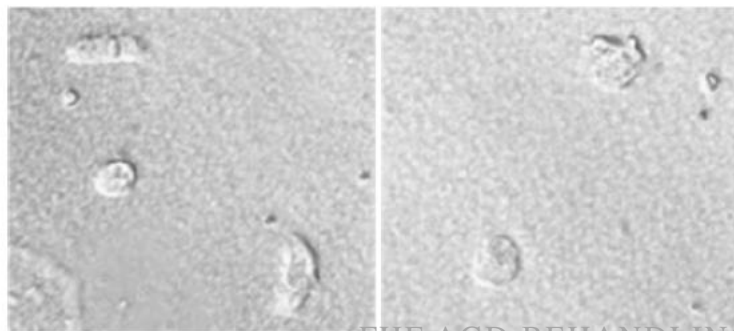


Figure 3. Stressed or damaged amoebae that has balled up several days after UV exposure (Photo: A. C. Wennberg)



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 ²] | 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 ²] | 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 |



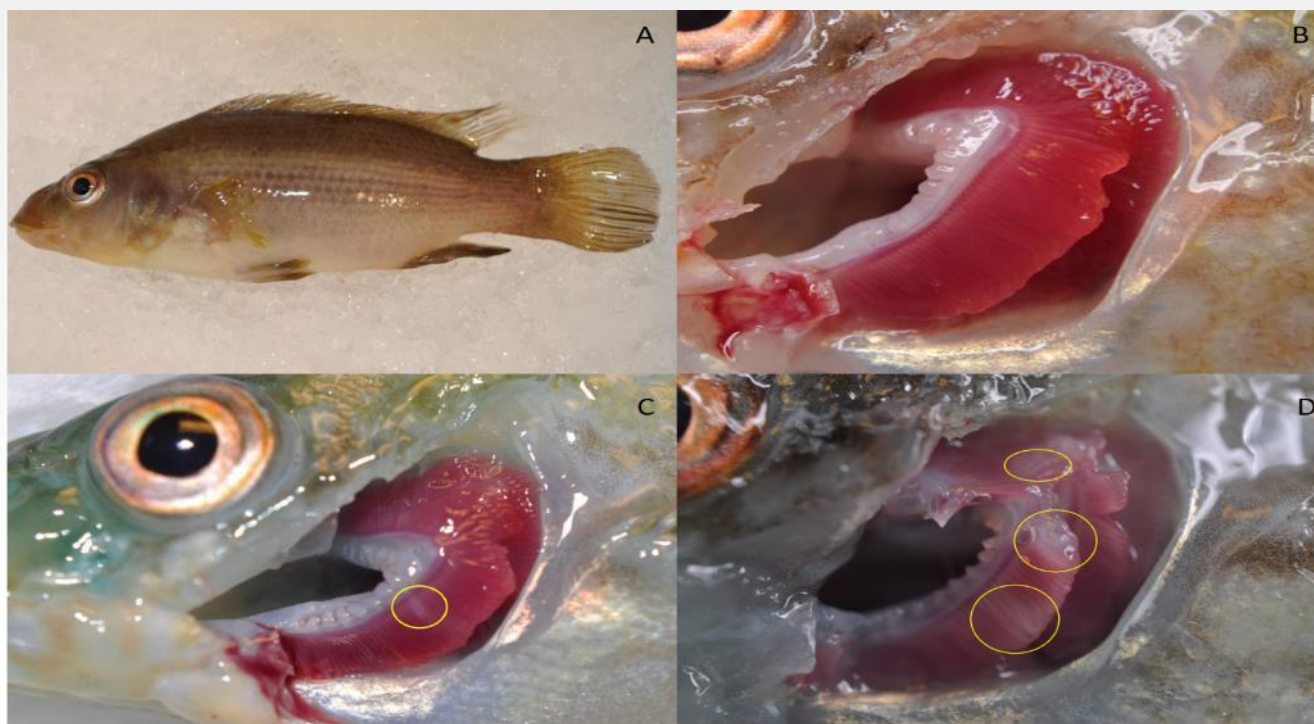


- 8 separate tanker (ca. 50 fisk per. tank)
- (700 L) med 400 L sjøvann – 13.6°C, 34,68 ‰, pH 7,98 - 8,02
- Medium pressure UV-lamper ble brukt til UV-bestråling av amøbekulturen
 - 10 sekunder for lav dose – 2 mJ/cm²
 - 90 sekunder for høy dose – 20 mJ/cm²

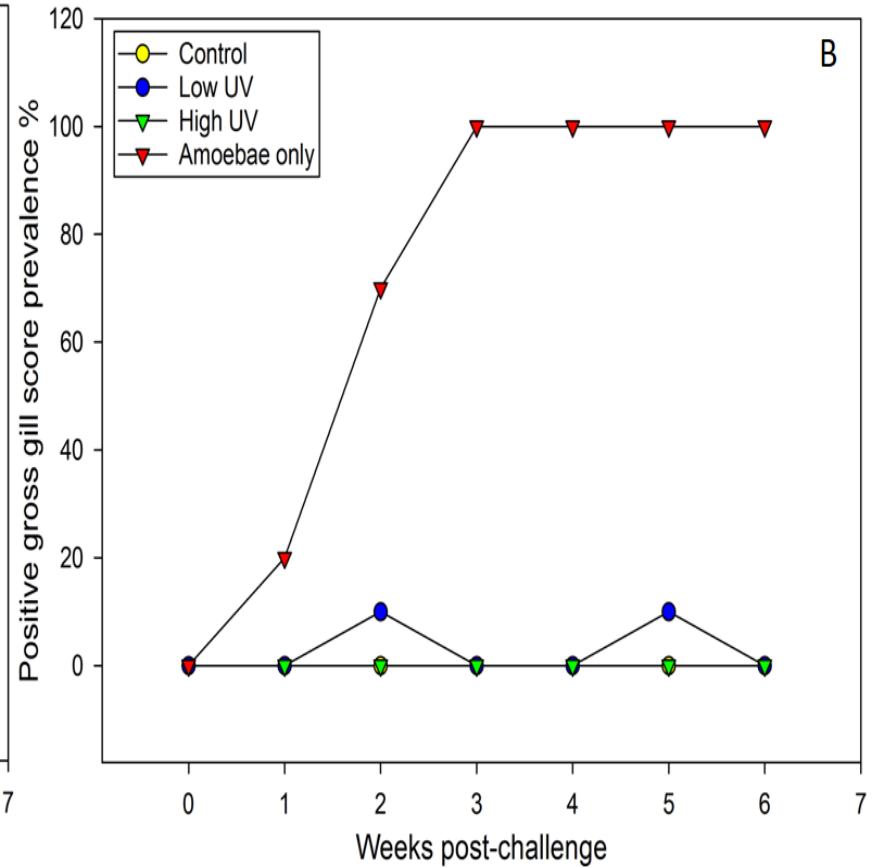
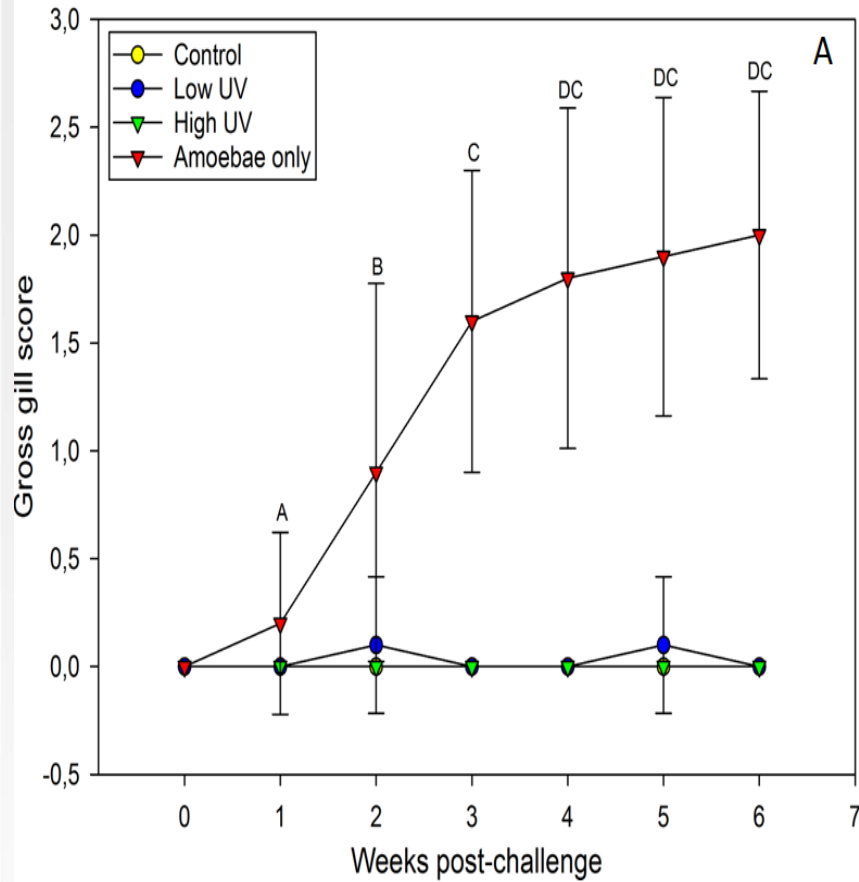
Karoppsett:

- ❖ Negative kontroller – 2 kar med 0 celler/L
- ❖ Høy dose UV-bestråling (20 mJ/cm²) – 2 kar med 1000 celler/L
- ❖ Lav dose UV-bestråling (2 mJ/cm²) – 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 |

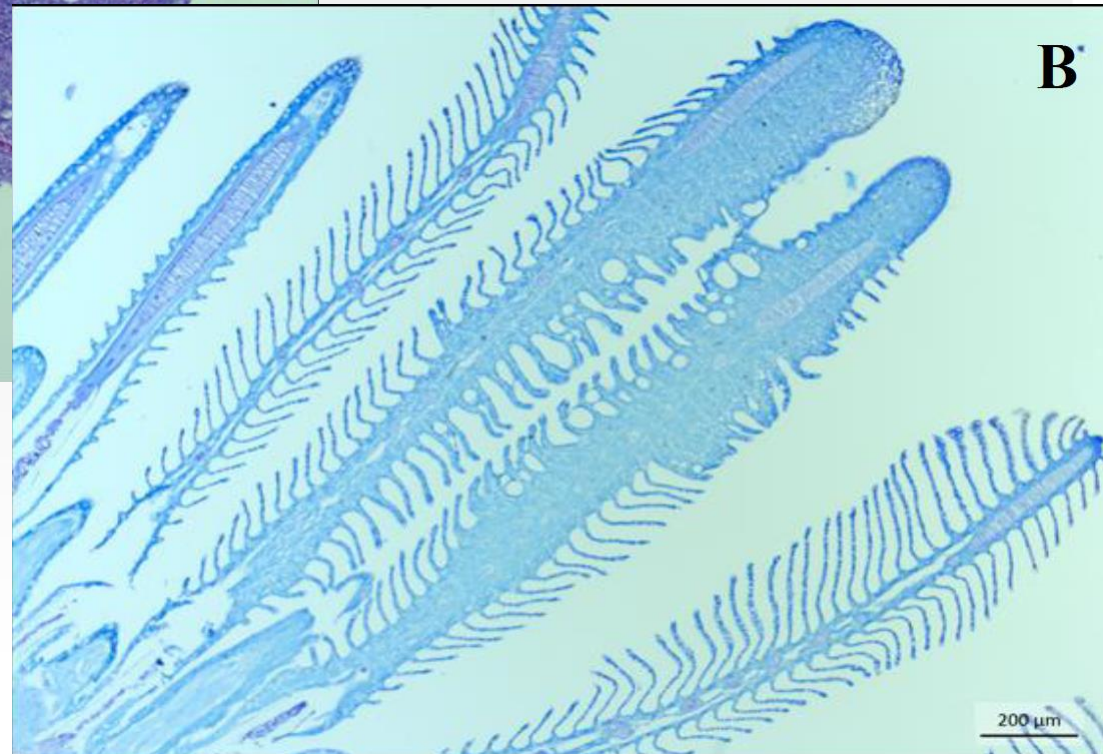
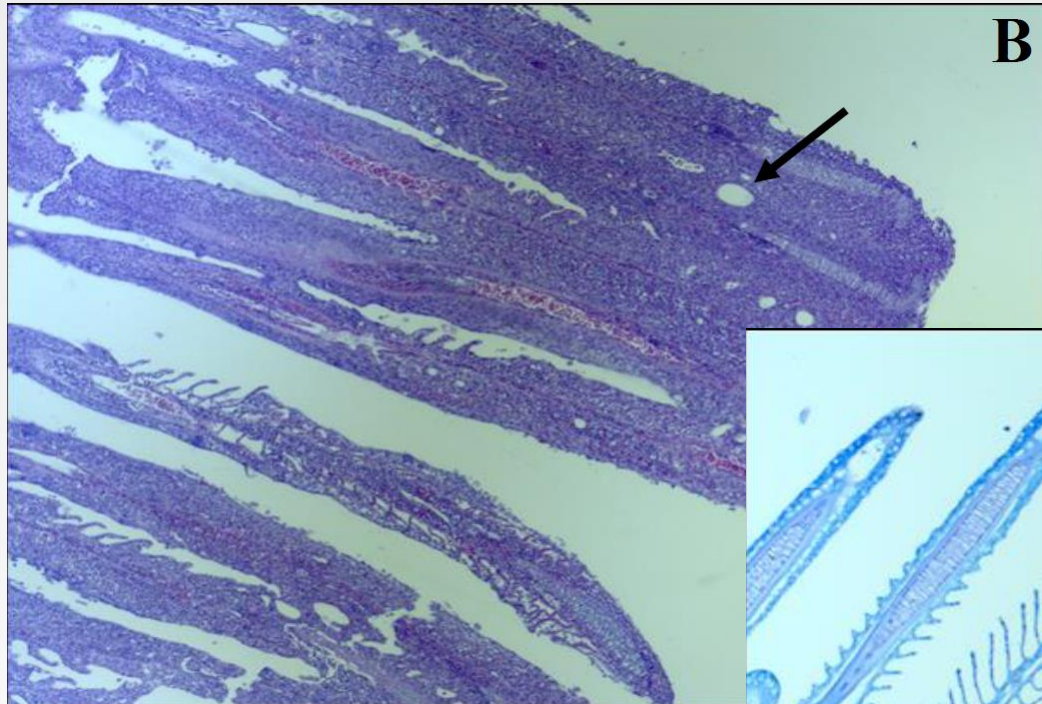


Gjellescore



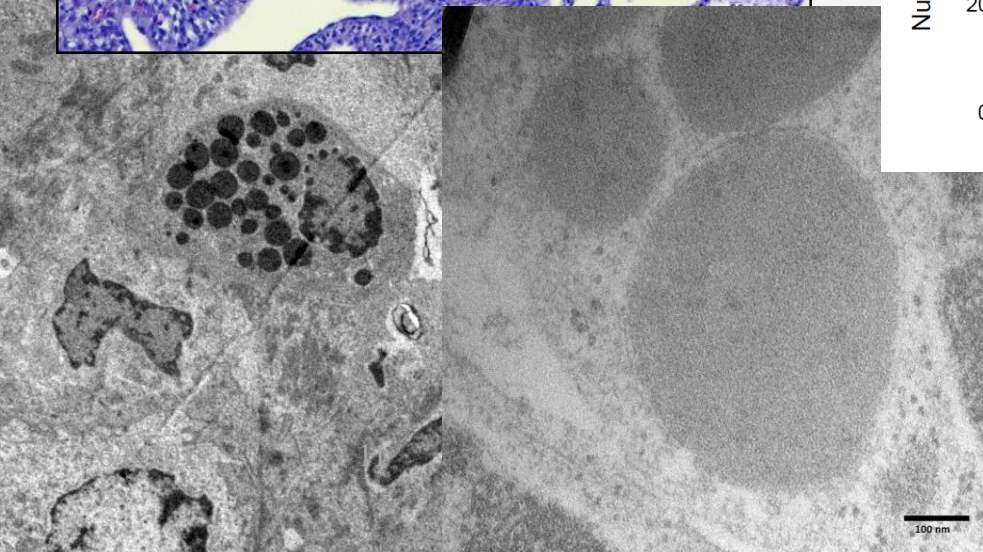
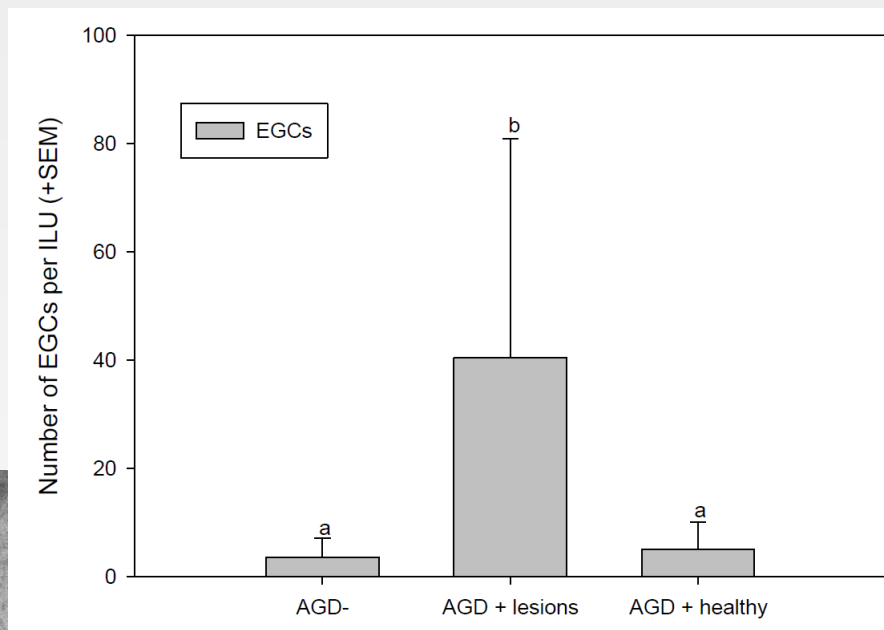
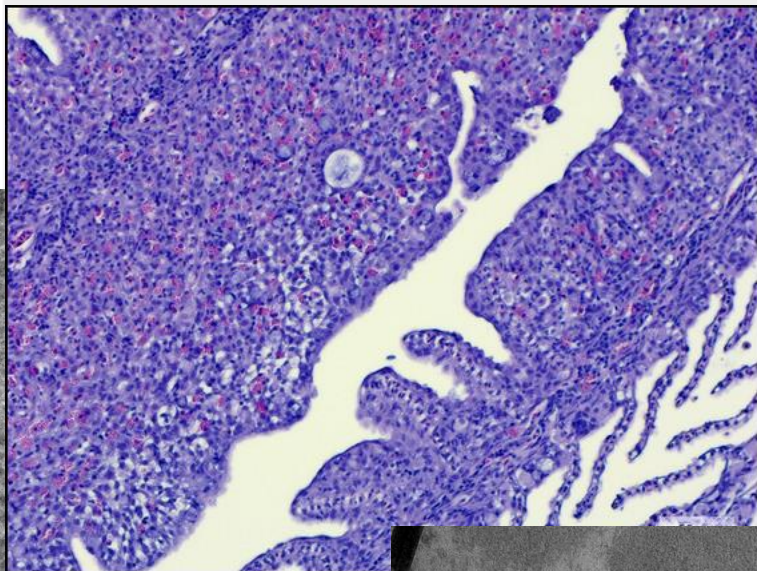


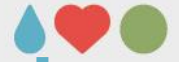
AGD i berglyt



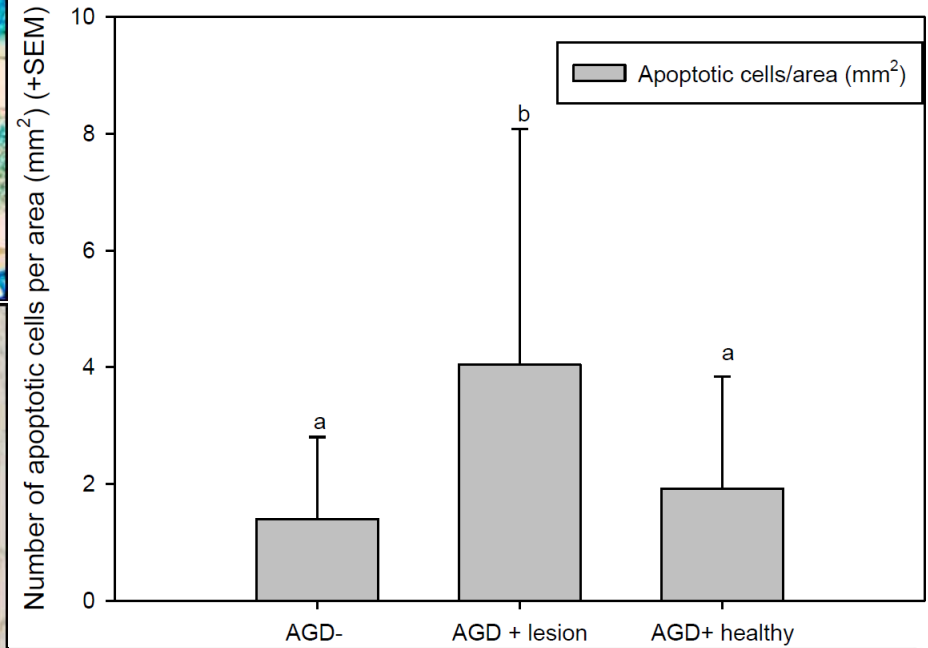
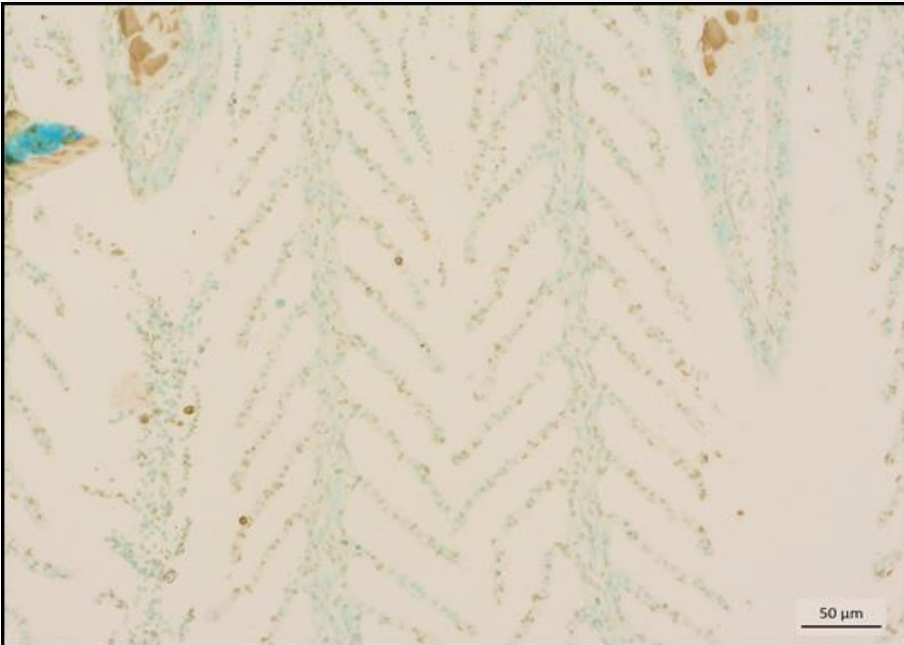
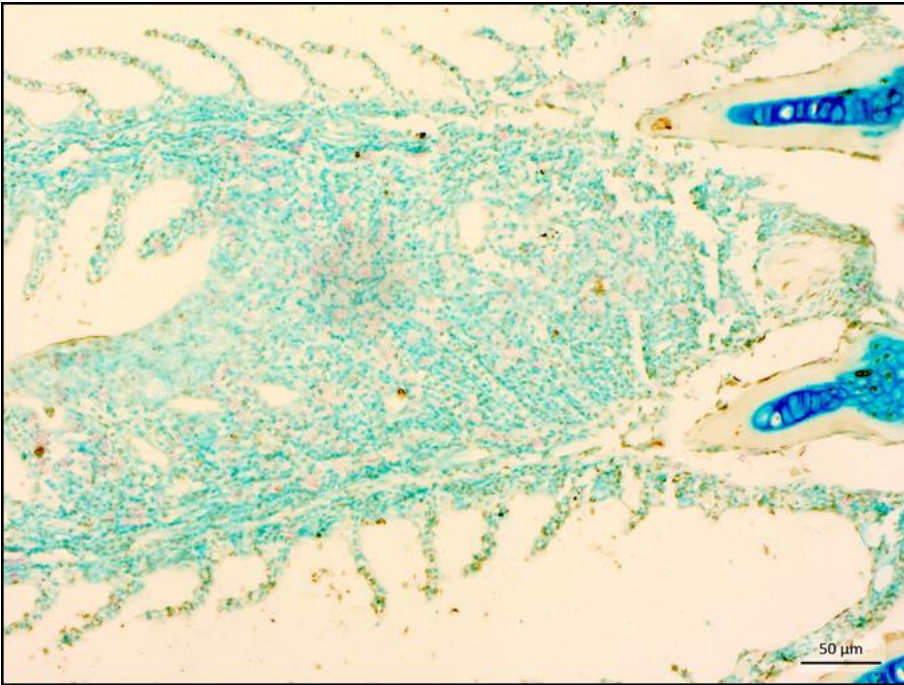


EGCs i lesioner som bergyldt

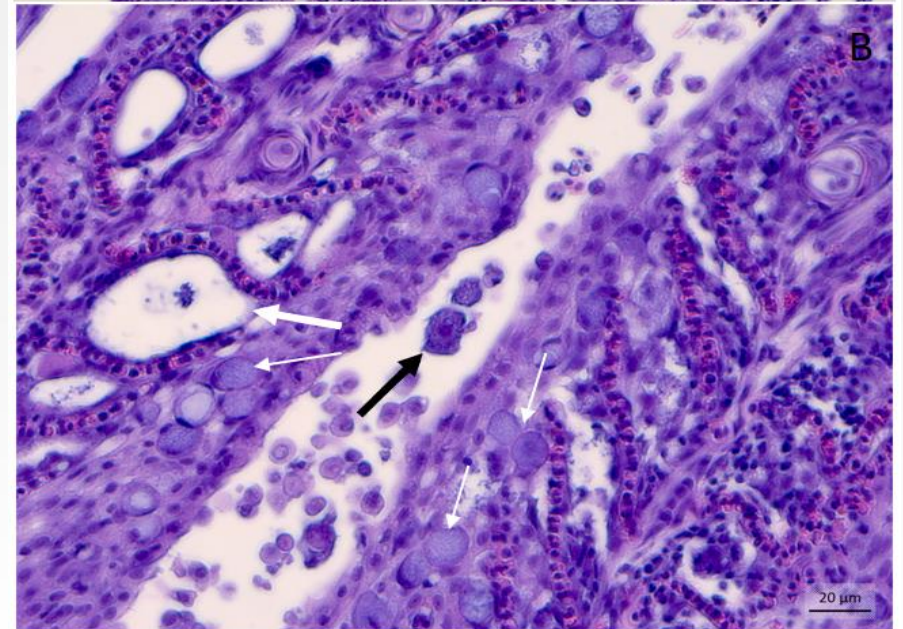
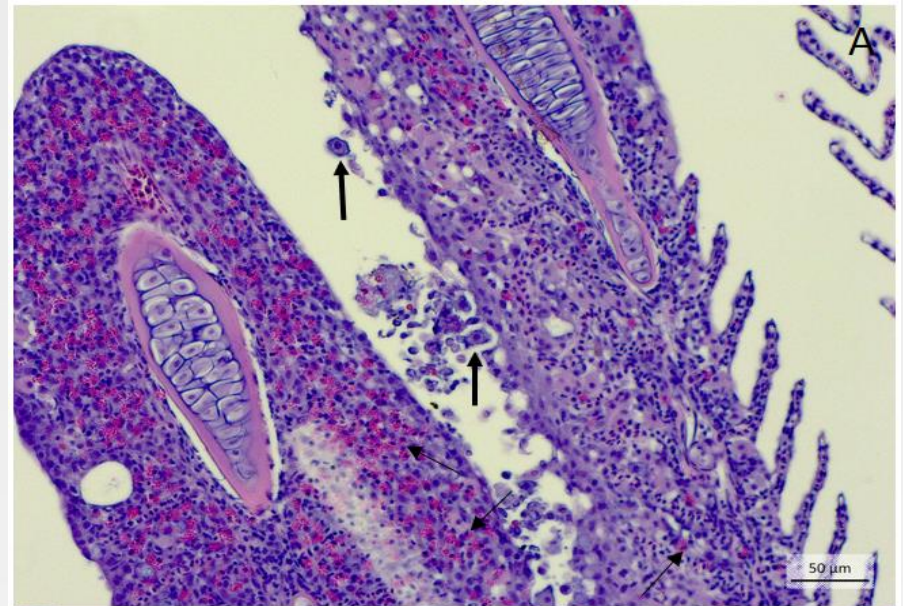
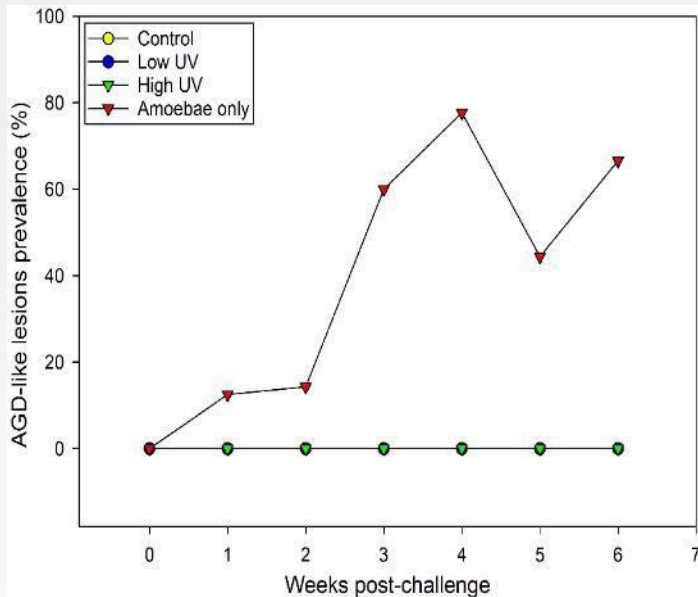




Apoptosis i AGD lesioner



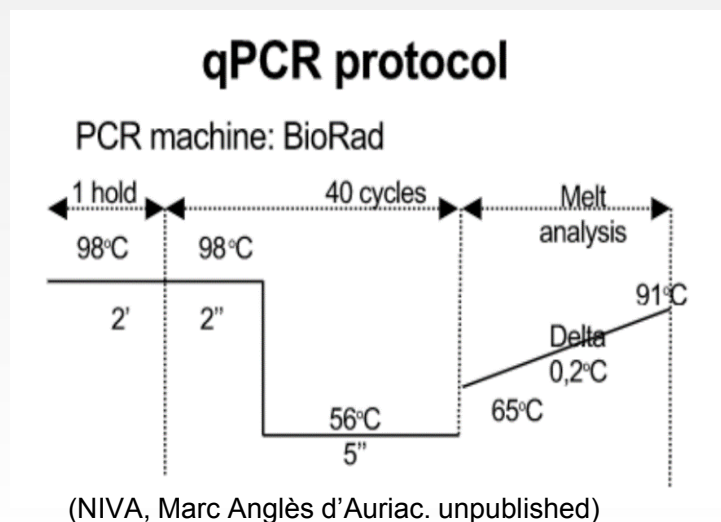
Histologi: AGD- lignende lesjoner

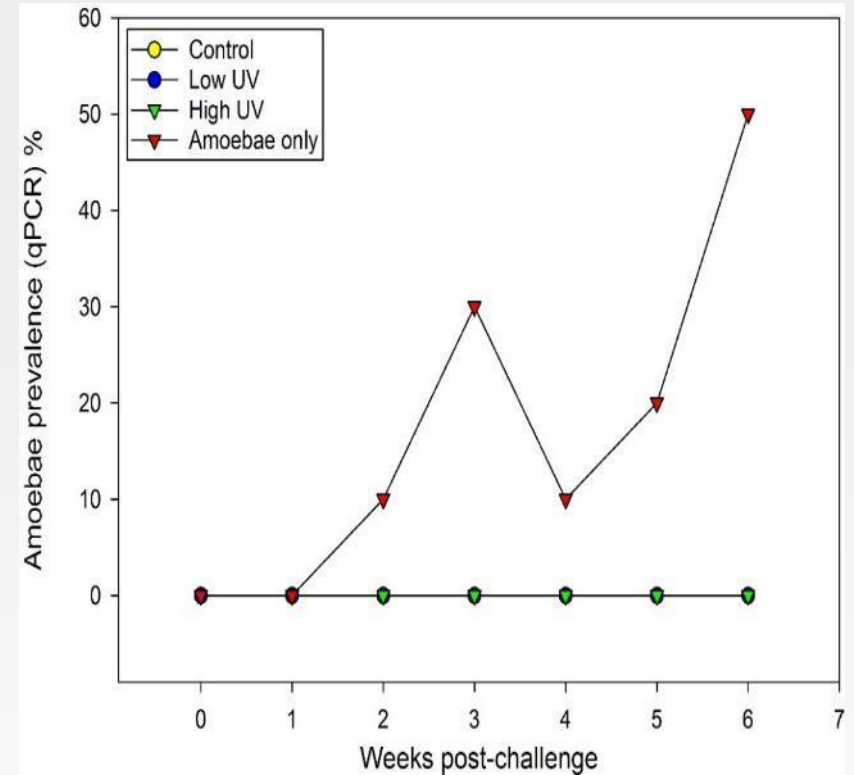
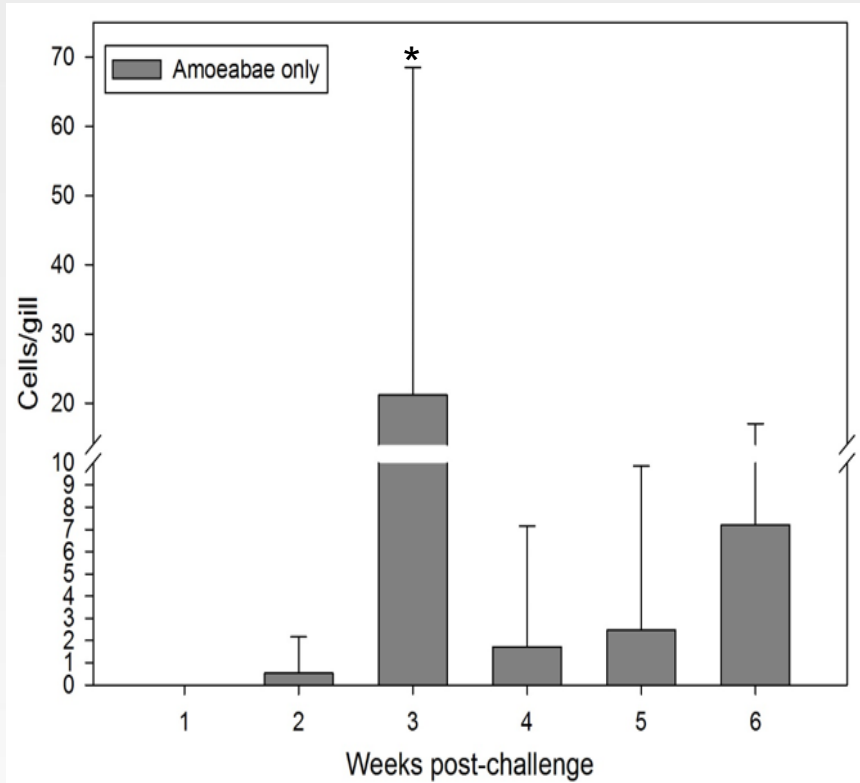




DNA-basert qPCR

- 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 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



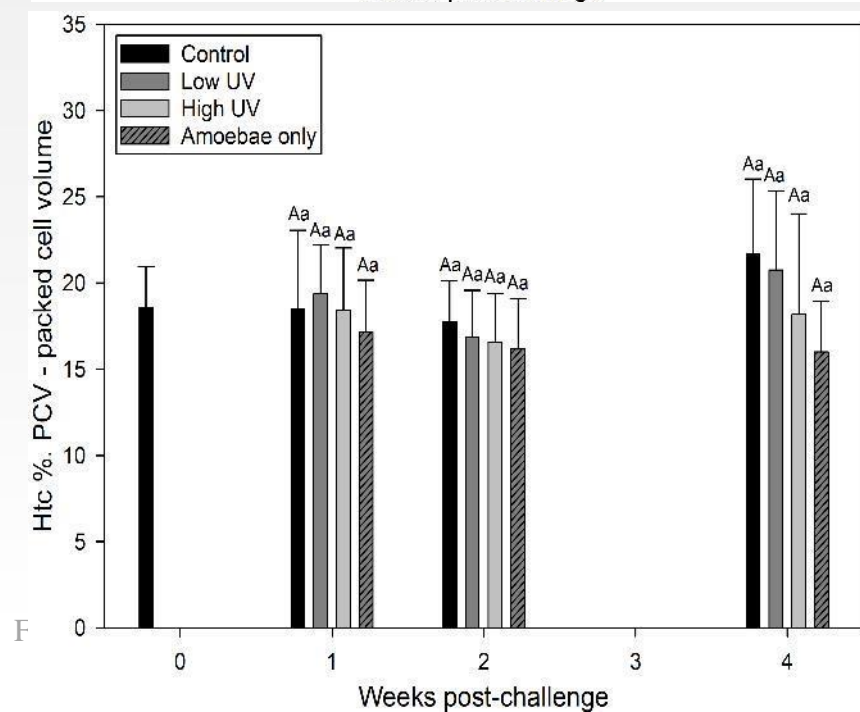
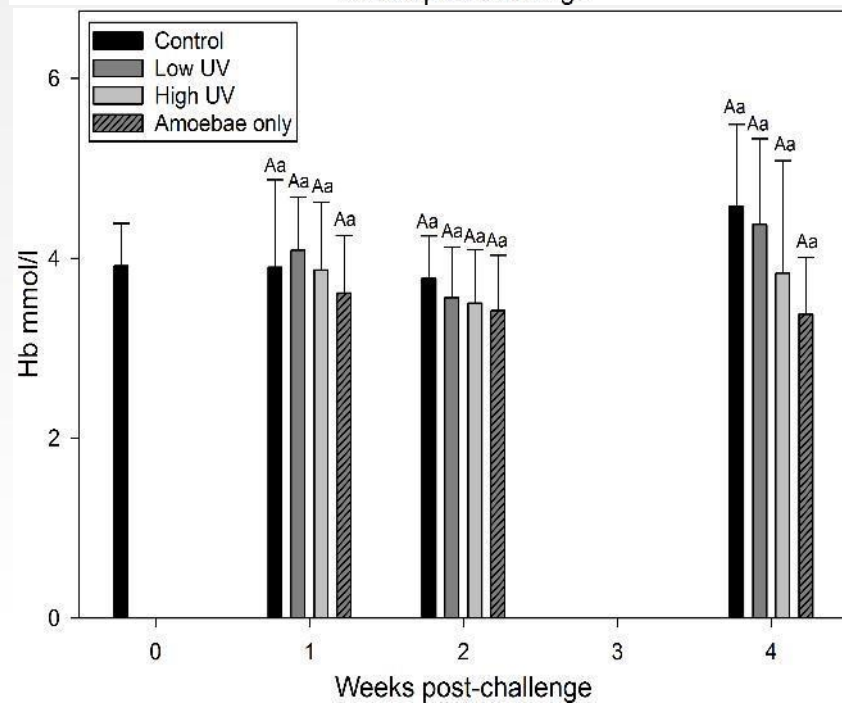
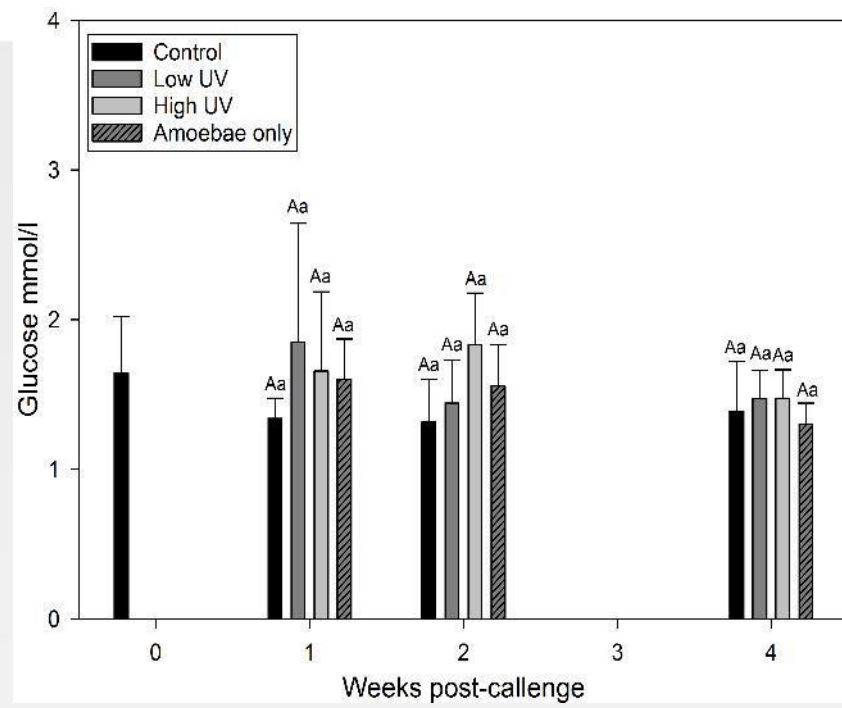
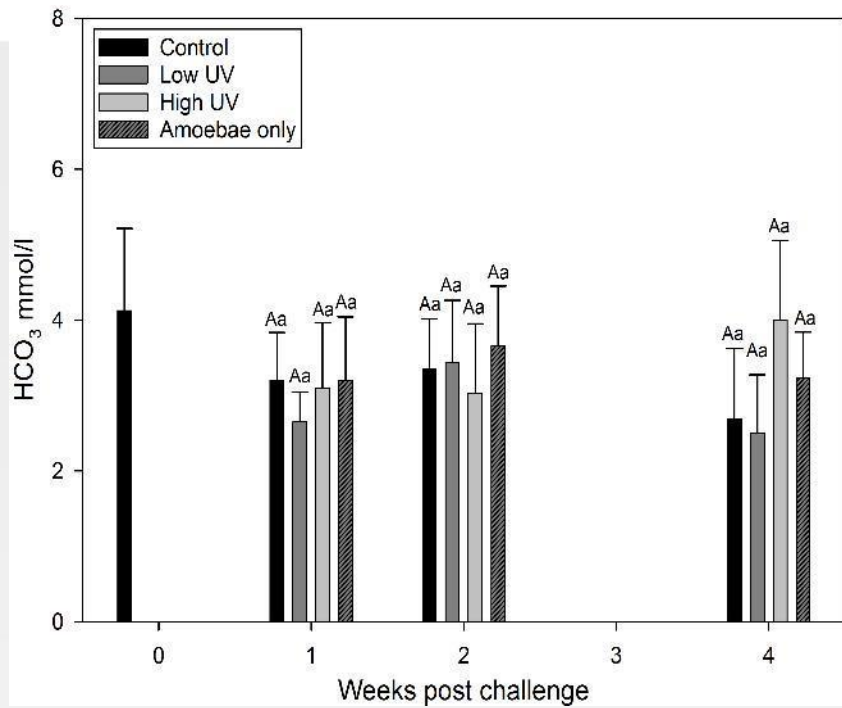
REPORT L. 7075-2016

AGD control and disinfection
in cleanerfish
Part 2: Challenge trial

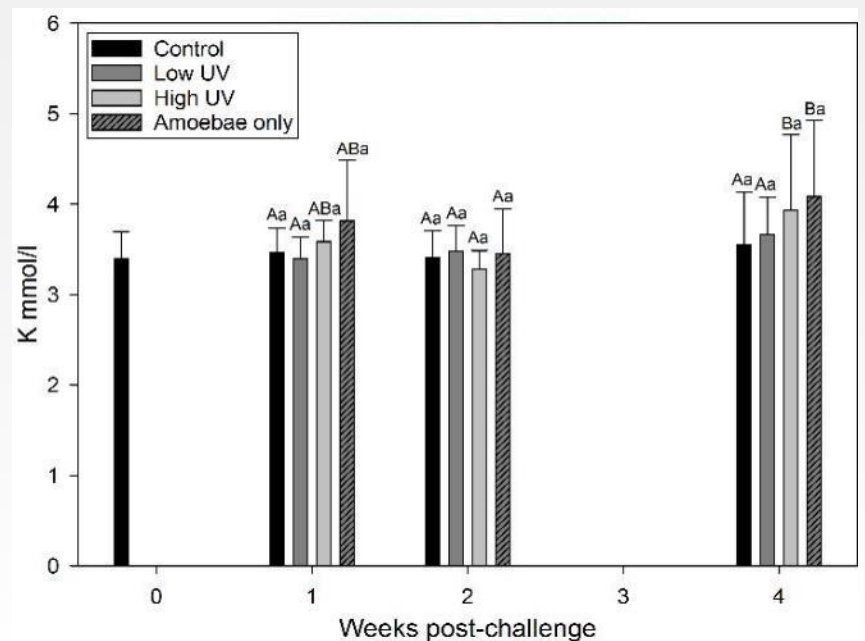
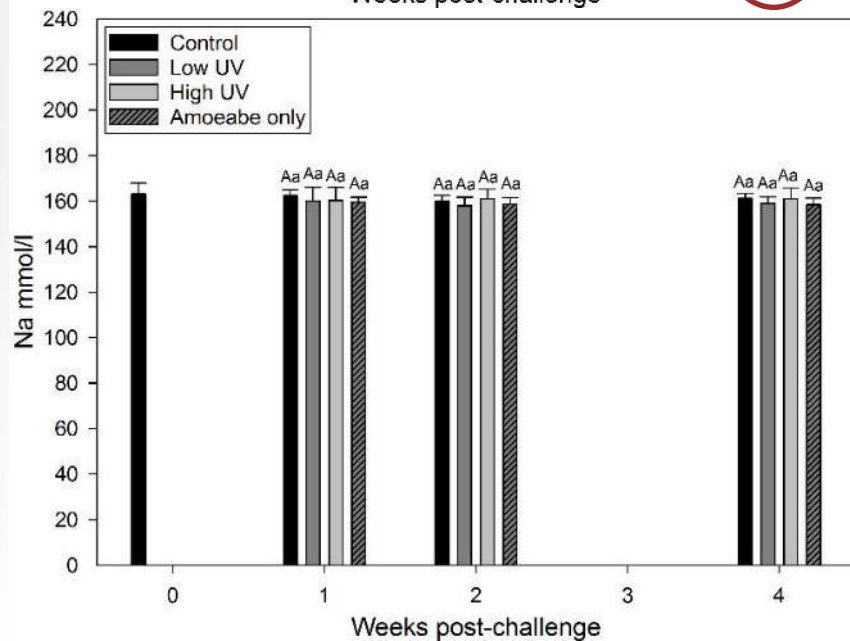
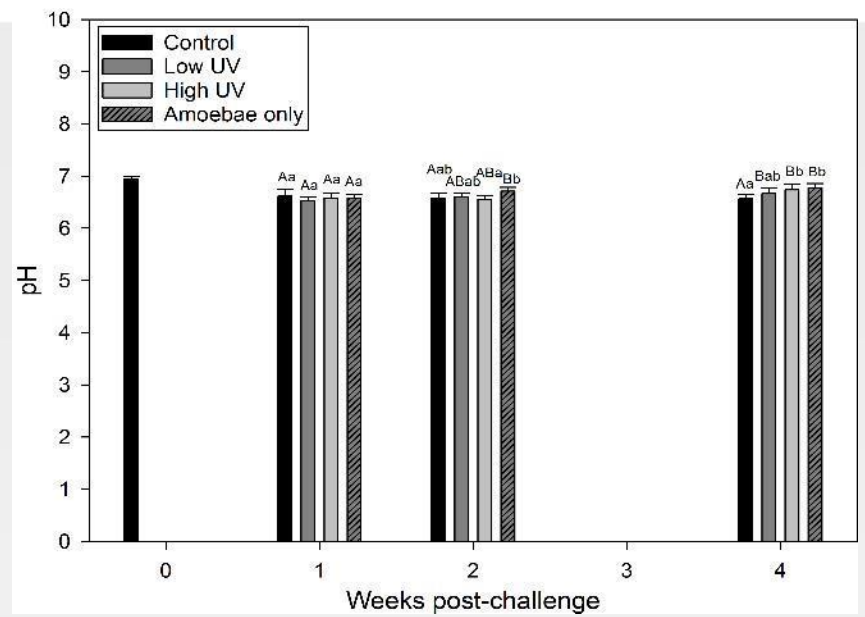
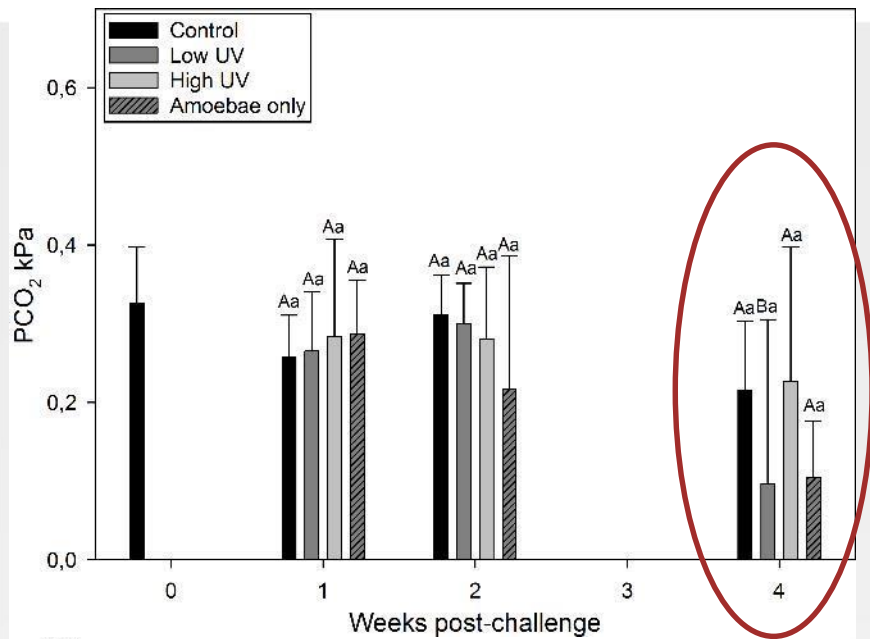
Forbyggende tiltak i landbaserte anlegg



- **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/cm²
 - Innaktivering av mikroorganismer skjer fra 2 mJ/cm² - >230 mJ/cm²
- Mest resistente: Virus og bakteriesporer



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

| Cell contents: ❖ Correlation Coefficient ❖ P Value ❖ Number of samples | Haemorrhagic lesions | Clubbing-lesions | Hyperplastic lesions | AGD-like lesions | Gross gill score |
|---|------------------------|------------------------|--|--|---|
| Na/K mmol/l | 0.123 0.277 80 | -0.183 0.103 80 | -0.246 0.0292 79 | -0.221 0.0491 79 | -0.206 0.0399 100 |
| HB mmol/l | 0.133 287 66 | 0.182 0.144 66 | 0.276 0.0262 65 | -0.0416 0.740 66 | -0.155 0.168 81 |
| HCO₃ mmol/l | -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 |
| PCO₂ kPa | -0.0949 0.429 58 | -0.0122 0.927 58 | -0.454 <0.001 57 | -0.397 0.00204 58 | -0.179 0.131 72 |
| pH | -0.0923 0.491 58 | 0.227 0.0869 58 | 0.305 0.0202 57 | 0.380 0.00322 58 | 0.321 <0.001 72 |
| Htc % | 0.135 0.279 66 | 0.182 0.144 66 | 0.279 0.0245 65 | -0.00261 0.983 67 | -0.153 0.172 81 |
| Glucose mmol/l | 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 |
| K mmol/l | -0.169 0.134 80 | 0.109 0.336 80 | 0.244 0.0303 79 | 0.270 0.0535 80 | 0.208 0.0376 100 |
| Na mmol/l | -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 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

