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

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REGIONALE FORSKNINGSFOND
marineharvest
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)

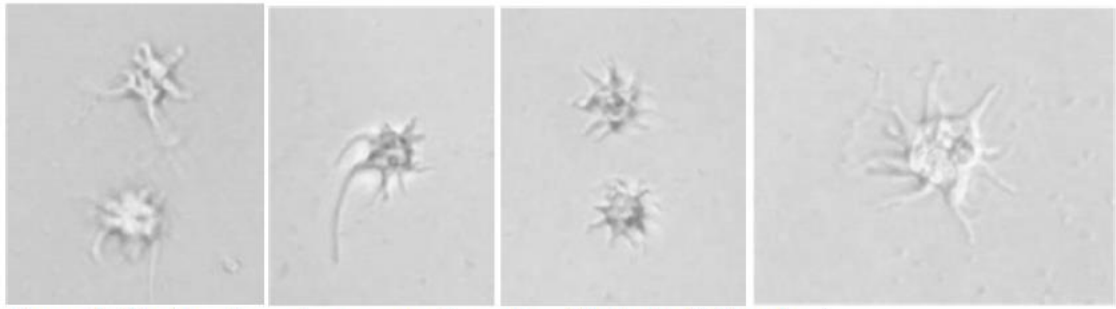


Figure 1. Floating stage of non-exposed amoebae (Photo: A. C. Wennberg)

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)

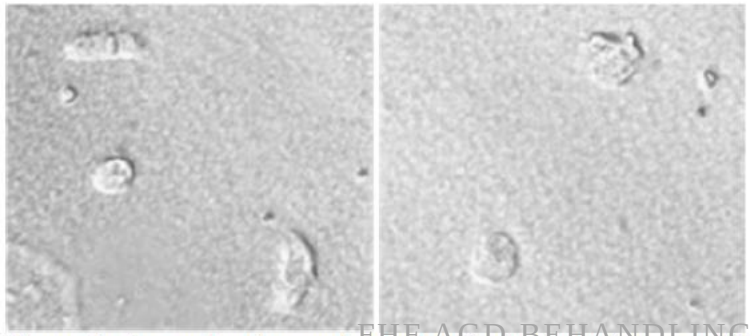


Figure 3. Stressed or damaged amoebae that has balled up several days after UV exposure (Photo: A. C. Wennberg)

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/cm²]	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/cm²]	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

Materiell og Metode

- 8 separate tanker (ca. 50 fisk per. tank)
- (700 L) med 400 L sjøvann – 13.6°C, 34,68 ‰, pH 7,98 - 8,02

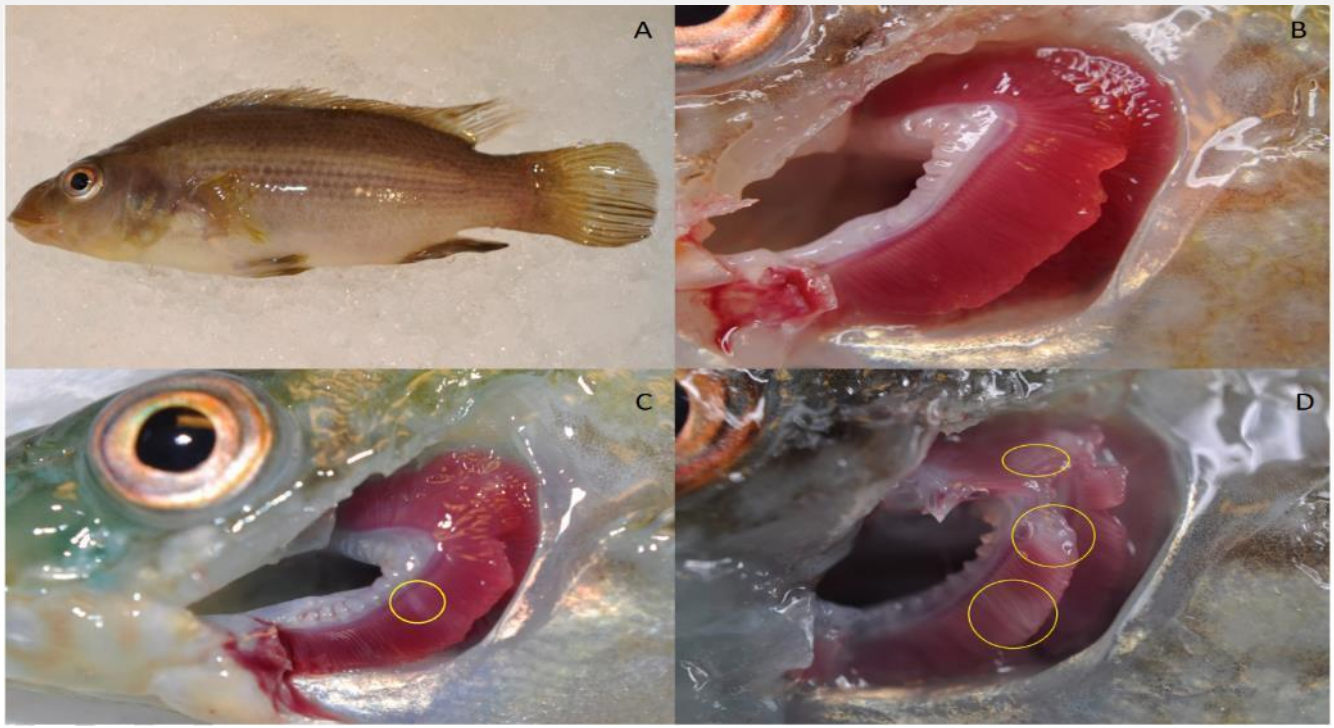
- Medium pressure UV-lamper ble brukt til UV-bestråling av amøbekulturen
  - 10 sekunder for lav dose – 2 mJ/cm^2
  - 90 sekunder for høy dose – 20 mJ/cm^2

Karoppsett:

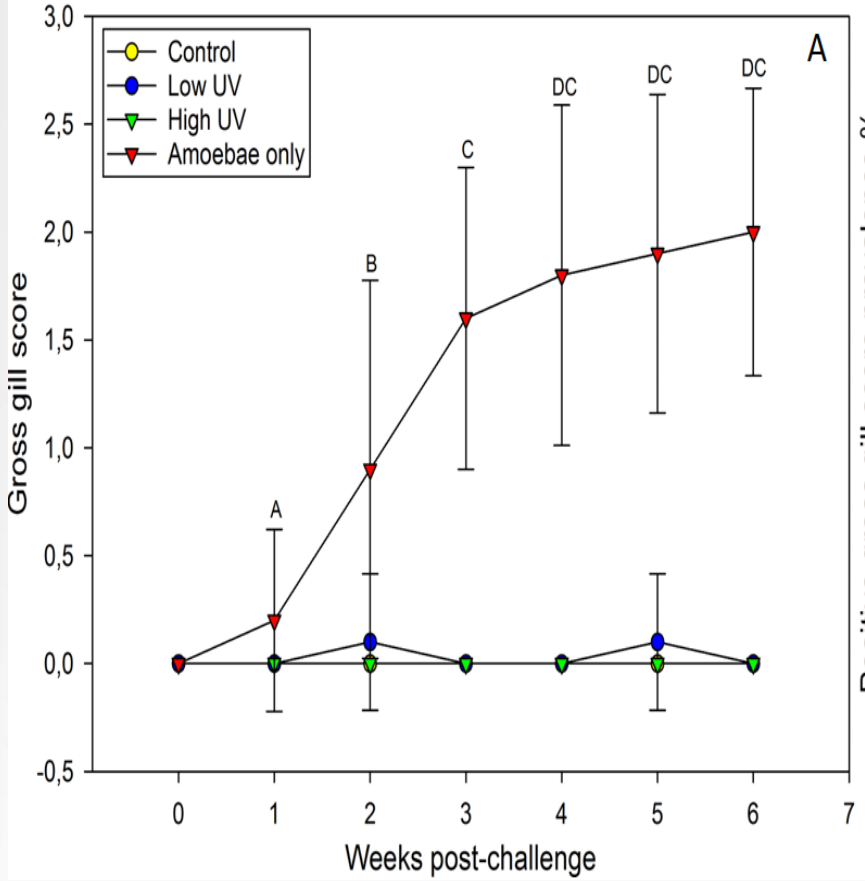
- Negative kontroller – 2 kar med 0 celler/L
- Høy dose UV-bestråling (20 mJ/cm^2) – 2 kar med 1000 celler/L
- Lav dose UV-bestråling (2 mJ/cm^2) – 2 kar med 1000 celler/L
- Positive kontroller (amøber som ikke var utsatt for noe UV-bestråling) – 1000 celler/L

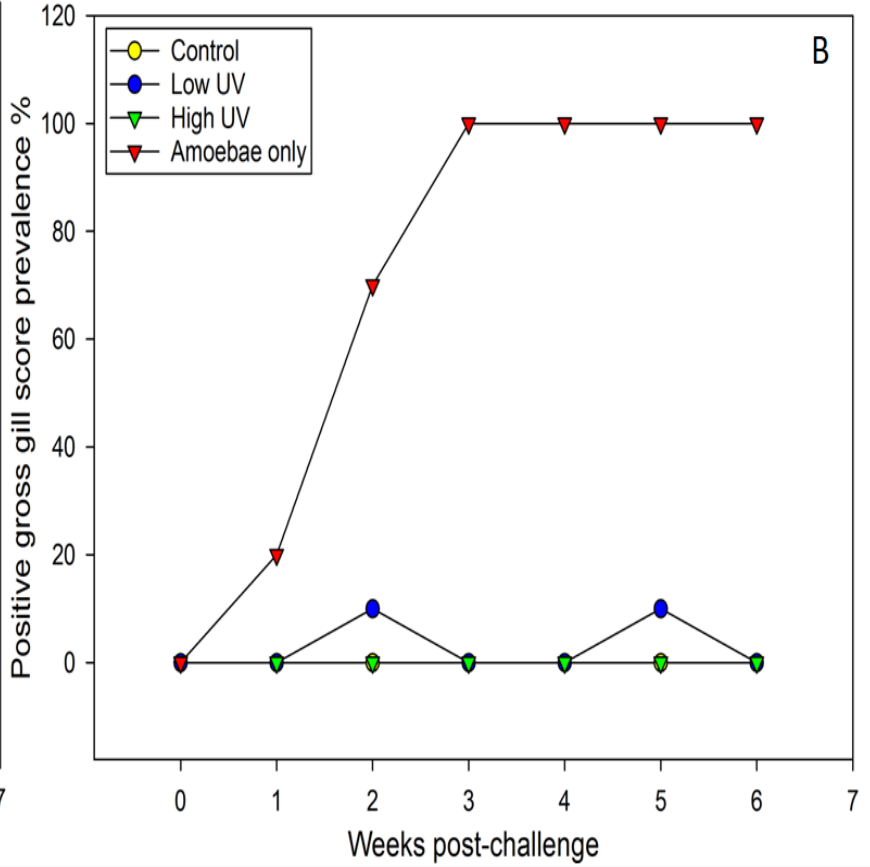
- Ukentlig prøver fra 5 fisk per kar – Gjellescore, gjellevev til histologi og qPCR og blod fra halevene for i-STAT-analyserer

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

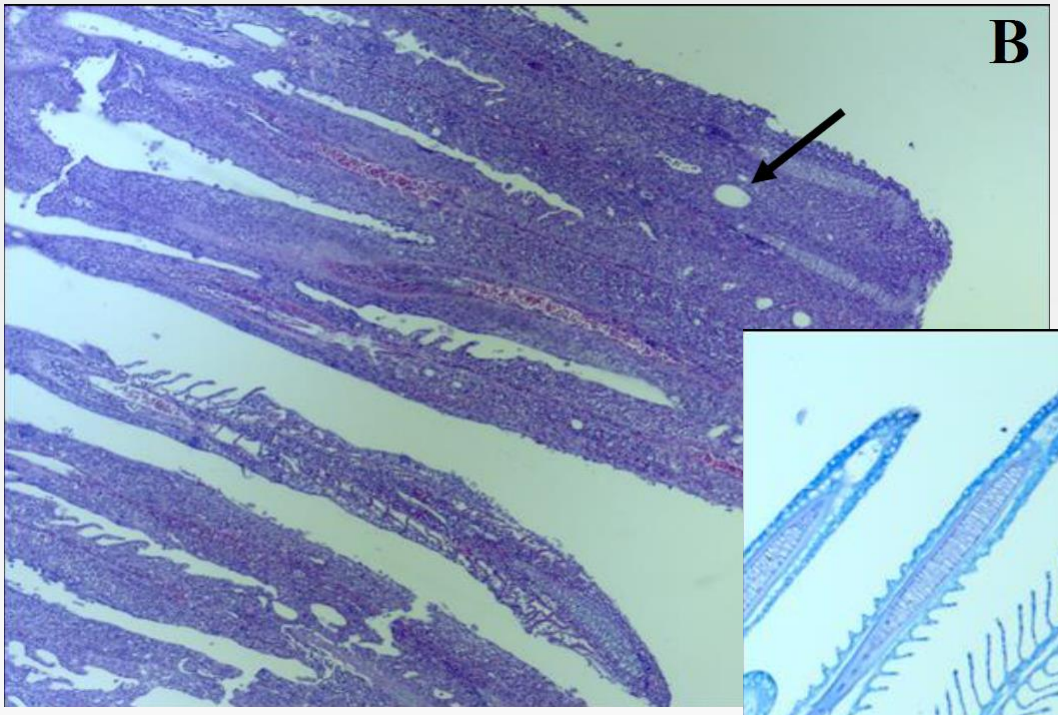


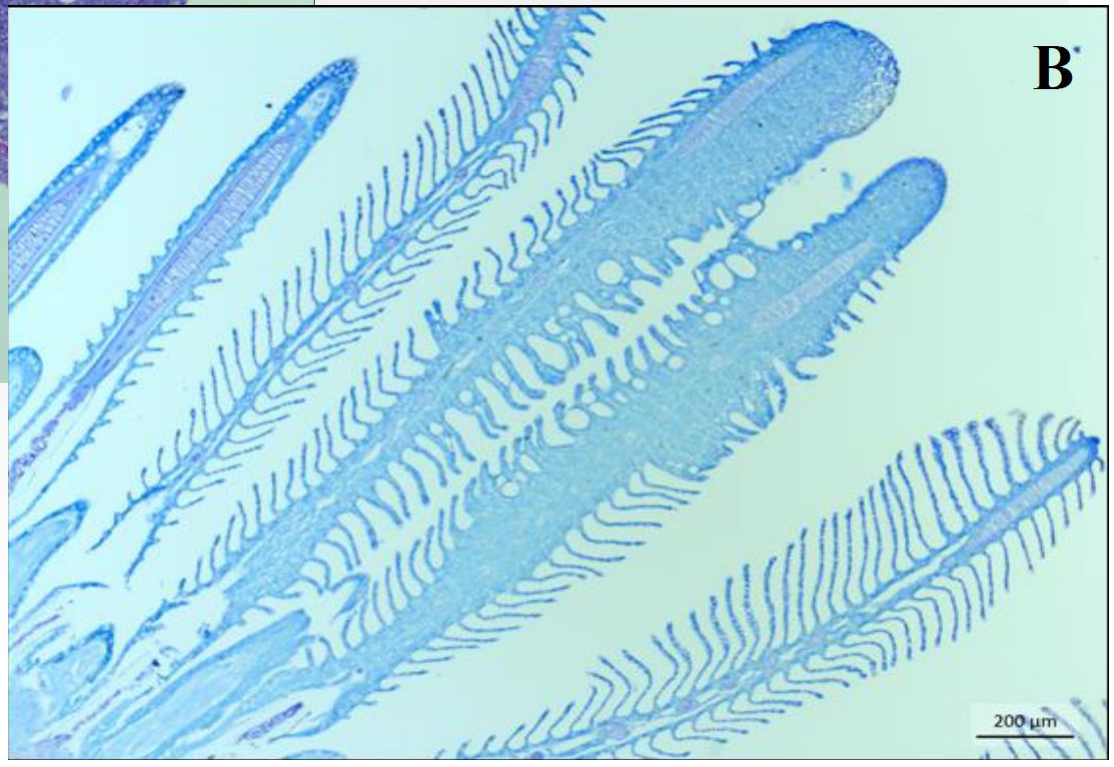
Gjellescore



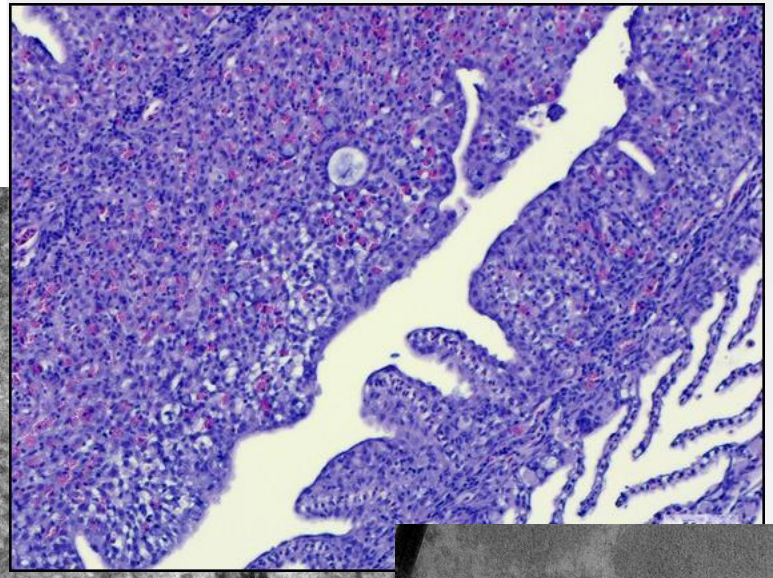


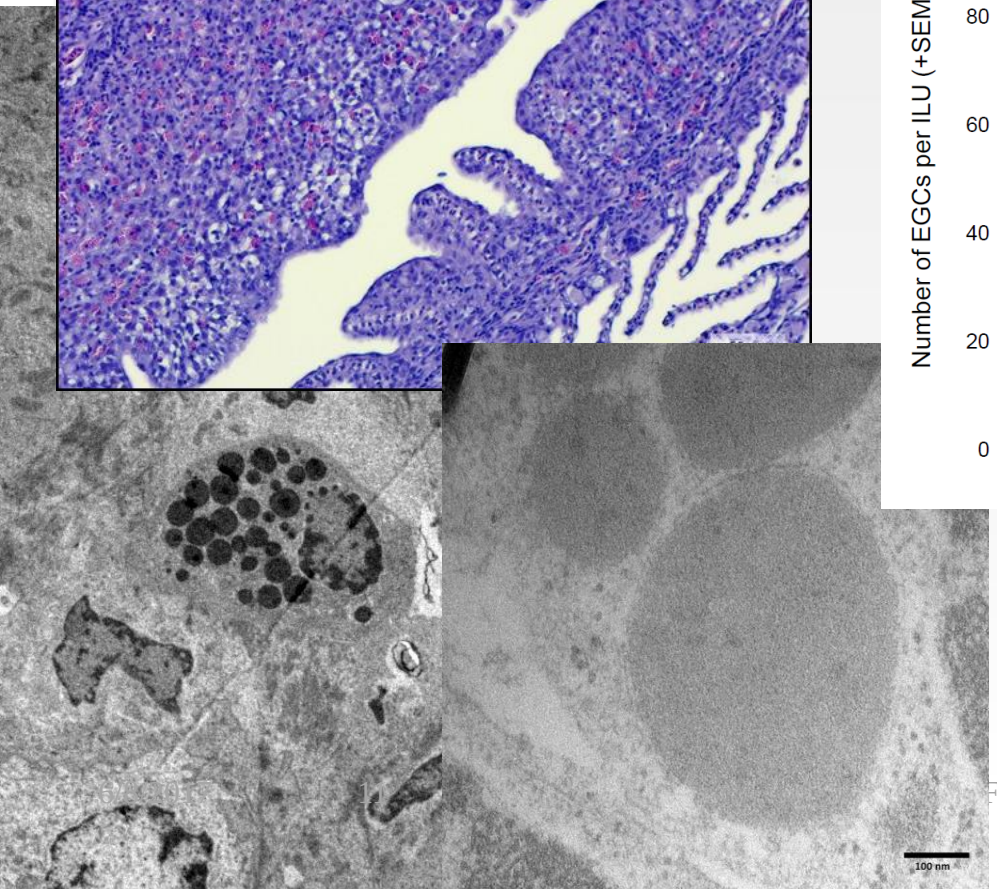
AGD i bergylt

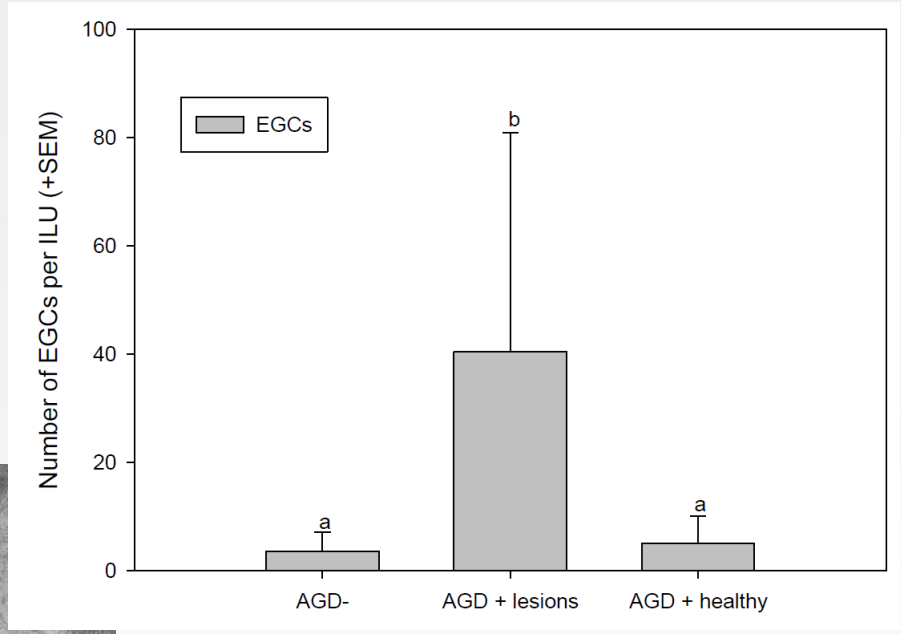




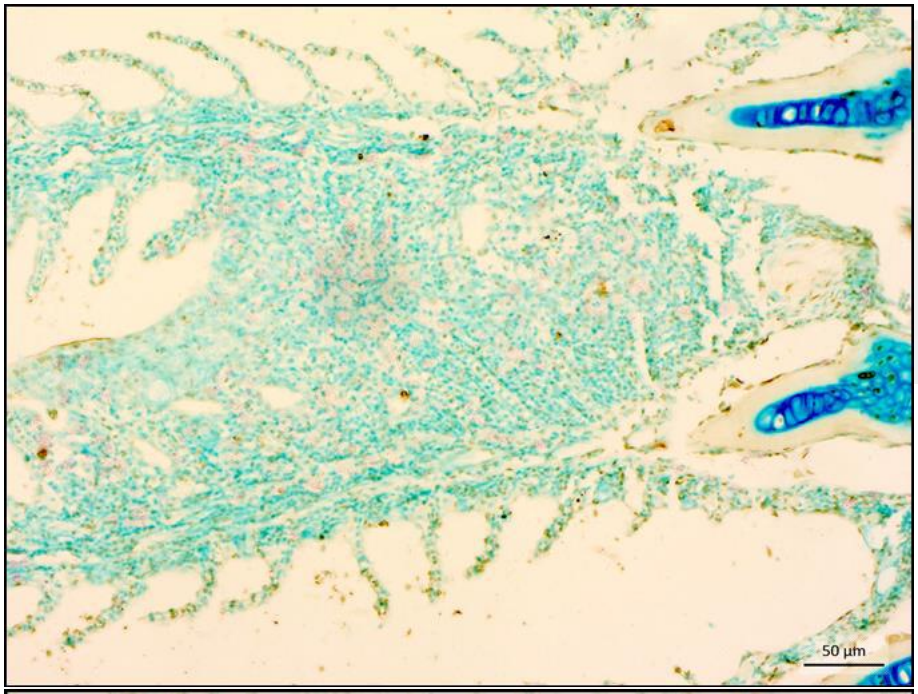
EGCs i lesioner som bergylt

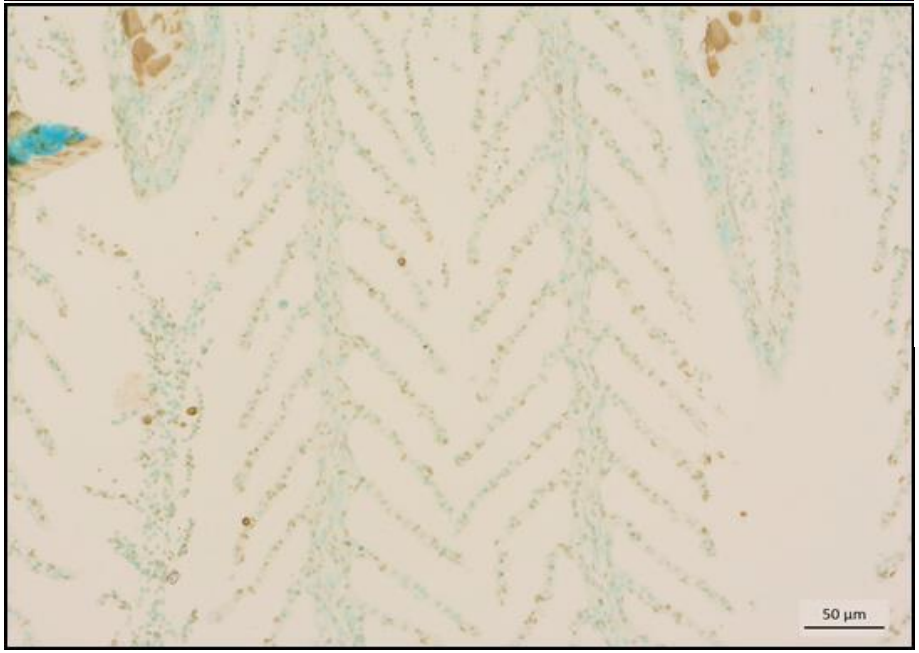


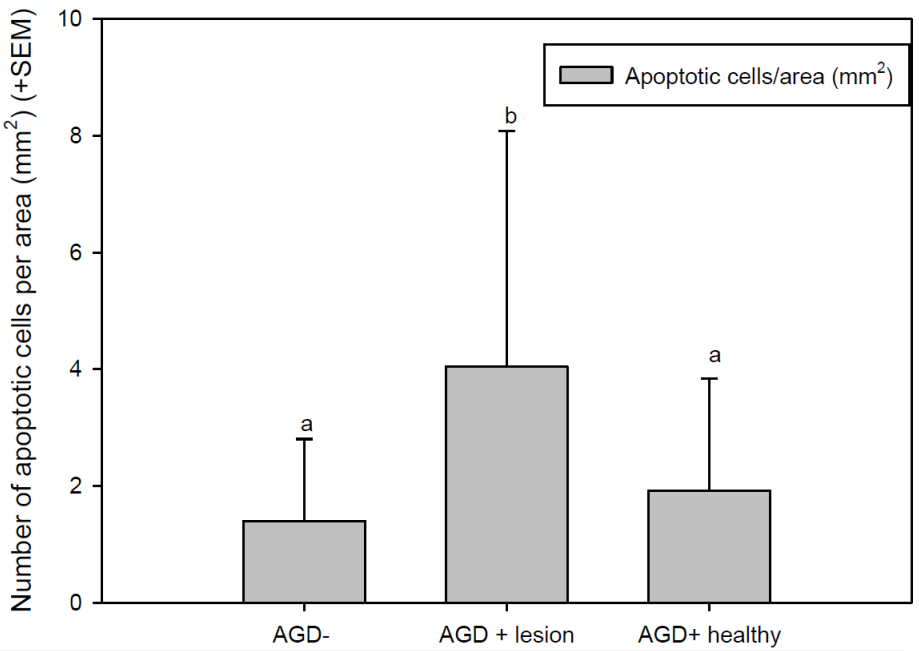




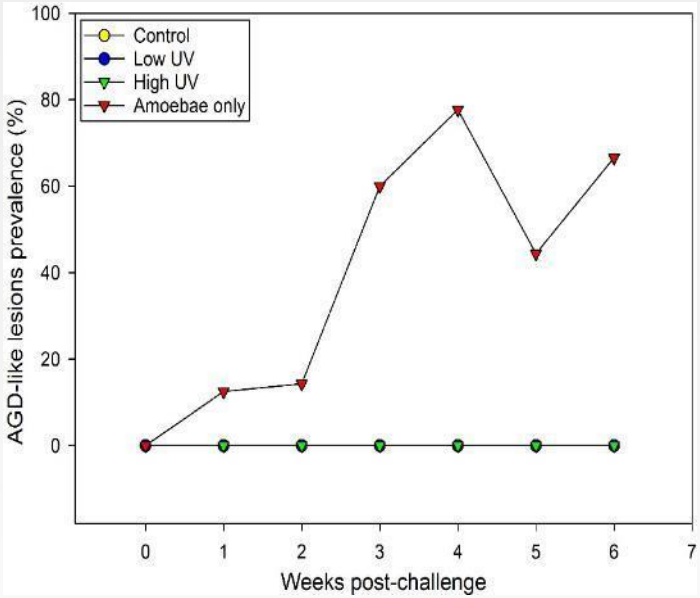
Apoptosis i AGD lesioner

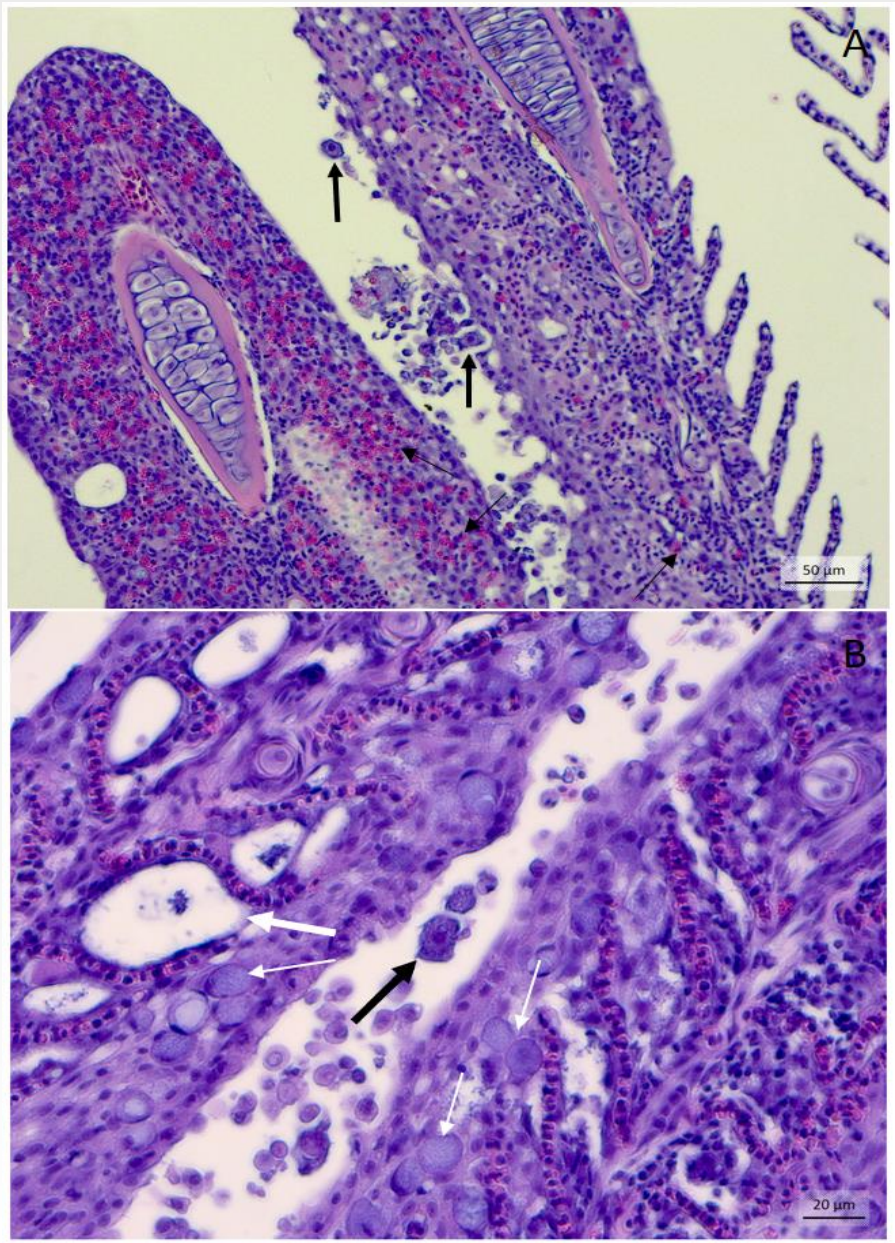






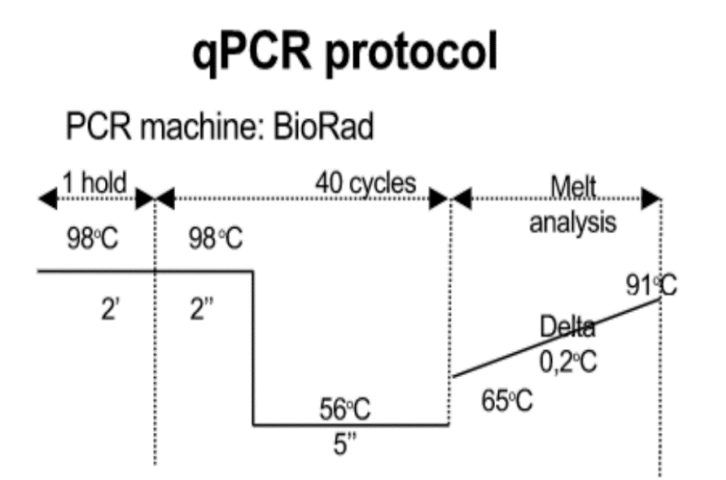
Histologi: AGD-lignende lesjoner





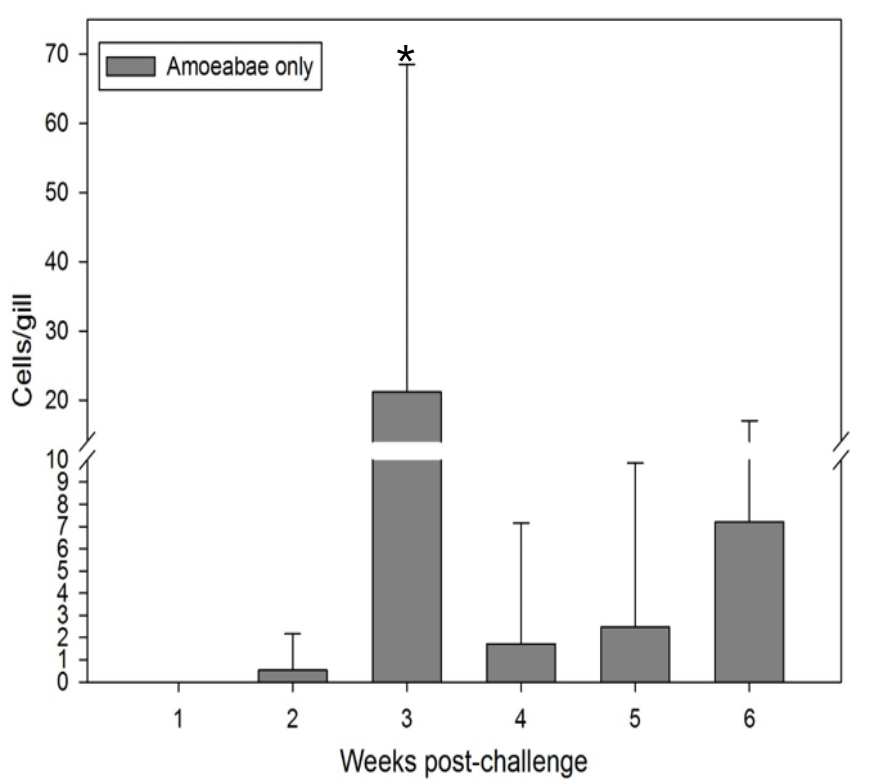
DNA-basert qPCR

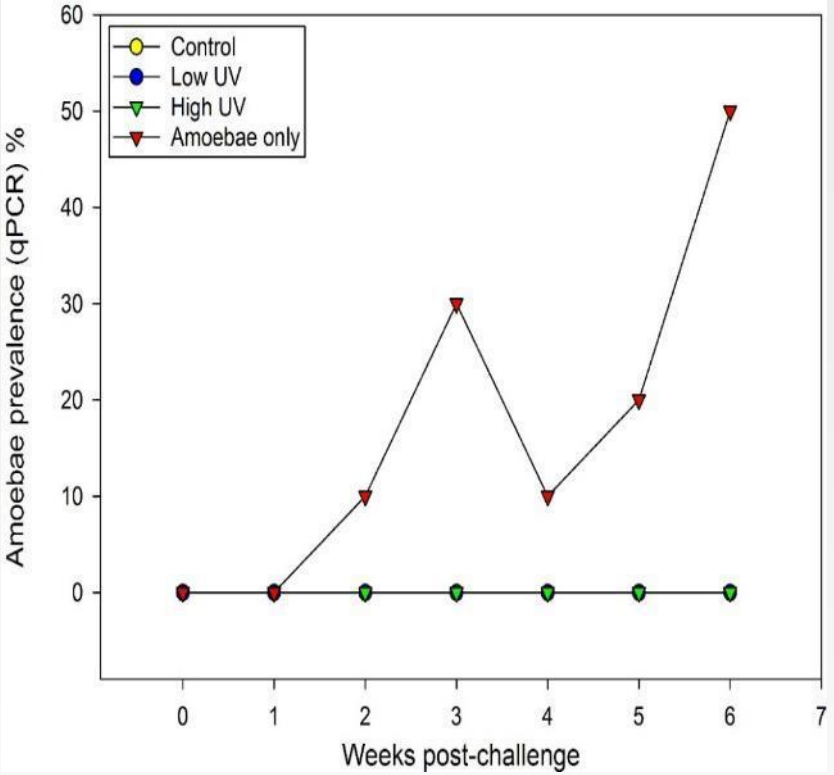
- Gjelleprøver sendt til NIVA, Oslo
- DNA basert qPCR for å kalkulere antall amøber per gjelleprøve
  - Kalkulering basert på kjent referensekultur
- Gene target *Neoparamoeba perurans*/18SrDNA
- Oligonucleotide primers (Bridle et al., 2010)



(NIVA, Marc Anglès d'Auriac. unpublished)

qPCR


Amoeabae only
*
Cells/gill
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
0
1
2
3
4
5
6
Weeks post-challenge


Control
Low UV
High UV
Amoebae only
Amoebae prevalence (qPCR) %
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
Weeks post-challenge

Oppsummering og diskusjon

- UV-radiering med enten lav eller høy dose ser ut til å inhibere amøben til å forårsake sykdom hos berggylt
  - 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

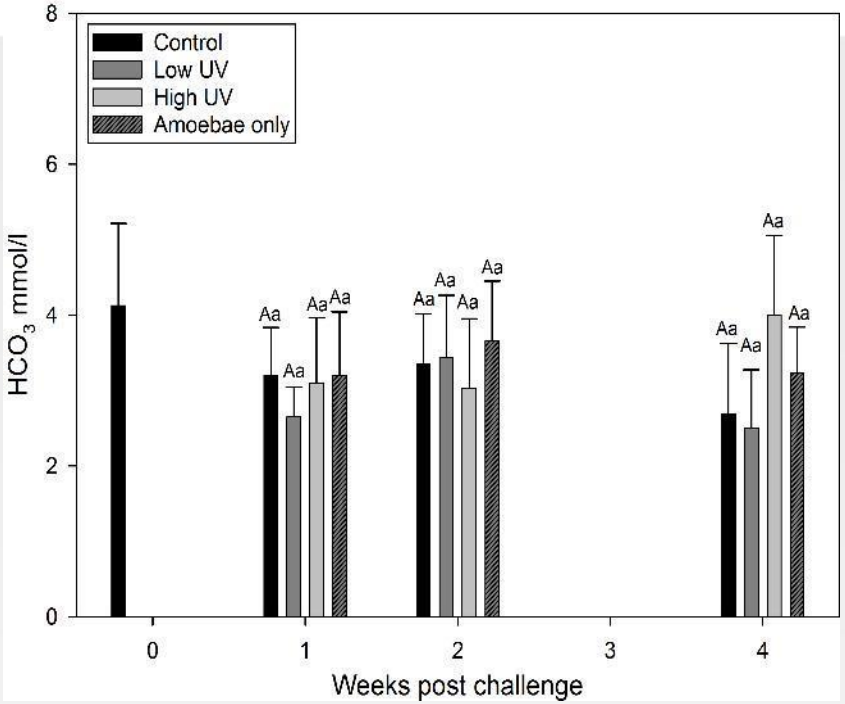
Disinfection of Paramoebae perurans
with UV and ozone
In situ dose-response testing

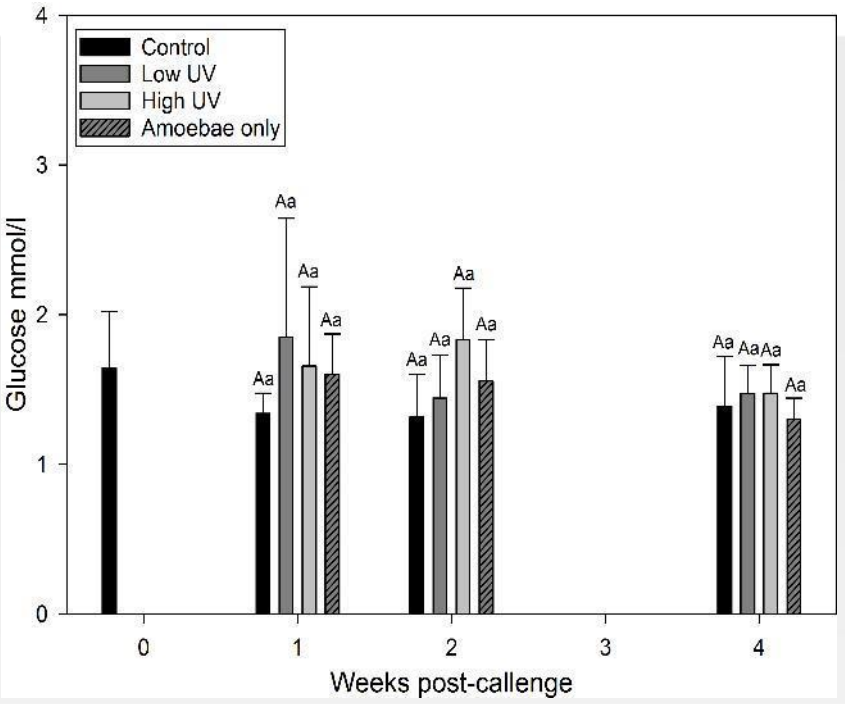
REPORT L. 7075-2016

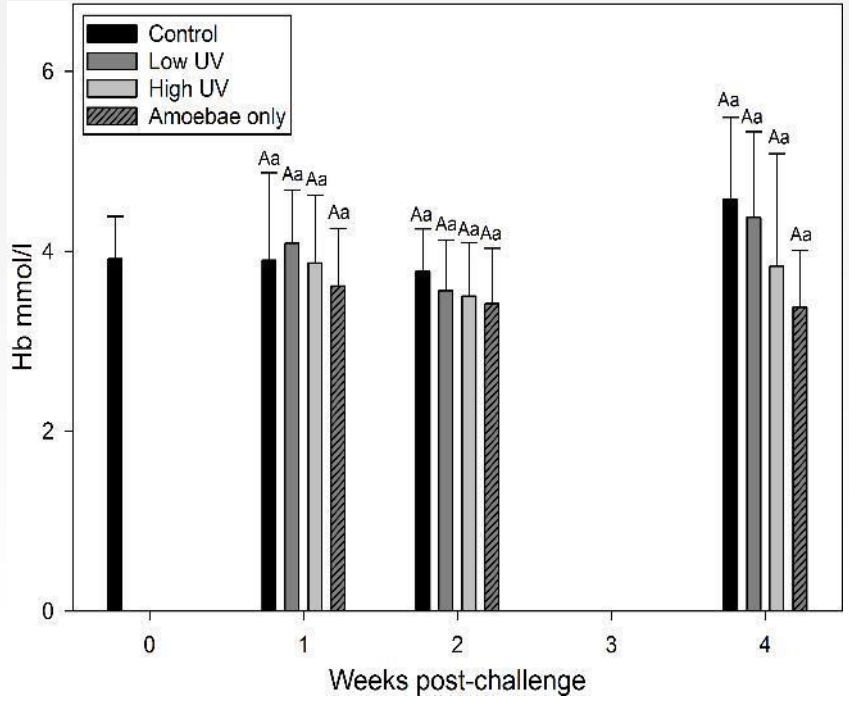
AGD control and disinfection
in cleanerfish
Part 2: Challenge trial

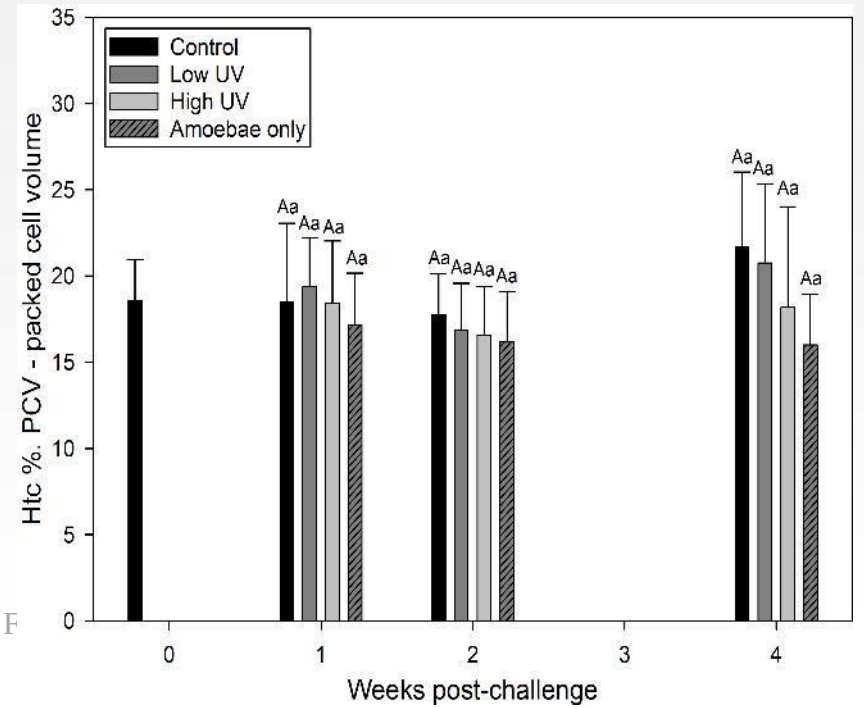
Forbyggende tiltak i landbaserte anlegg

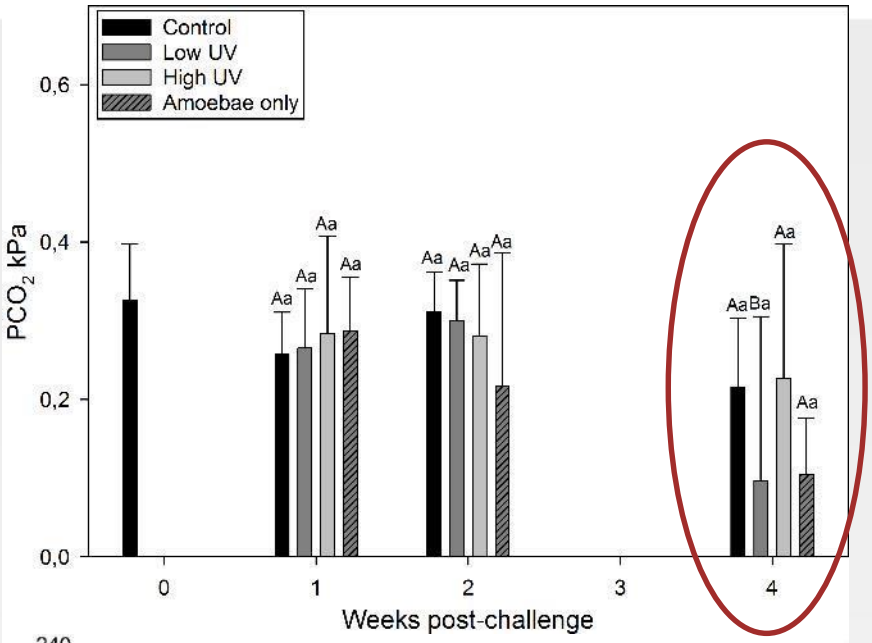
- **UV-bestråling**
- Denaturerer DNA og RNA til mikroorganismer
- Effekt påvirkes av turbiditet, UV-gjennomtrenging (Uvt), partikler, og gjennomstrømning
- Minimumsdose for inntaksvann i Norge – 25 mJ/cm^2
    - Innaktivering av mikroorgansimer skjer fra 2 mJ/cm^2 - >230 mJ/cm^2
- Mest resistente: Virus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

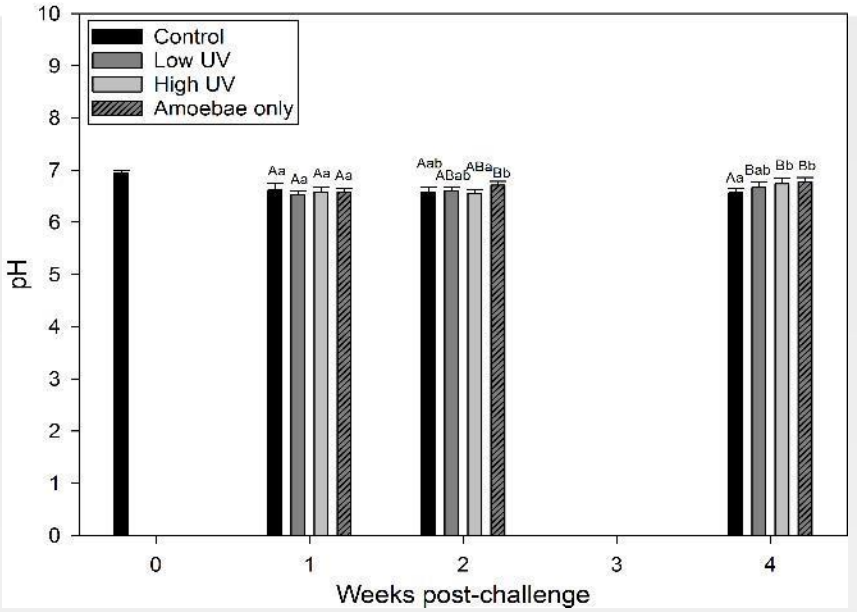

Control
Low UV
High UV
Amoebae only
HCO_3 mmol/l
Weeks post challenge

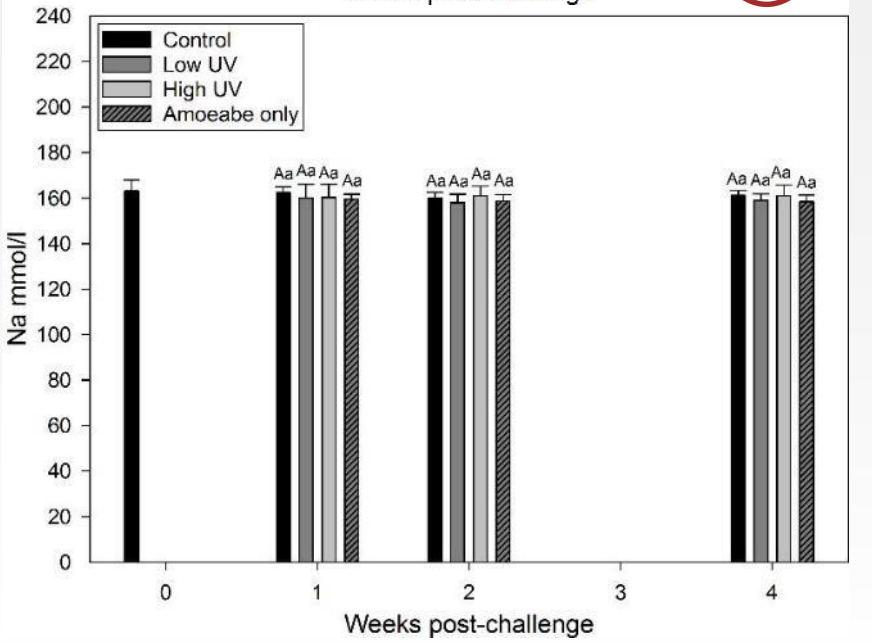

Control
Low UV
High UV
Amoebae only
Glucose mmol/l
Weeks post-callenge

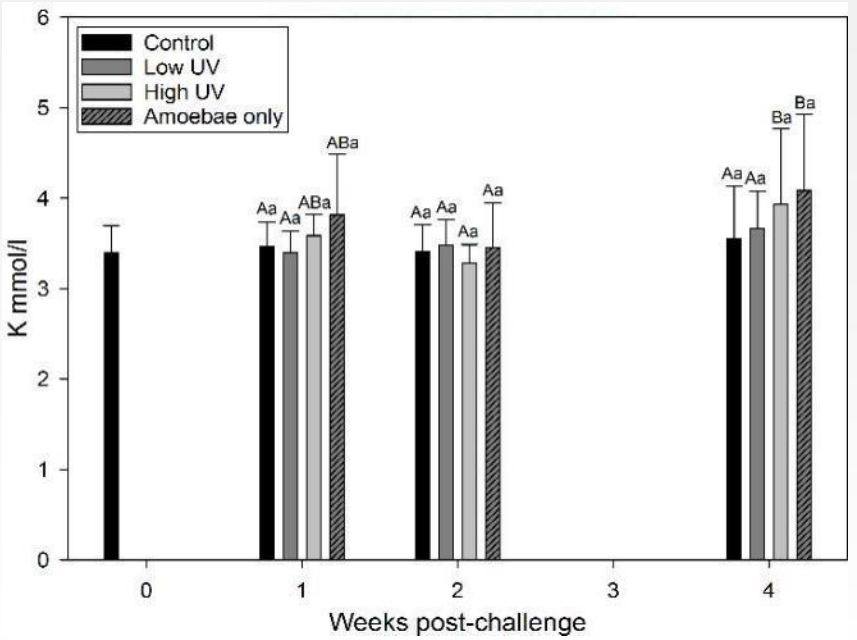

Control
Low UV
High UV
Amoebae only
Hb mmol/l
Weeks post-challenge


Control
Low UV
High UV
Amoebae only
Htc %. PCV - packed cell volume
Weeks post-challenge


Control
Low UV
High UV
Amoebae only
PCO_2 kPa
0,0
0,2
0,4
0,6
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Ba
Aa
Aa
0
1
2
3
4
Weeks post-challenge


Control
Low UV
High UV
Amoebae only
pH
0
1
2
3
4
5
6
7
8
9
10
Aa
Aa
Aa
Aa
Aab
ABab
ABa
Bb
Aa
Bab
Bb
Bb
0
1
2
3
4
Weeks post-challenge


Control
Low UV
High UV
Amoeabe only
Na mmol/l
0
20
40
60
80
100
120
140
160
180
200
220
240
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
0
1
2
3
4
Weeks post-challenge


Control
Low UV
High UV
Amoebae only
K mmol/l
0
1
2
3
4
5
6
Aa
Aa
ABa
ABa
Aa
Aa
Aa
Aa
Aa
Aa
Ba
Ba
0
1
2
3
4
Weeks post-challenge

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
HCO_3 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
PCO_2 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	-0322 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

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

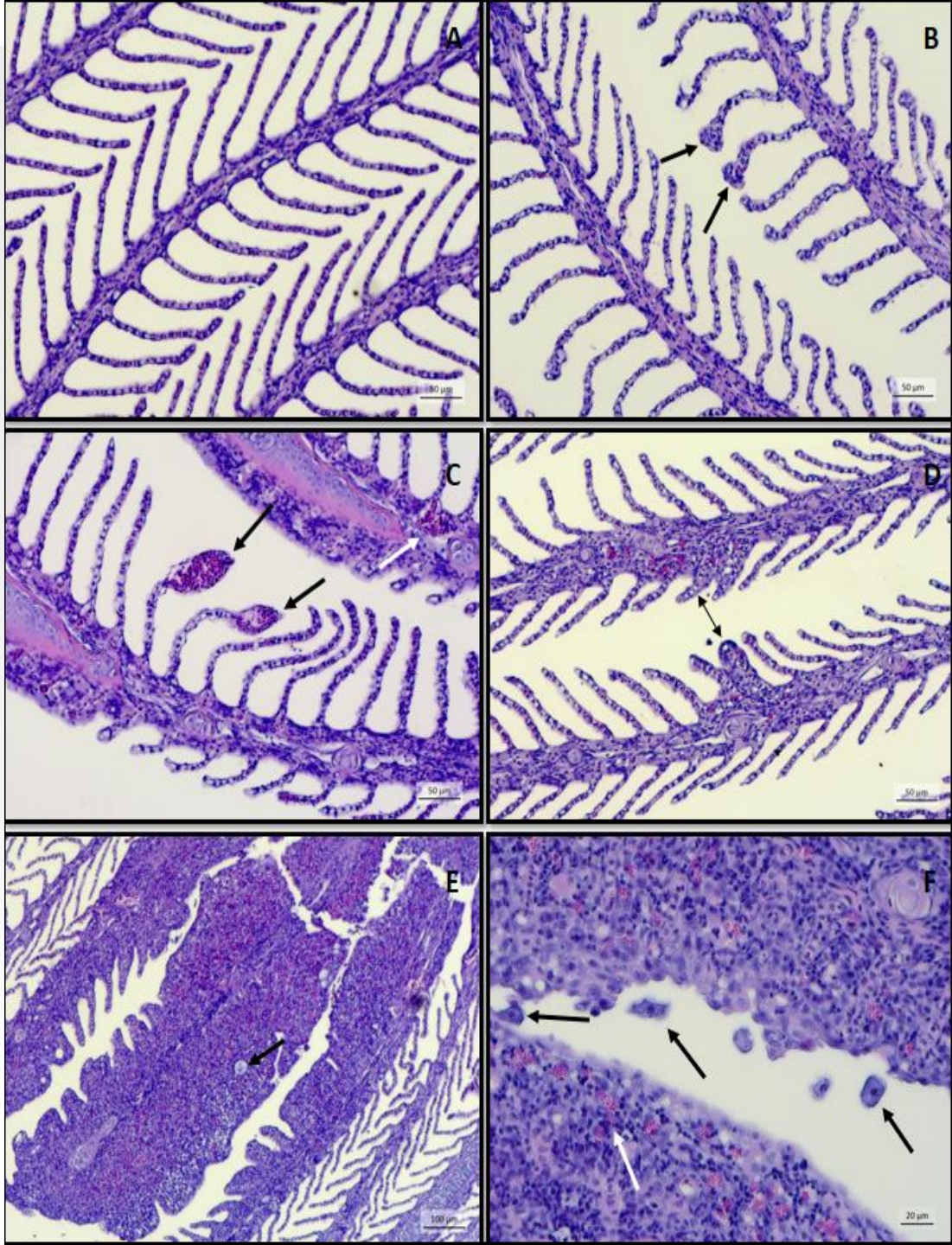
Konkluderende bemerkninger

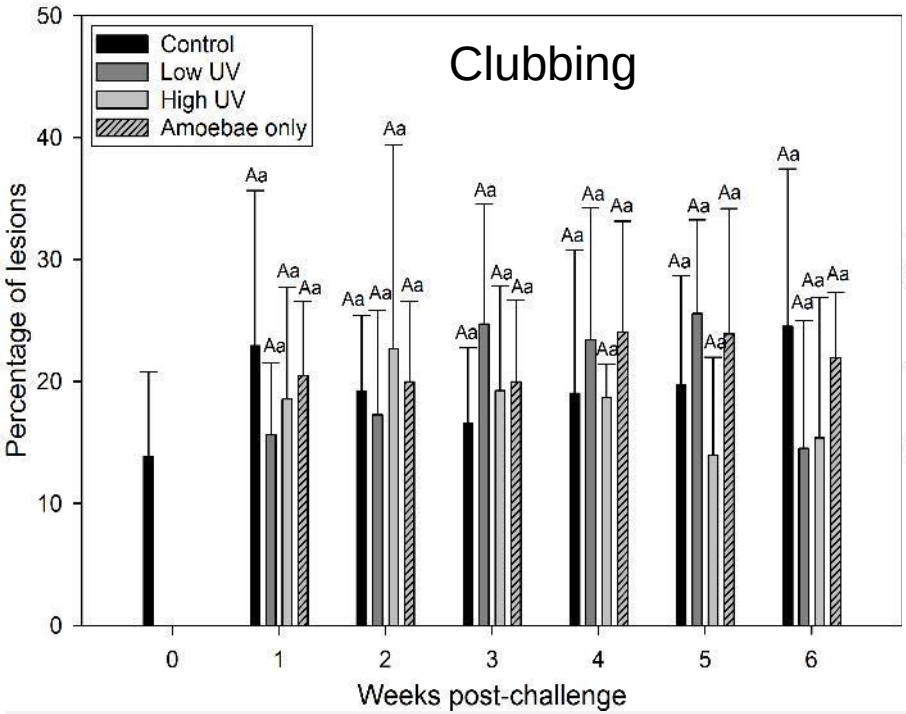
- UV-dosen bør overstige 2 mJ/cm^2 for å inhibere amøben til å forårsake sykdom hos berggylt

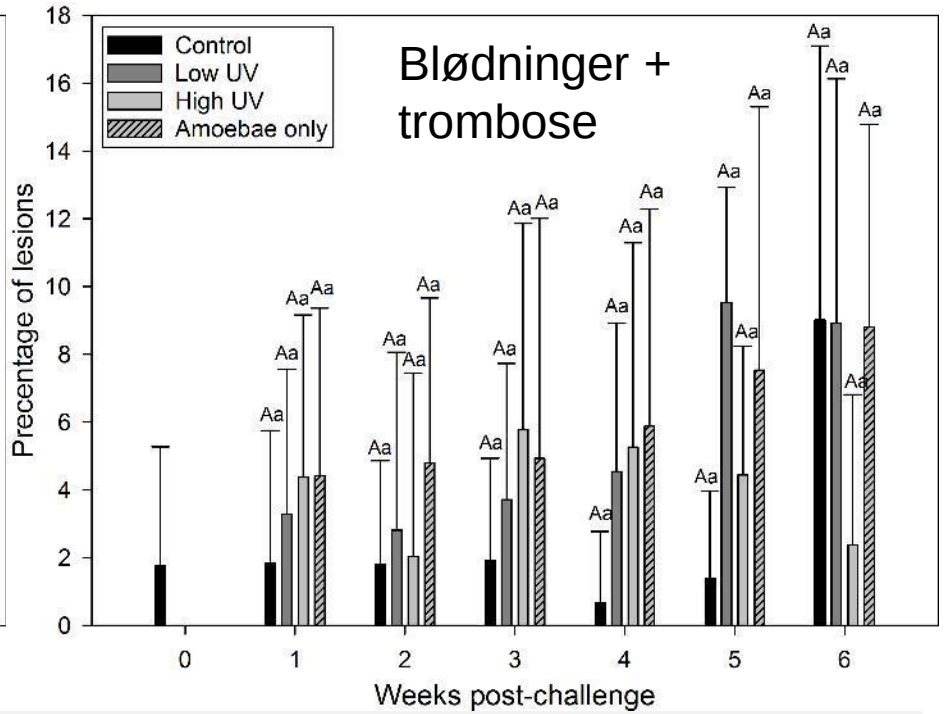
Histologisk score

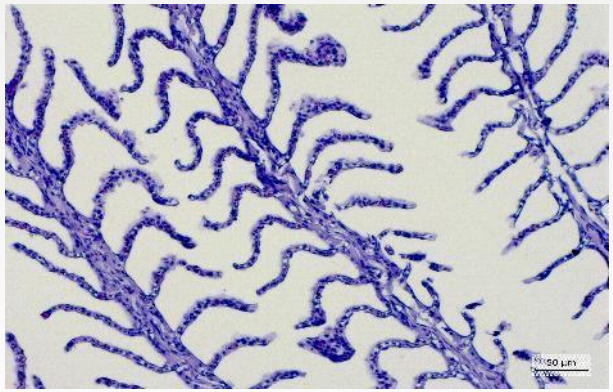
- Prosentandel filamenter med en eller flere gitte lesjoner
  - Clubbing
  - Blødninger, trombose og telangiectasis
  - Hyperplasi
  - AGD-lignende lesjoner

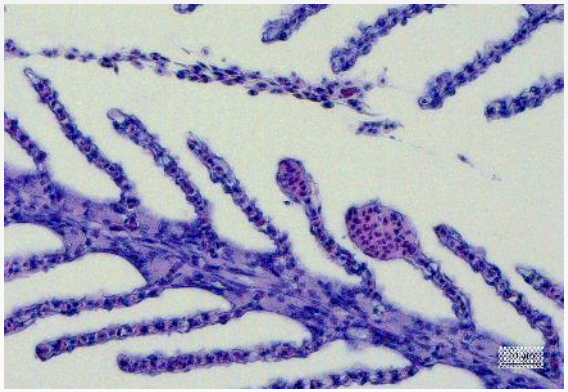
- 75 % velorienterte filamenter for evaluering

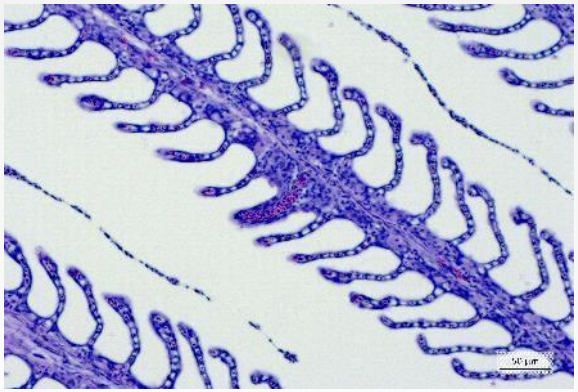


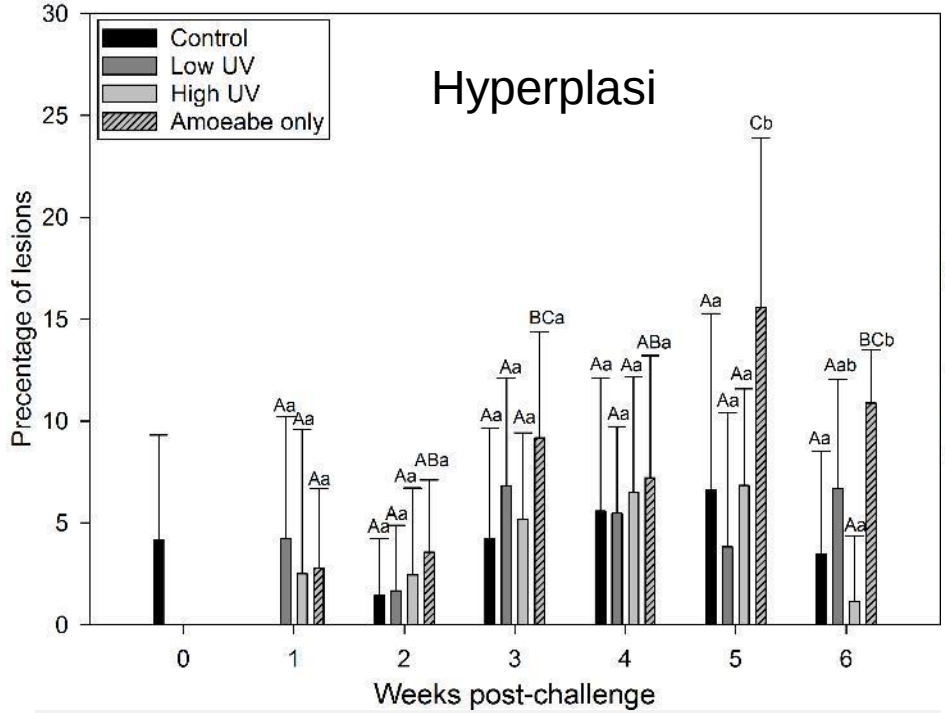

Clubbing
Control
Low UV
High UV
Amoebae only
Percentage of lesions
Weeks post-challenge
0
10
20
30
40
50
0
1
2
3
4
5
6
Aa


Blødninger + trombose
Control
Low UV
High UV
Amoebae only
Precentage of lesions
Weeks post-challenge
0
2
4
6
8
10
12
14
16
18
0
1
2
3
4
5
6
Aa





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Low UV
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Precentage of lesions
Weeks post-challenge
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BCa
Cb
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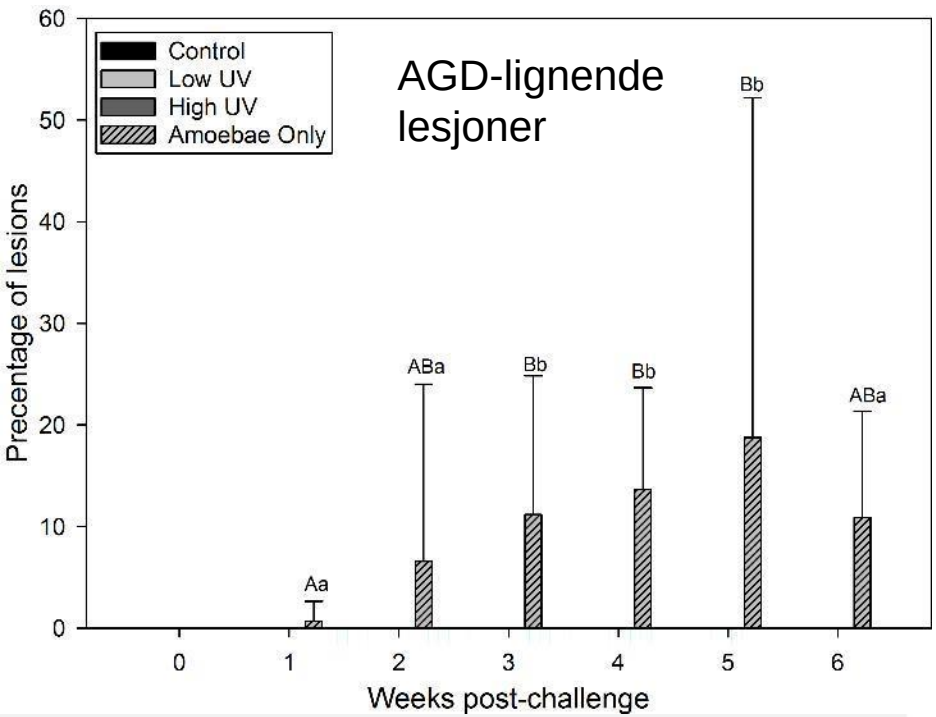
BCb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Precentage of lesions
Weeks post-challenge
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ABa
Bb