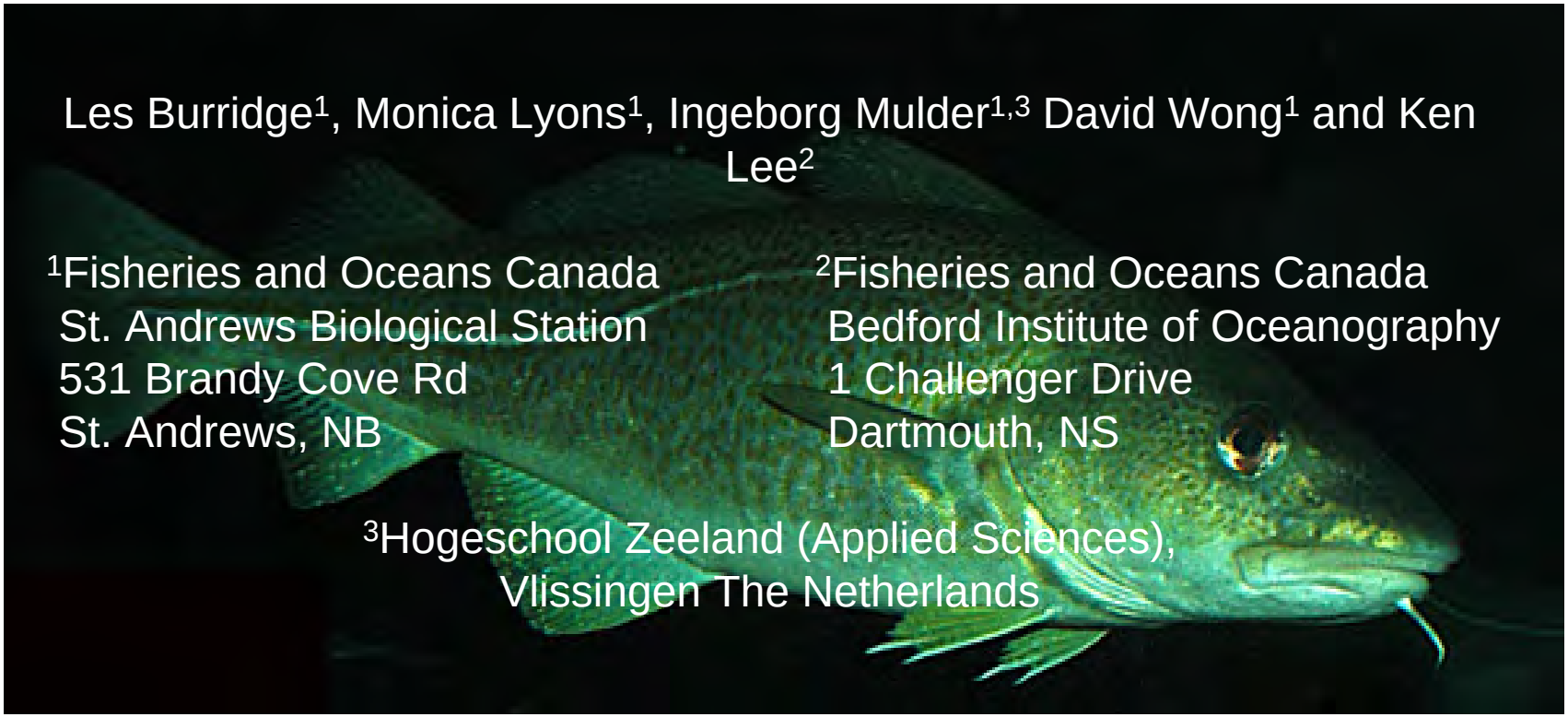
The effects of WAF or CEWAF on the Fertilization and Hatching Success of Atlantic cod eggs.



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WAF & CEWAF

- Water Accommodated Fraction (WAF) of oil
  - Weathered Alaskan North Slope (ANS) and Medium South American (MESA) crude oil.
- Chemically Enhanced Water Accommodated Fraction (CEWAF) of oil
  - ANS and SPC-1000 or Corexit 9500
  - MESA and SPC-1000 or Corexit 9500

- Chemical dispersants may be used to disperse oil after spills in the ocean
- Therefore questions about effects on marine species
- Cod are commercially important
- Cod are batch spawners & release gametes over broad geographic range
- Cod release gametes into the open ocean at various depths and over a fairly long period of time

Methods

- 1 part MESA or ANS in 9 parts filtered SW
- magnetic stirrer & baffled flask for 18 h; settle for 1 hr
- Exposure Temp = 4 - 6˚C
- 5 concentrations from 0% - 25% v/v


11/04


09/20/2008

- **Prepare WAF**
- **1 part dispersant for 20 parts oil stir for 1 hr.**
- **Settle for 60 min**
- **ExposureTemp = 4 - 6 ˚C**
- **5 concentrations 0% - 0.5% v/v.**


GYROTORY

Water analysis

- "General" analysis by fluorometry. To confirm presence and range of "concentrations"
- Calibration line developed by serial dilution of oil in hexane
- Water samples (4 or 50 mL) extracted in hexane and Quantified using the oil std curve.
- Full GC and GC-MS workup in progress

Representative Water Analysis

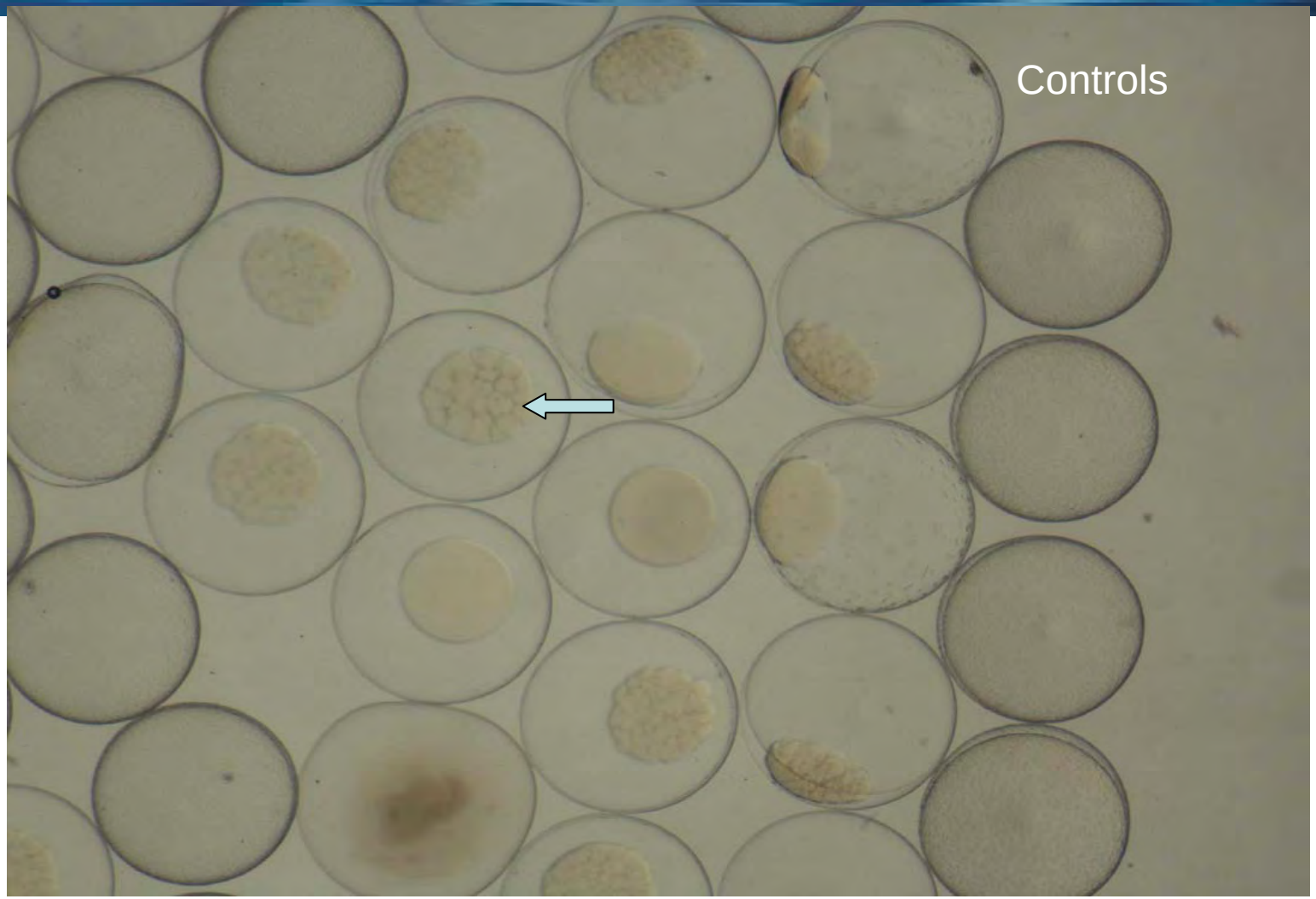
	WAF (ANS) 75% WAF	WAF (MESA) 75% WAF	CEWAF (ANS + SPC) 64% CEWAF	CEWAF (ANS + Corexit) 64% CEWAF	CEWAF (MESA + SPC) 64% CEWAF	CEWAF (MESA + Corexit) 64% CEWAF
Σ Alkanes ($ng \cdot L^{-1}$)	15682.72	38758.82	235767.0987	256876.74	319336.39	390536.17
ΣMethylated PAHs ($ng \cdot L^{-1}$)	435.64	1560.72	39885.81	47741.42	45500.43	53302.38
Σ PAHs ($ng \cdot L^{-1}$)	607.74	1423.62	4732.04	5607.61	4707.93	5396.91
TPH ($\mu g \cdot mL^{-1}$)	0.38	0.40	4.82	4.12	5.36	5.81

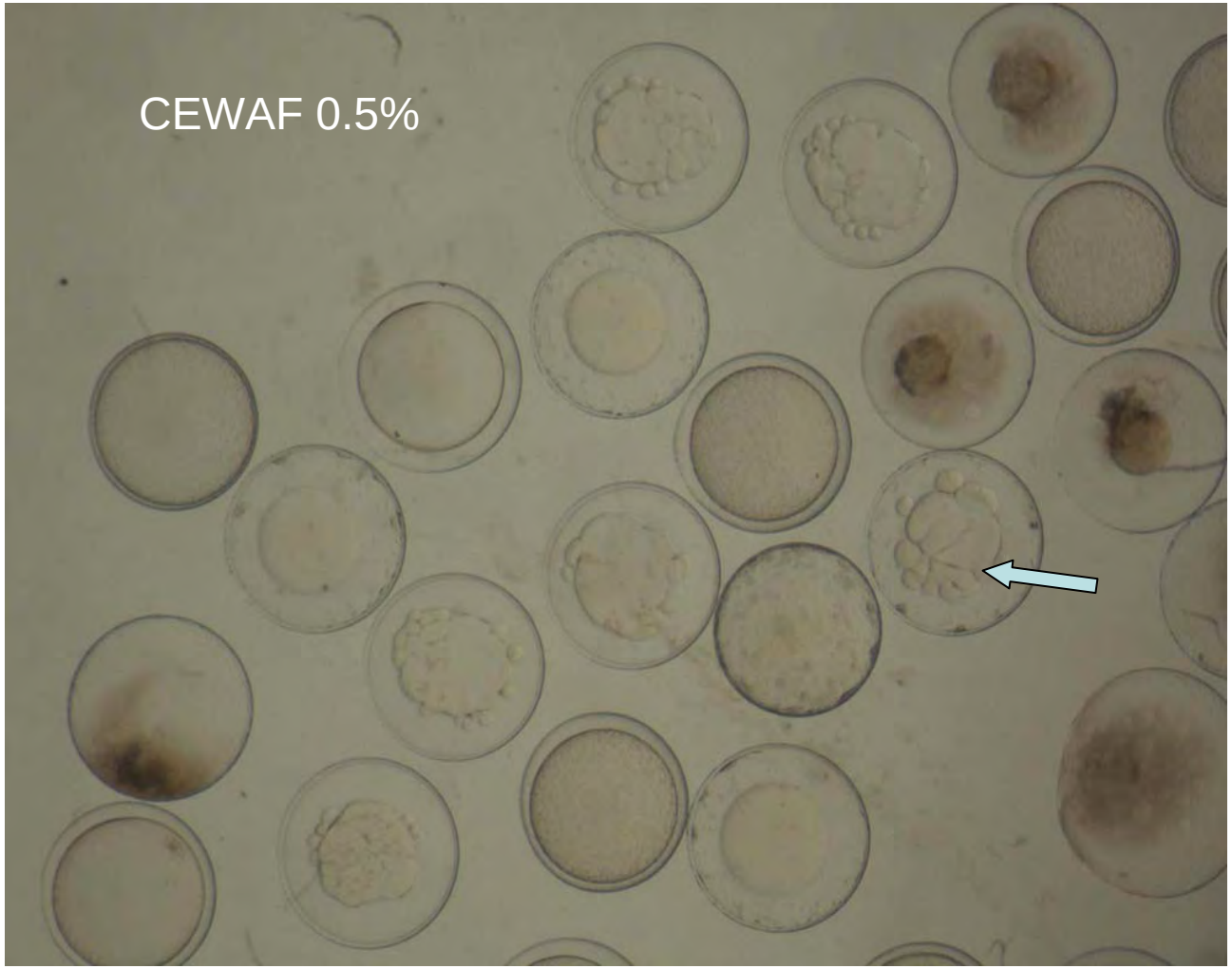
- Eggs and milt manually stripped from mature cod.
- Studies conducted with gametes from single set of parents

Methods

- 500 µL milt mixed in 200 mL of WAF or CEWAF.
- Eggs (25 ml) added and stirred (5 sec)
- Eggs “hardened” for 5 min, washed and transferred to 200mL of WAF or CEWAF.
- At T=24 h assess fertilisation success (subsample X 3)
- Successfully fertilised eggs transferred to 96 well plates and held at 6 C for 20 days.
- Assessed timing and success of hatch to larvae.


02/26/2008


Controls


CEWAF 0.5%

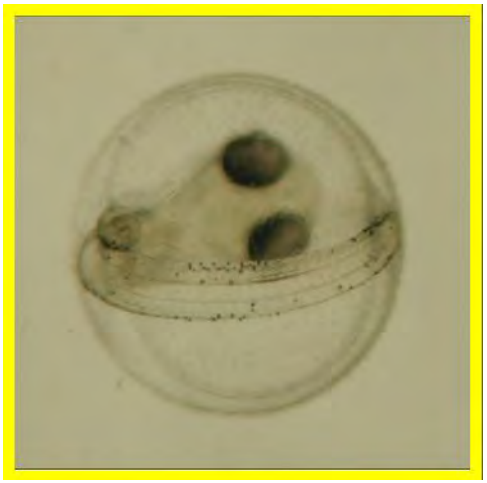
“Normal” fertilisation

- Quality of gametes is variable, especially eggs
- Fertilisation success is therefore variable and can be quite low.
- We accept rates as low as 10% (reluctantly)
- Regardless of actual fertilisation success in controls it is considered 100 for our comparisons and success in treated is reported in relative form.

4 Days

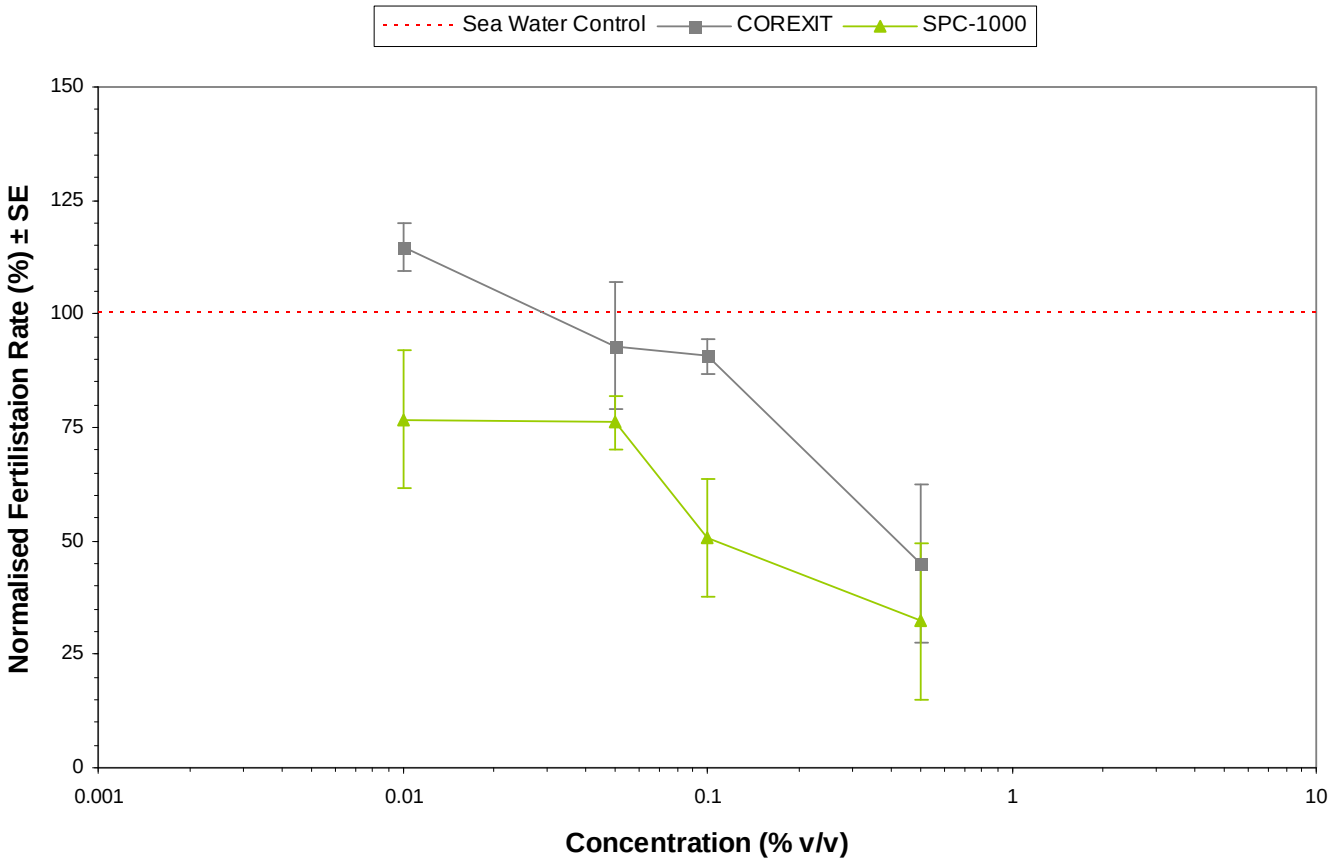
8 Days

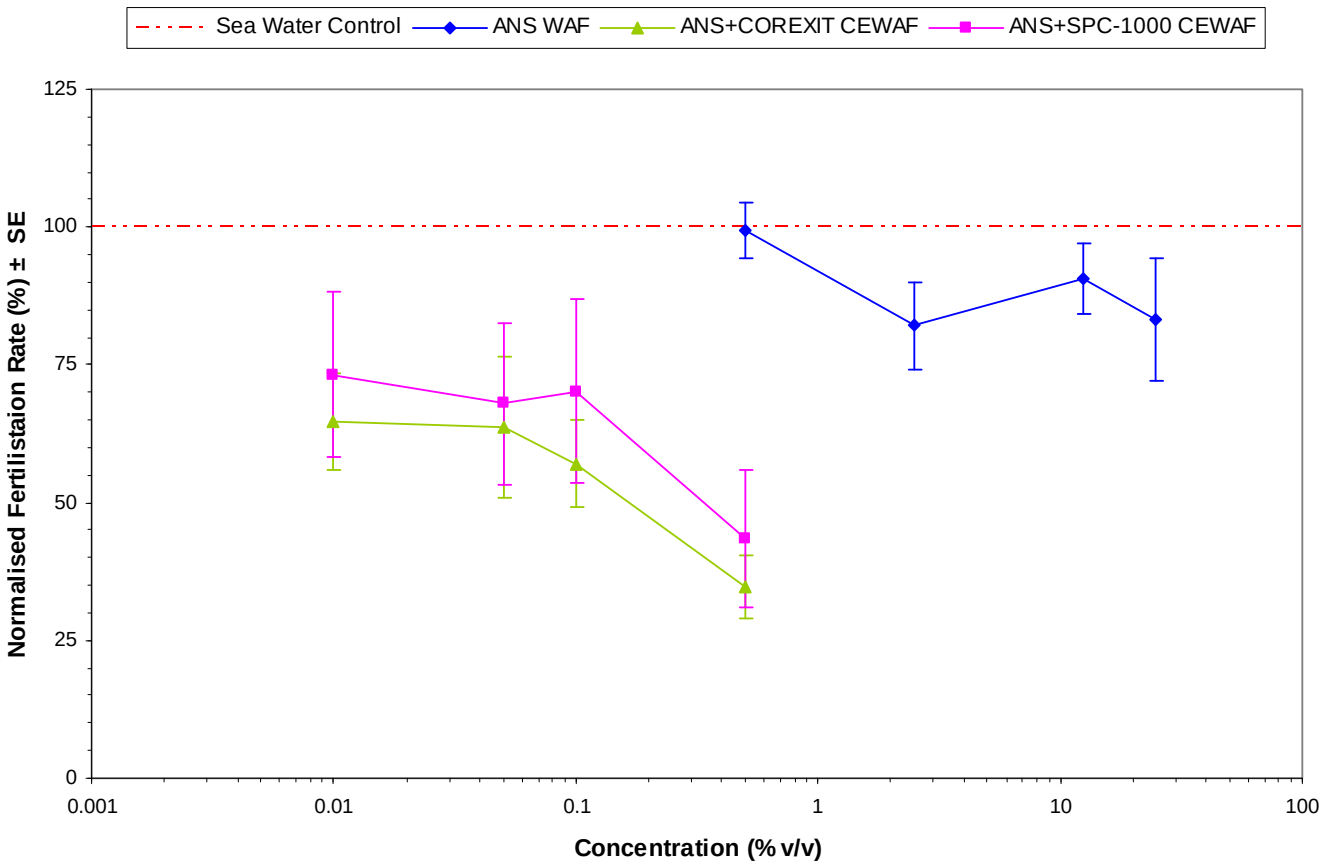
16 Days



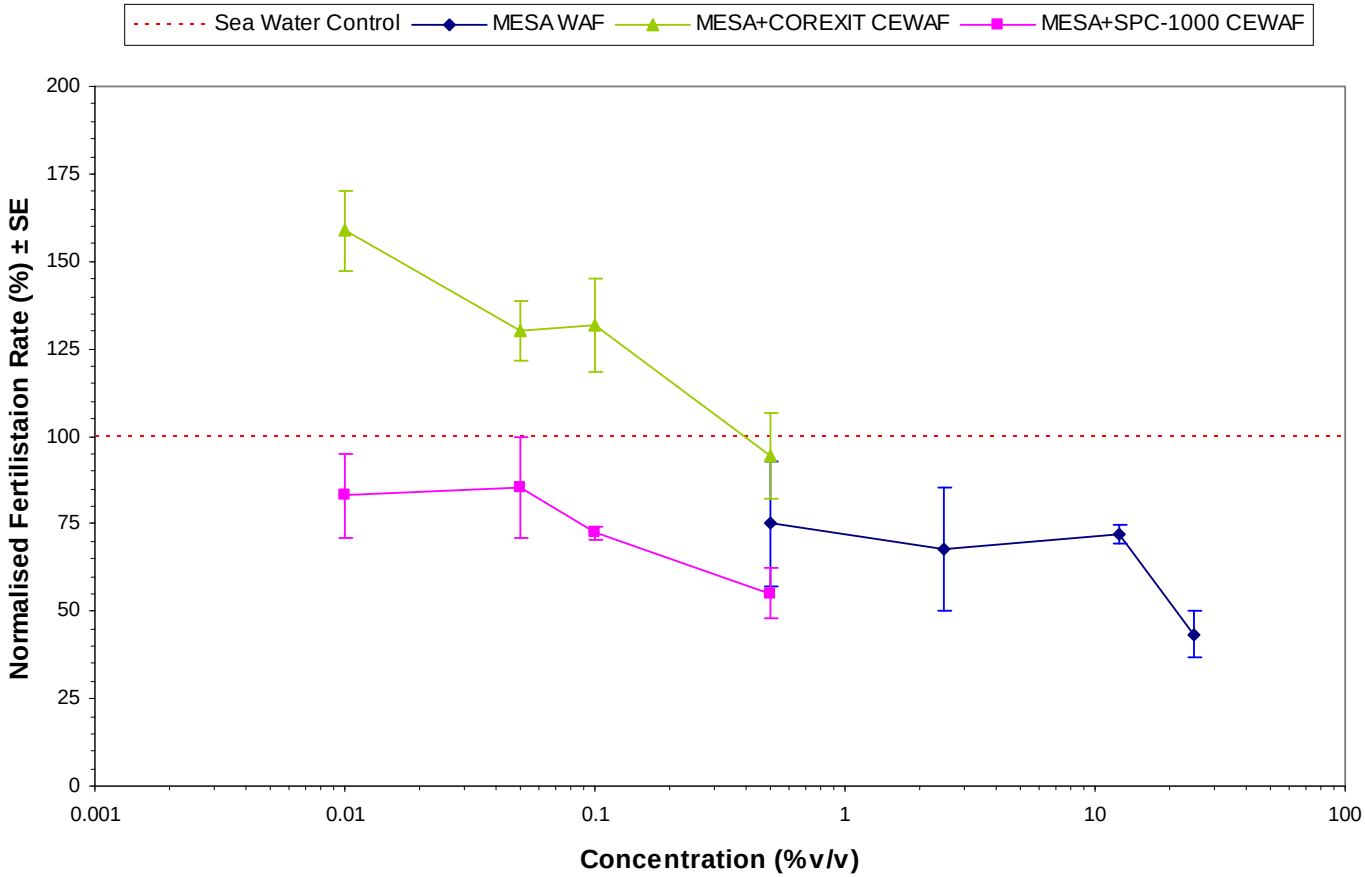
Post Hatch

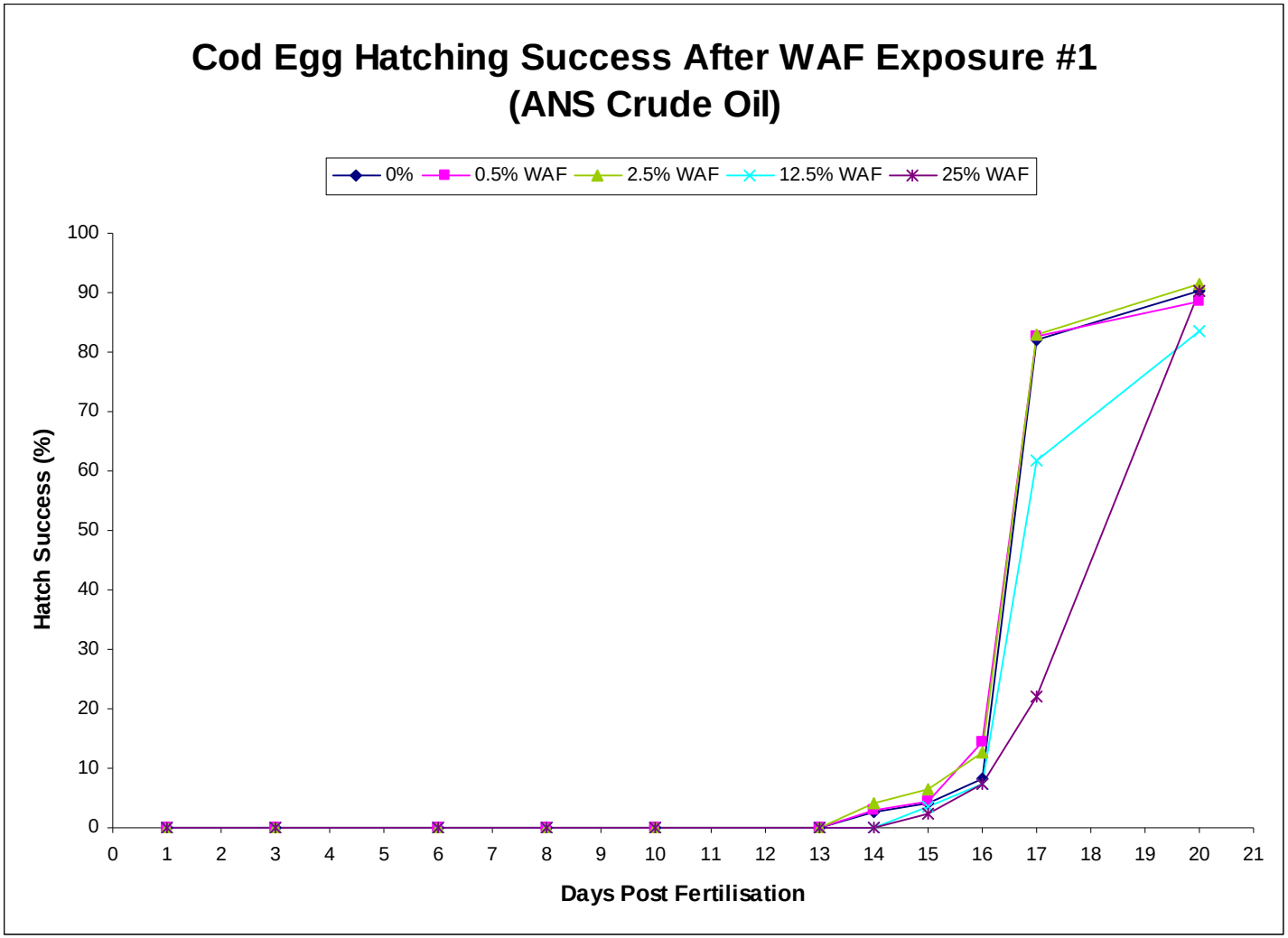
Cod Egg Fertilisation Rate in COREXIT and SPC-1000 Dispersants (Mean ± SE) Normalised Against Sea Water Control

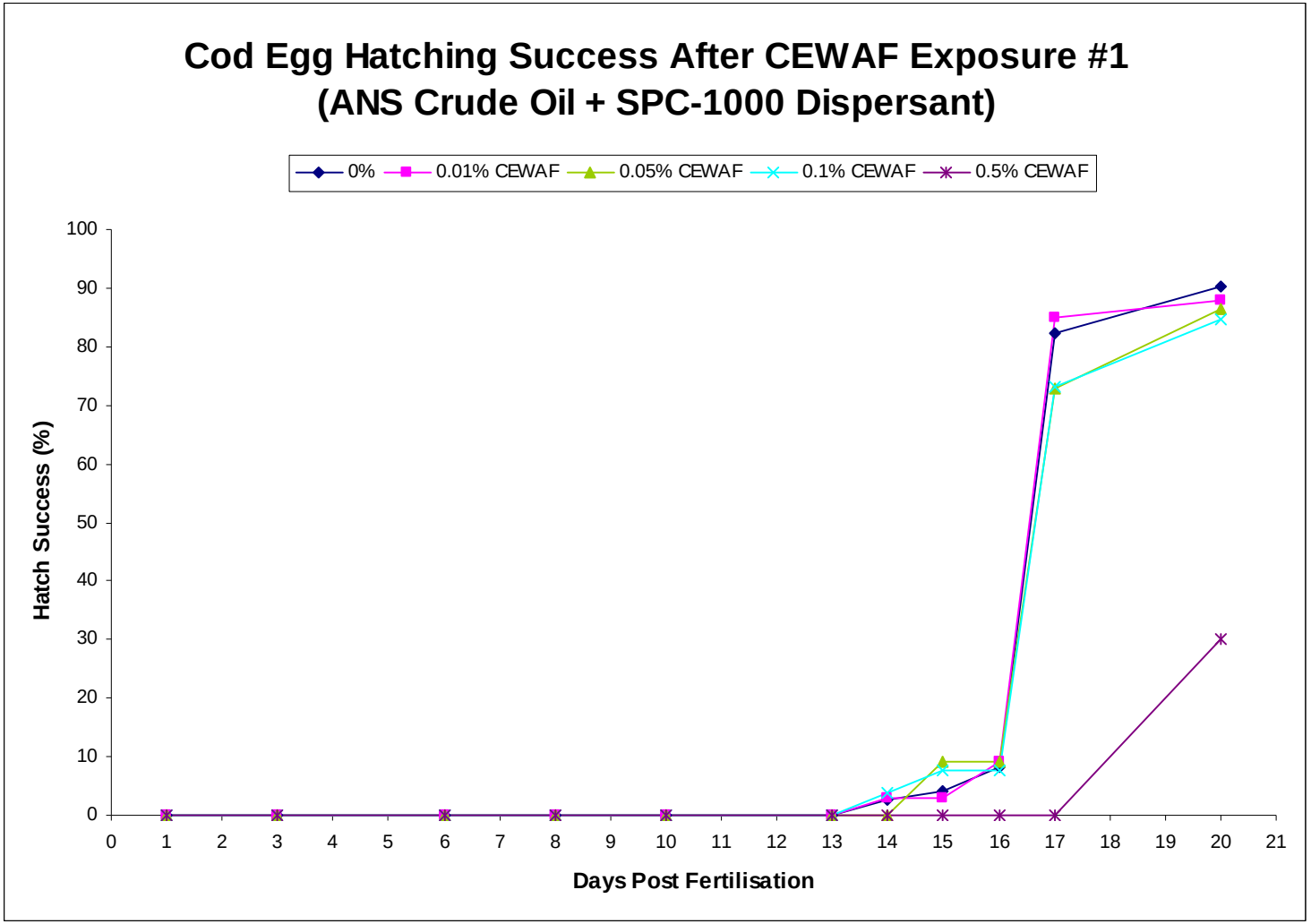


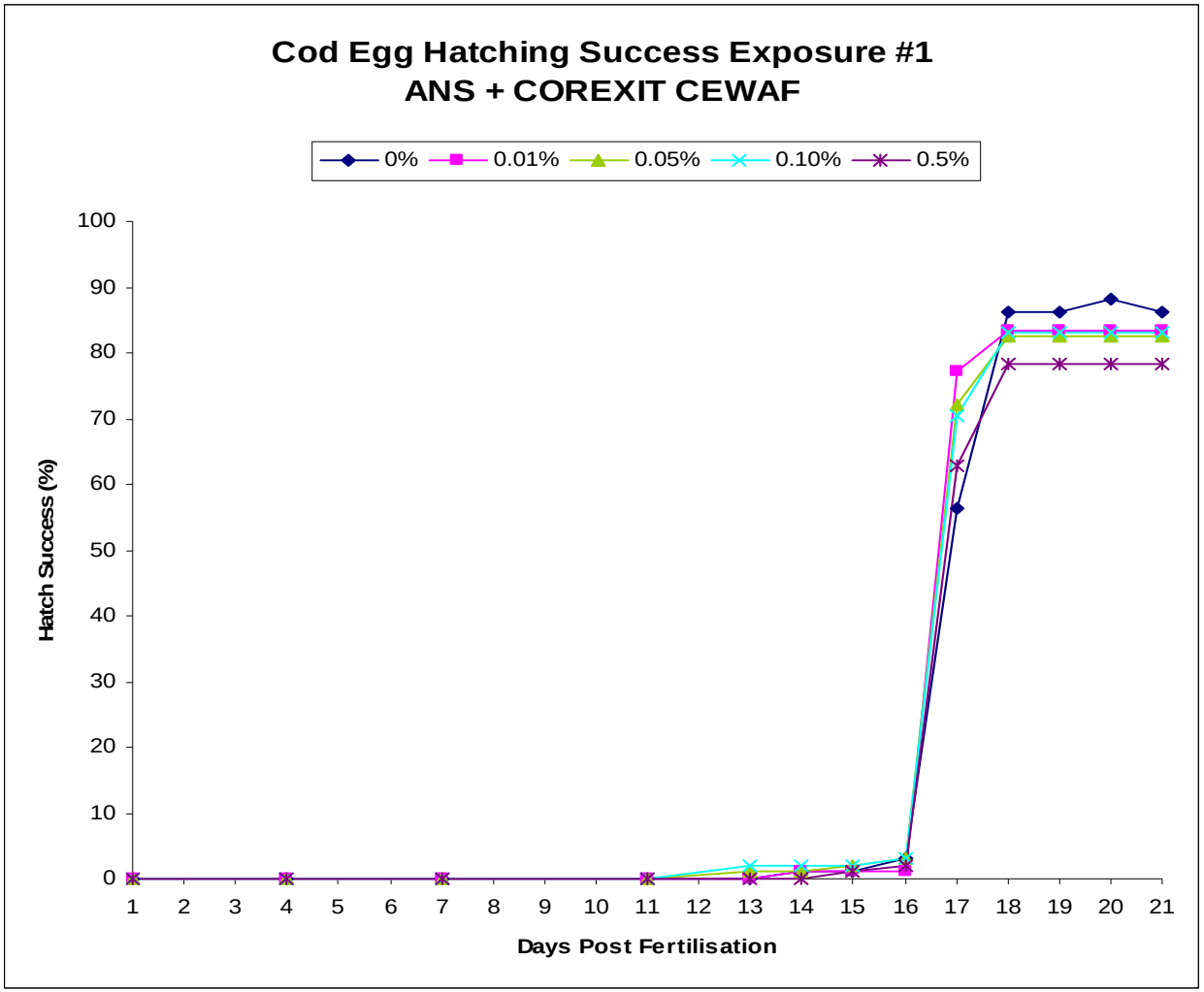

Cod Egg Fertilisation Rate in ANS WAF and CEWAF (Mean ± SE)
Normalised Against Sea Water Control
Sea Water Control
ANS WAF
ANS+COREXIT CEWAF
ANS+SPC-1000 CEWAF
Normalised Fertilistaion Rate (%) ± SE
125
100
75
50
25
0
0.001
0.01
0.1
1
10
100
Concentration (% v/v)

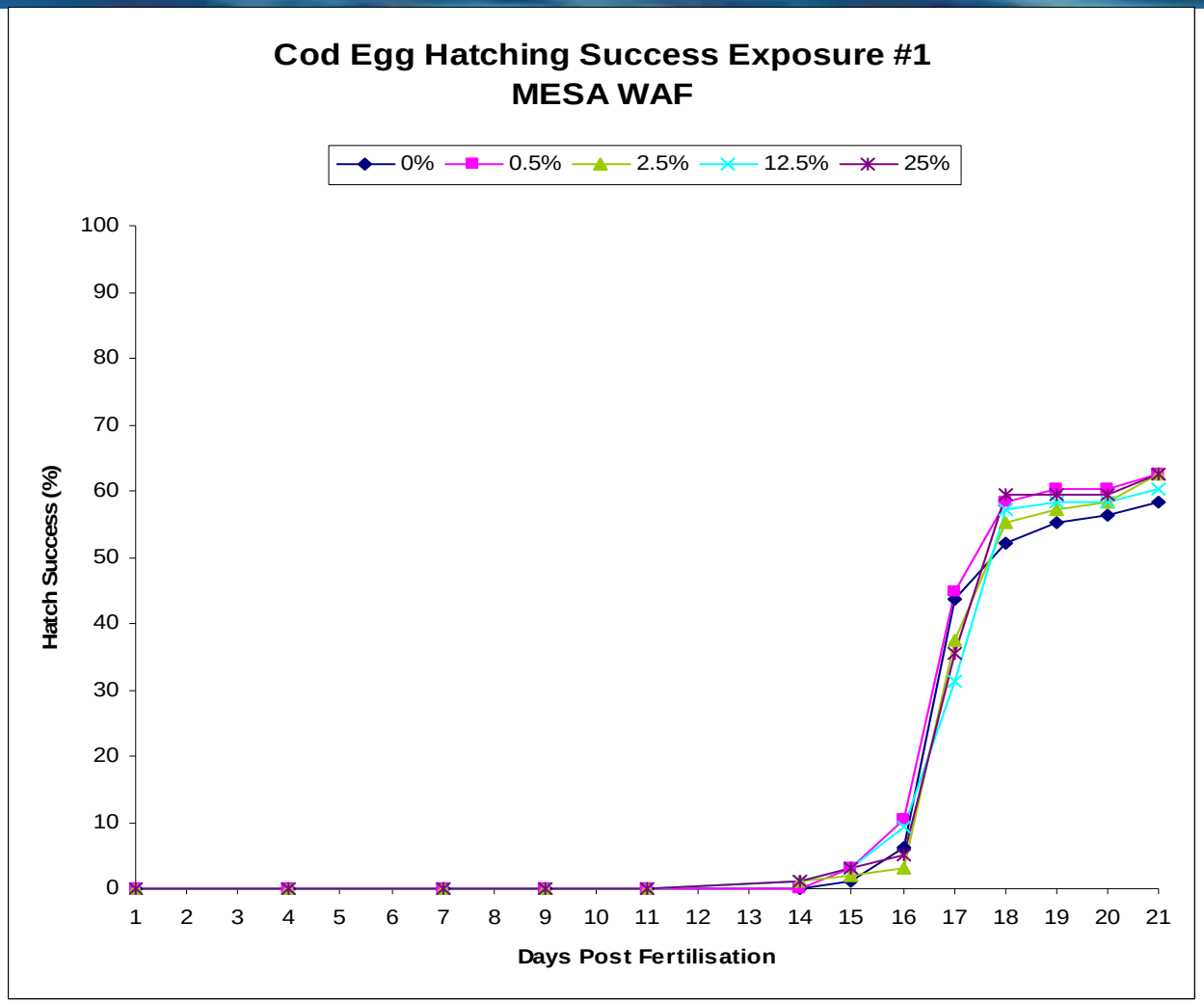
Cod Egg Fertilisation Rate in MESA WAF and CEWAF (Mean ± SE) Normalised Against Sea Water Control

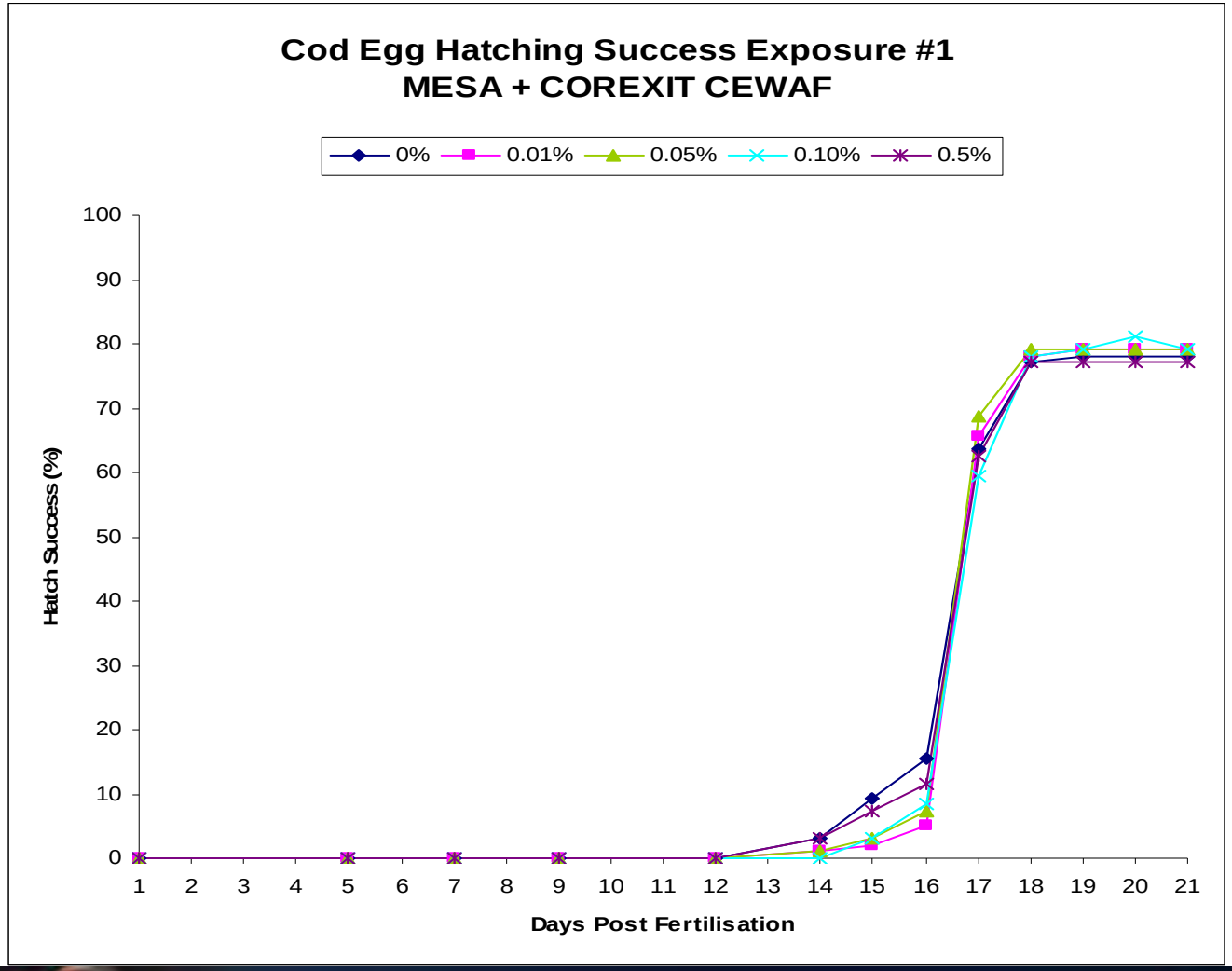


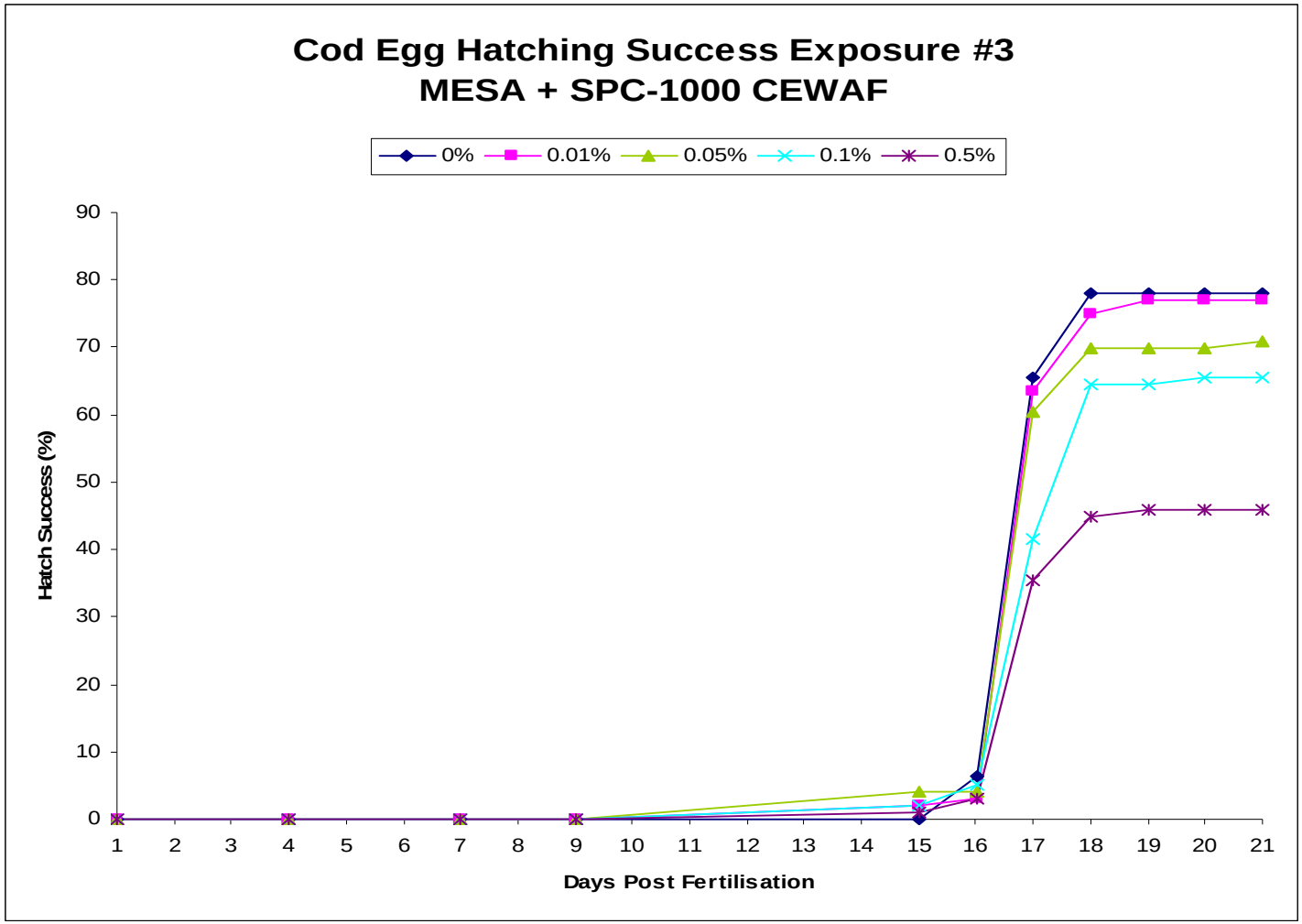

Cod Egg Hatching Success After WAF Exposure #1
(ANS Crude Oil)
0%
0.5% WAF
2.5% WAF
12.5% WAF
25% WAF
Hatch Success (%)
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days Post Fertilisation

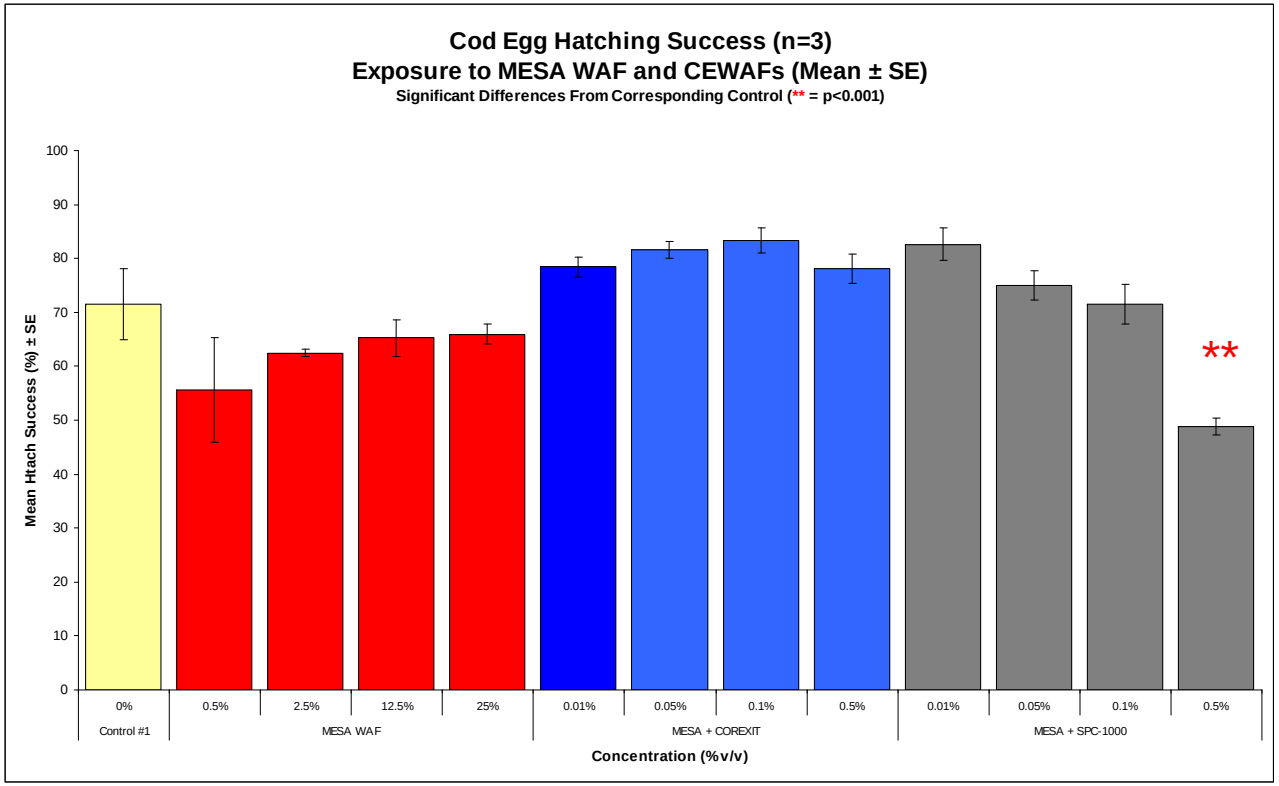

Cod Egg Hatching Success After CEWAF Exposure #1
(ANS Crude Oil + SPC-1000 Dispersant)
0%
0.01% CEWAF
0.05% CEWAF
0.1% CEWAF
0.5% CEWAF
Hatch Success (%)
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days Post Fertilisation

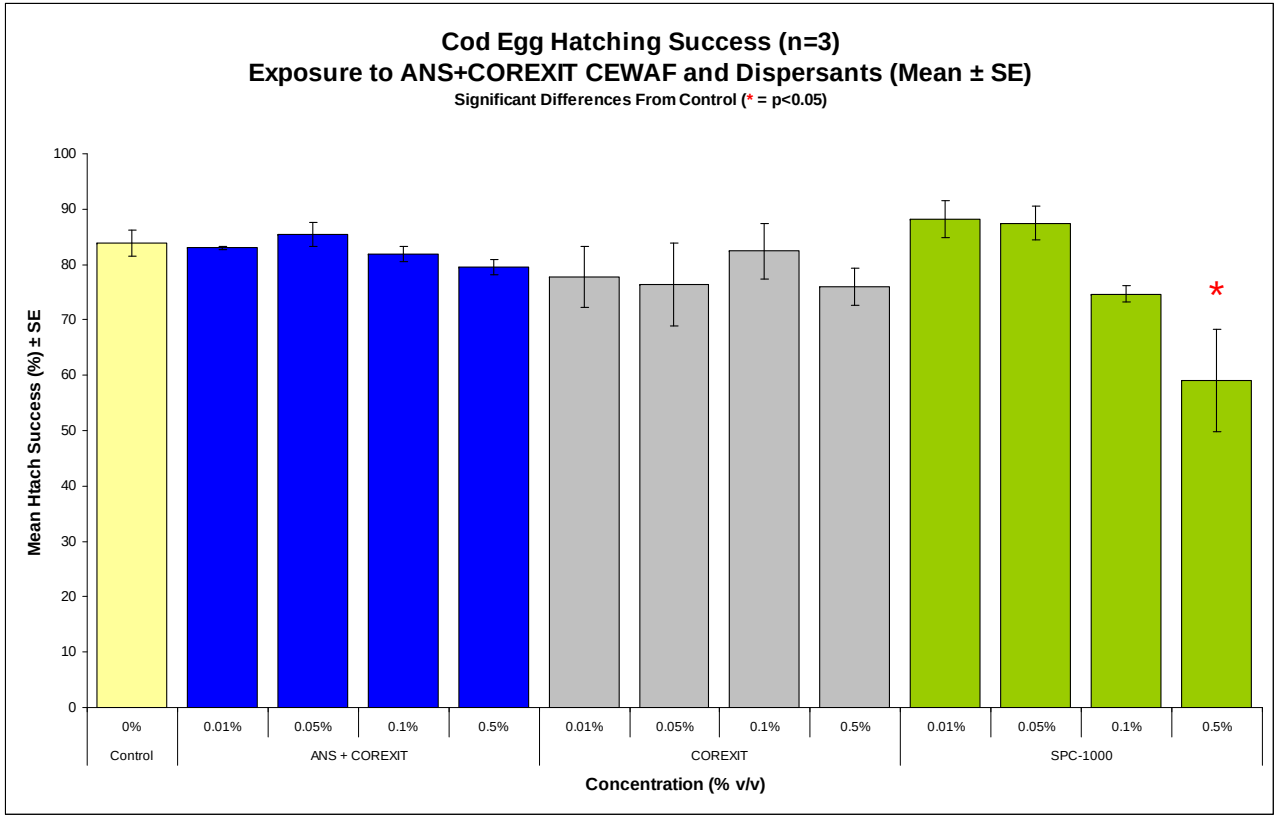

Cod Egg Hatching Success Exposure #1
ANS + COREXIT CEWAF
0%
0.01%
0.05%
0.10%
0.5%
Hatch Success (%)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
Days Post Fertilisation


Cod Egg Hatching Success Exposure #1
MESA WAF
0%
0.5%
2.5%
12.5%
25%
Hatch Success (%)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
Days Post Fertilisation


Cod Egg Hatching Success Exposure #1
MESA + COREXIT CEWAF
0%
0.01%
0.05%
0.10%
0.5%
Hatch Success (%)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
Days Post Fertilisation

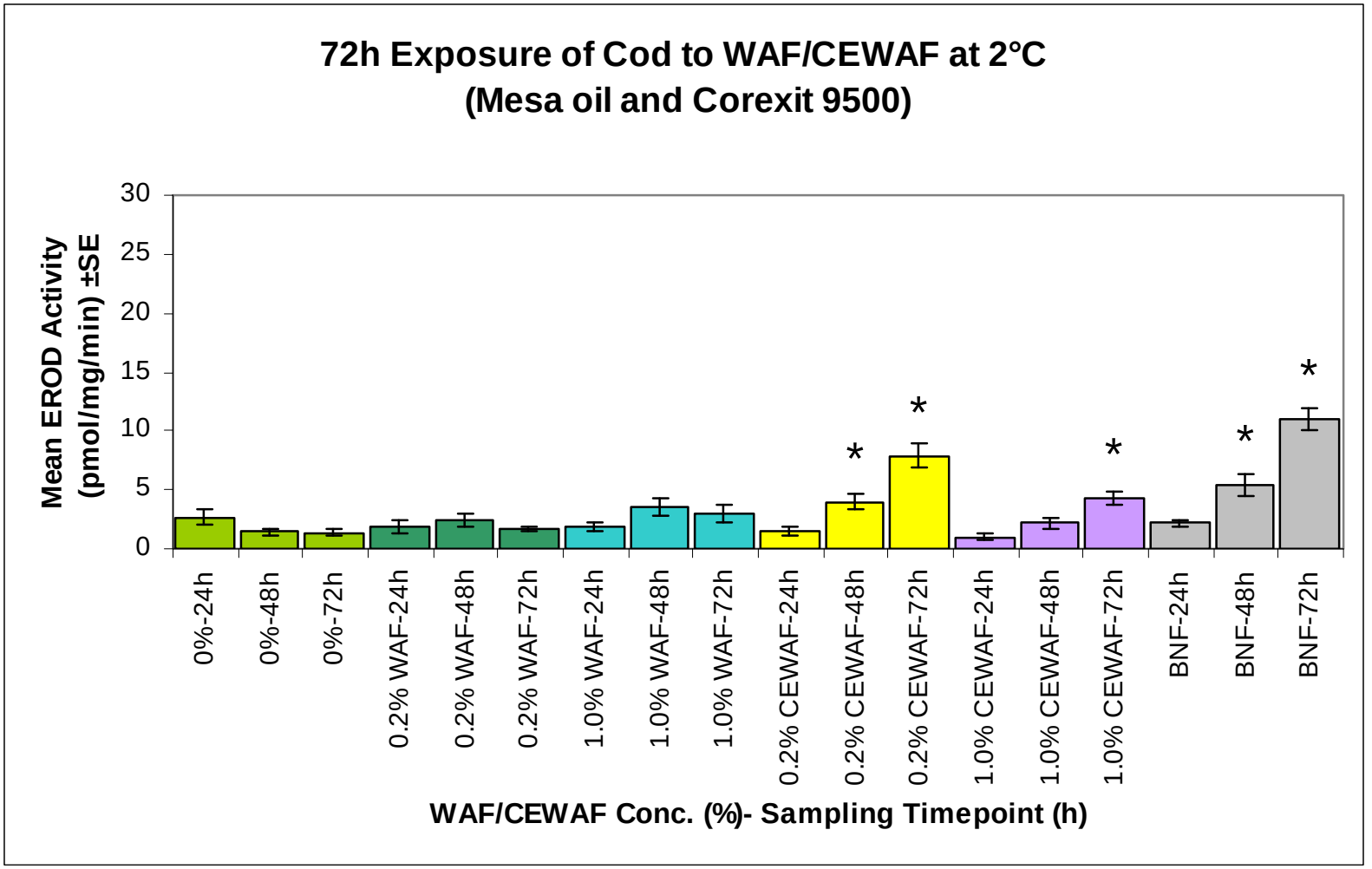

Cod Egg Hatching Success Exposure #3
MESA + SPC-1000 CEWAF
0%
0.01%
0.05%
0.1%
0.5%
Hatch Success (%)
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
Days Post Fertilisation

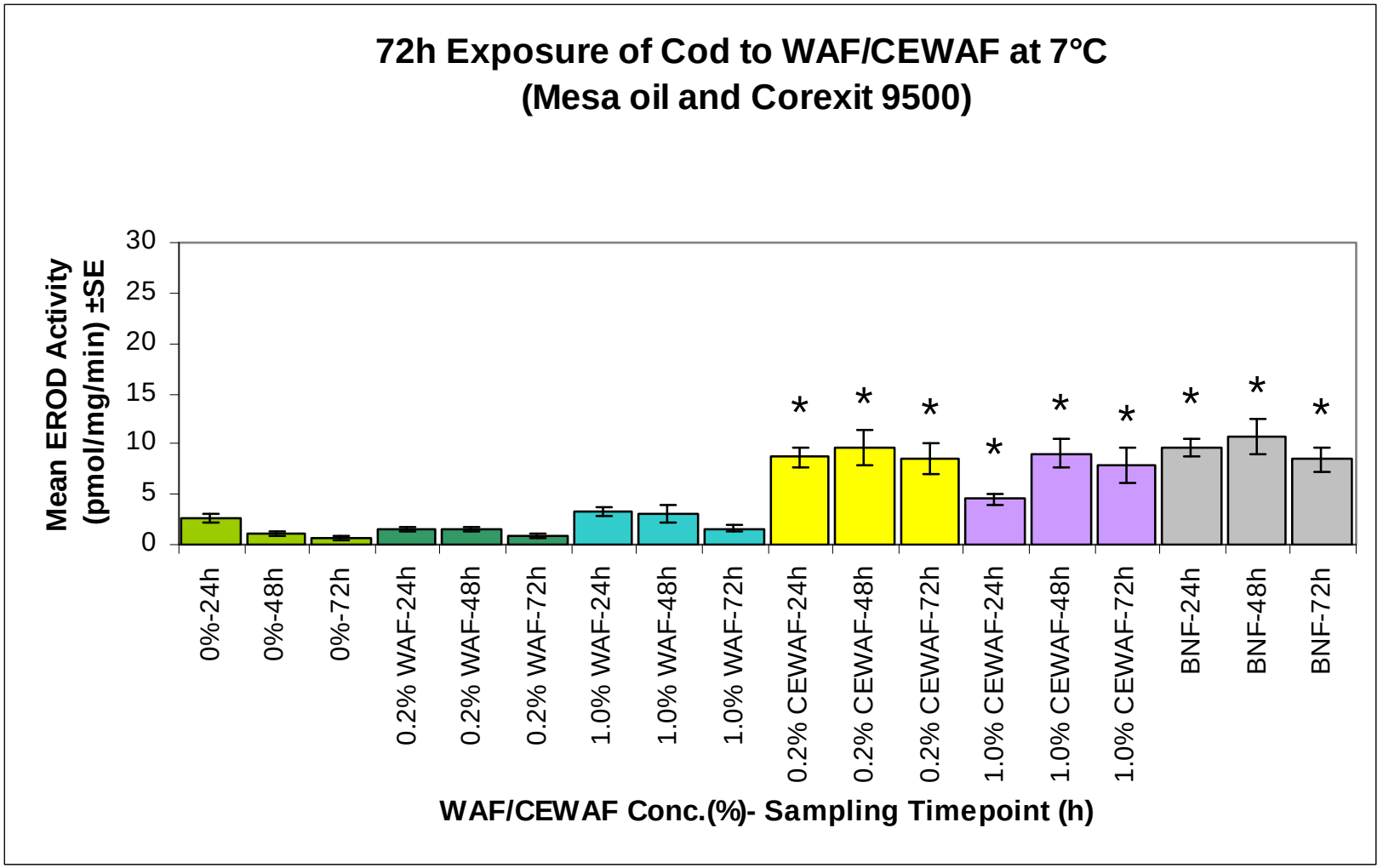

Cod Egg Hatching Success (n=3)
Exposure to MESA WAF and CEWAFs (Mean ± SE)
Significant Differences From Corresponding Control (** = p<0.001)
Mean Htach Success (%) ± SE
100
90
80
70
60
50
40
30
20
10
0
**
0%
0.5%
2.5%
12.5%
25%
0.01%
0.05%
0.1%
0.5%
0.01%
0.05%
0.1%
0.5%
Control #1
MESA WAF
MESA + COREXIT
MESA + SPC-1000
Concentration (%v/v)

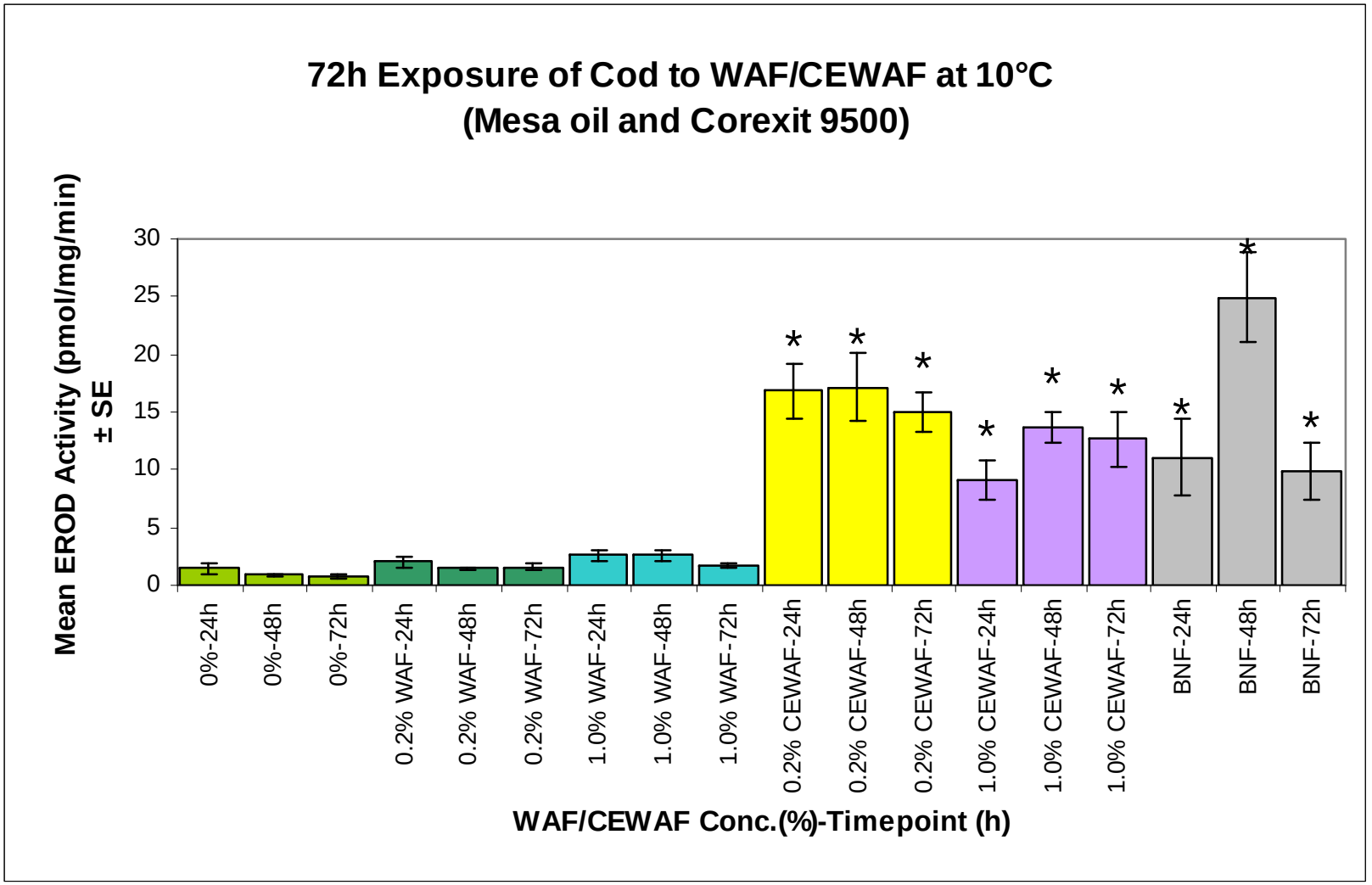

Cod Egg Hatching Success (n=3)
Exposure to ANS+COREXIT CEWAF and Dispersants (Mean ± SE)
Significant Differences From Control (* = p<0.05)
Mean Htach Success (%) ± SE
100
90
80
70
60
50
40
30
20
10
0
*
0%
0.01%
0.05%
0.1%
0.5%
0.01%
0.05%
0.1%
0.5%
0.01%
0.05%
0.1%
0.5%
Control
ANS + COREXIT
COREXIT
SPC-1000
Concentration (% v/v)

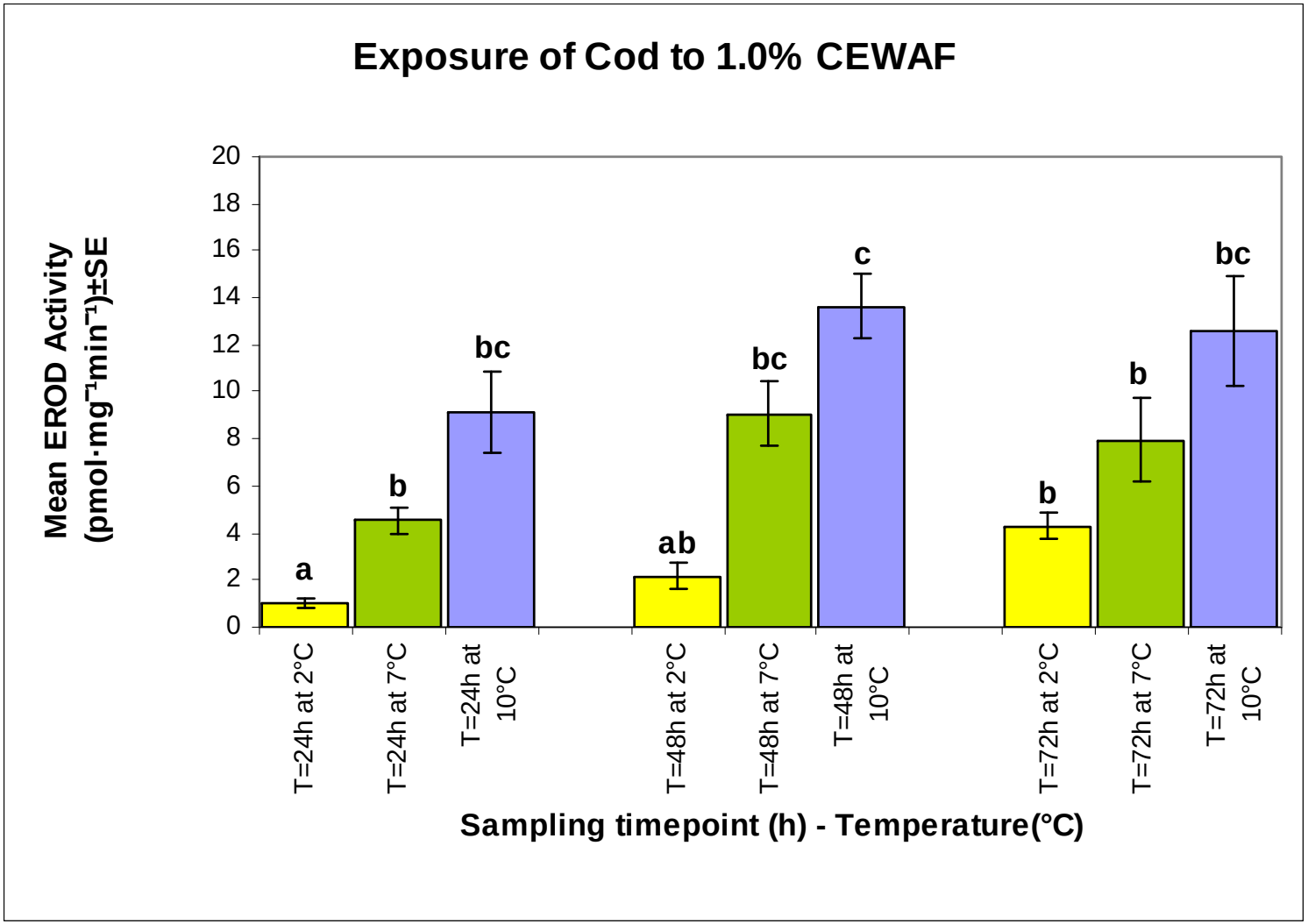
Conclusions

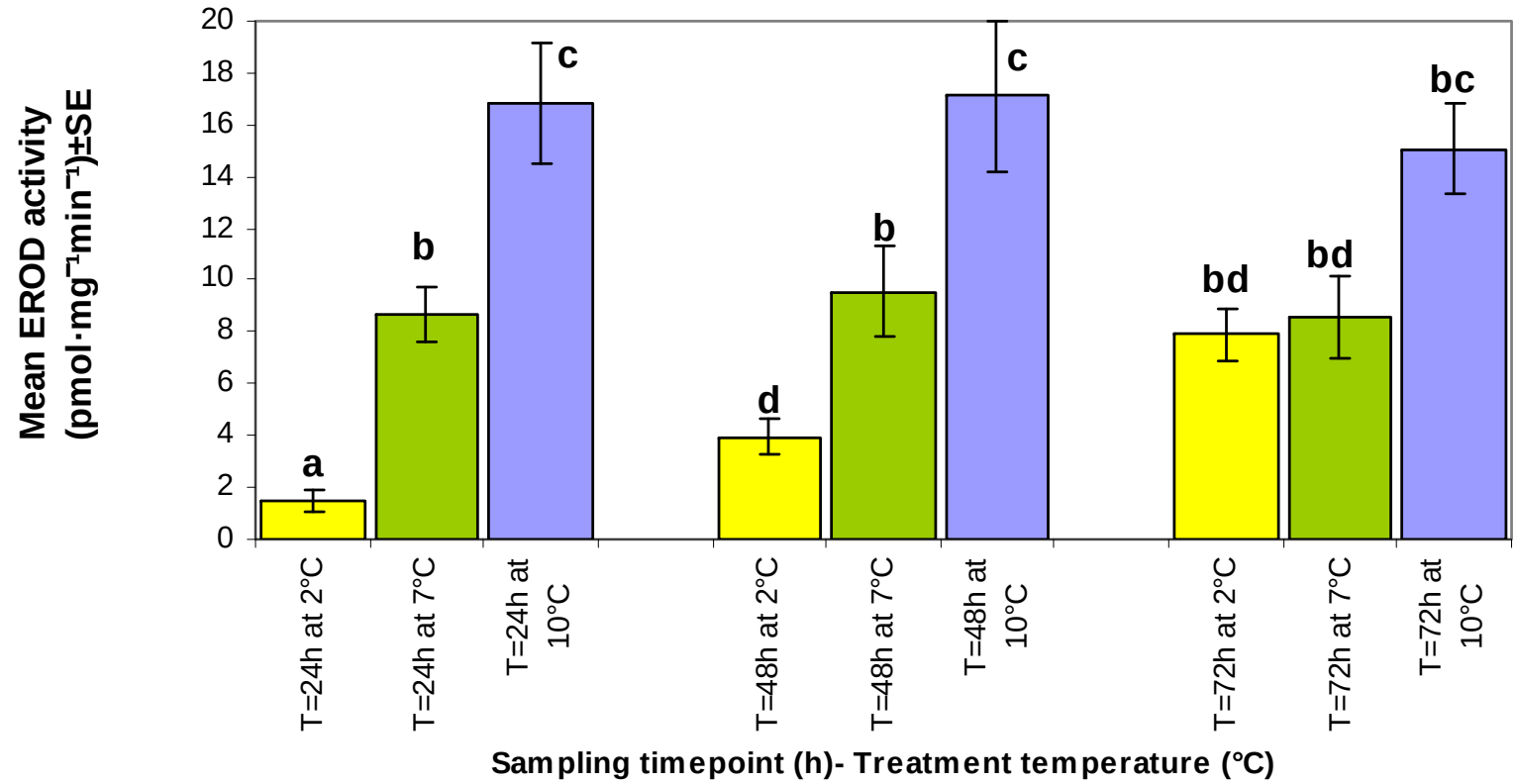
- Fertilisation in the presence of WAF only affected at percentage dilutions, therefore at (probably) unrealistically high concentrations.
- Fertilisation in the presence of CEWAF or dispersants affected at all concentrations tested. Not always negative (MESA/Corexit)
- If fertilisation is successful, hatching success unaffected except in the presence of SPC-1000.


72h Exposure of Cod to WAF/CEWAF at 2°C
(Mesa oil and Corexit 9500)
Mean EROD Activity
(pmol/mg/min) ±SE
30
25
20
15
10
5
0
0%-24h
0%-48h
0%-72h
0.2% WAF-24h
0.2% WAF-48h
0.2% WAF-72h
1.0% WAF-24h
1.0% WAF-48h
1.0% WAF-72h
0.2% CEWAF-24h
0.2% CEWAF-48h
0.2% CEWAF-72h
1.0% CEWAF-24h
1.0% CEWAF-48h
1.0% CEWAF-72h
BNF-24h
BNF-48h
BNF-72h
*
WAF/CEWAF Conc. (%)- Sampling Timepoint (h)


72h Exposure of Cod to WAF/CEWAF at 7°C
(Mesa oil and Corexit 9500)
Mean EROD Activity
(pmol/mg/min) ±SE
30
25
20
15
10
5
0
0%-24h
0%-48h
0%-72h
0.2% WAF-24h
0.2% WAF-48h
0.2% WAF-72h
1.0% WAF-24h
1.0% WAF-48h
1.0% WAF-72h
0.2% CEWAF-24h
0.2% CEWAF-48h
0.2% CEWAF-72h
1.0% CEWAF-24h
1.0% CEWAF-48h
1.0% CEWAF-72h
BNF-24h
BNF-48h
BNF-72h
WAF/CEWAF Conc.(%)- Sampling Timepoint (h)


72h Exposure of Cod to WAF/CEWAF at 10°C
(Mesa oil and Corexit 9500)
Mean EROD Activity (pmol/mg/min)
± SE
30
25
20
15
10
5
0
0%-24h
0%-48h
0%-72h
0.2% WAF-24h
0.2% WAF-48h
0.2% WAF-72h
1.0% WAF-24h
1.0% WAF-48h
1.0% WAF-72h
0.2% CEWAF-24h
0.2% CEWAF-48h
0.2% CEWAF-72h
1.0% CEWAF-24h
1.0% CEWAF-48h
1.0% CEWAF-72h
BNF-24h
BNF-48h
BNF-72h
WAF/CEWAF Conc.(%)-Timepoint (h)


Exposure of Cod to 1.0% CEWAF
Mean EROD Activity (pmol·mg⁻¹min⁻¹)±SE
20
18
16
14
12
10
8
6
4
2
0
a
b
bc
ab
bc
c
b
b
bc
T=24h at 2°C
T=24h at 7°C
T=24h at 10°C
T=48h at 2°C
T=48h at 7°C
T=48h at 10°C
T=72h at 2°C
T=72h at 7°C
T=72h at 10°C
Sampling timepoint (h) - Temperature(°C)


Exposure of Cod to 0.2% CEWAF
Mean EROD activity (pmol·mg⁻¹min⁻¹)±SE
20
18
16
14
12
10
8
6
4
2
0
a
b
c
d
b
c
bd
bd
bc
T=24h at 2°C
T=24h at 7°C
T=24h at 10°C
T=48h at 2°C
T=48h at 7°C
T=48h at 10°C
T=72h at 2°C
T=72h at 7°C
T=72h at 10°C
Sampling timepoint (h)- Treatment temperature (°C)

What is this telling us?

- WAF and CEWAF are getting into the water regardless of temperature, i.e. reduced EROD is not a result of reduced exposure.
- Fish “take up” WAF and CEWAF in a temperature-dependent manner, i.e. despite solution renewal there is no WAF or CEWAF in water at higher temperatures
- It is likely the metabolic rate of the fish that matters. Although dispersion, droplet size and bioavailabilty must also be considered.

What is this telling us? (cont'd)

- WAF is not causing an induction of EROD activity at any temperature. This could be related to mixing
- Results confirm intuitive thinking: Temperature affects the response of cod to WAF and CEWAF. Why needs to be confirmed.

Thank You

- Ken MacKeigan, Jessica Whitehead at SABS
- Funded by PWSRCAC, PERD, ESRF