Effects of substrate size and cleaning regime on growth and survival of captive-bred juvenile freshwater pearl mussels, *Margaritifera margaritifera* (Linnaeus, 1758)

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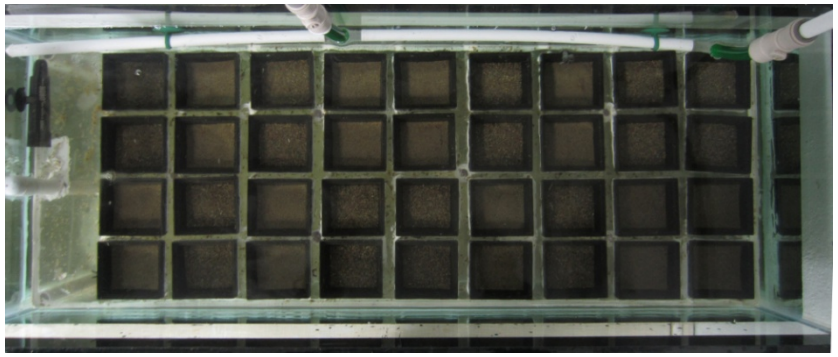
What we'll cover

- Experiment 1 – Effect of substrate size and cleaning regime on survival and size
- Experiment 2 – Investigating environmental factors affecting survival and size
  - Flow rate
  - Interstitial space
  - Dissolved oxygen
  - Ammonia concentration

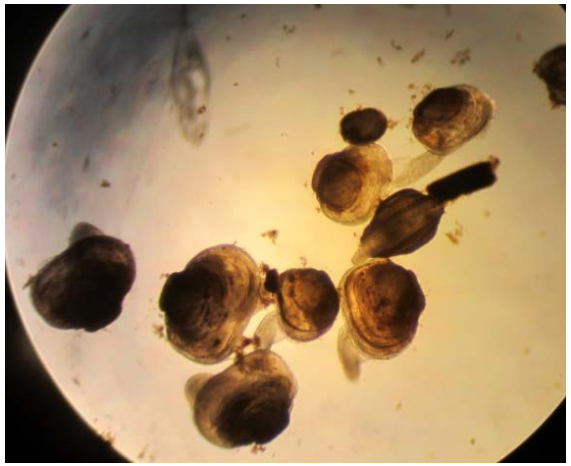
Initial experiment – Exp 1

- In 2012, 3600 juveniles (9 replicates) divided into 4 treatments:
  - 0.25-1 mm cleaned weekly
  - 0.25-1 mm cleaned monthly
  - 1-2 mm cleaned weekly
  - 1-2 mm cleaned monthly



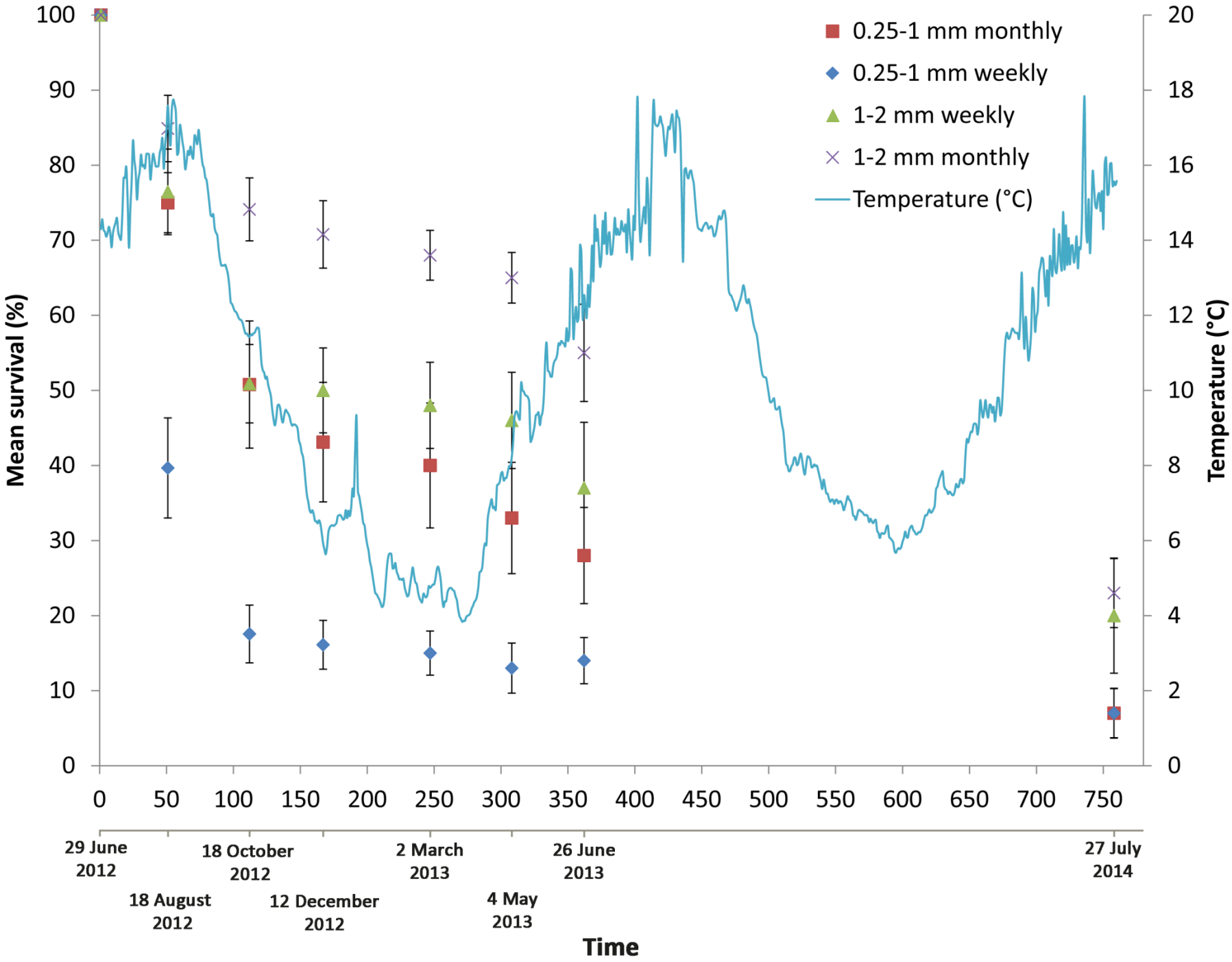
Exp 1 - Method

- Cleaning regime maintained for 25 months
- Substrate emptied into a glass dish, elutriated and poured through a 0.18 mm sieve to catch juveniles
- Sampled every 2 months for growth and survival during first 12 months, then final check at 25 months



Exp 1 - Results

- Survival

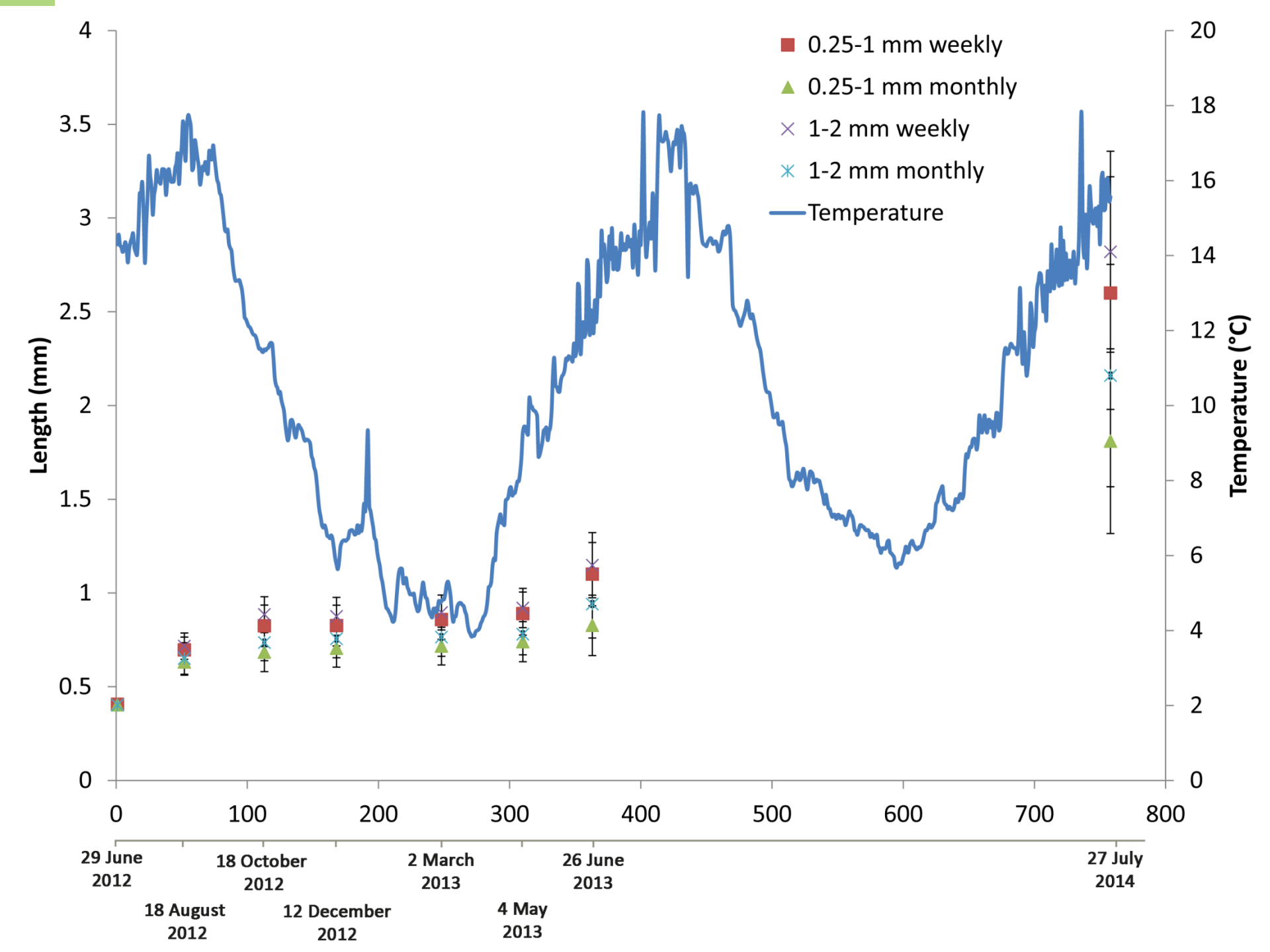

0.25-1 mm monthly
0.25-1 mm weekly
1-2 mm weekly
1-2 mm monthly
Temperature (°C)
Mean survival (%)
Temperature (°C)
Time
0
50
100
150
200
250
300
350
400
450
500
550
600
650
700
750
29 June 2012
18 August 2012
18 October 2012
12 December 2012
2 March 2013
4 May 2013
26 June 2013
27 July 2014

Exp 1 - Results

- Survival
  - Significantly ($P < 0.001$) different on all sampling occasions
  - On day 362 all treatments were significantly different from each other but by day 758 two groupings became obvious, larger and smaller substrate
  - 1-2 mm monthly > 1-2 mm weekly > 0.25-1 mm monthly > 0.25-1 mm weekly
  - Mortality slowed over winter but did not stop completely

Exp 1 - Results

- Size


0.25-1 mm weekly
0.25-1 mm monthly
1-2 mm weekly
1-2 mm monthly
Temperature
Length (mm)
Temperature (°C)
0
0.5
1
1.5
2
2.5
3
3.5
4
0
2
4
6
8
10
12
14
16
18
20
0
100
200
300
400
500
600
700
800
29 June 2012
18 August 2012
18 October 2012
12 December 2012
2 March 2013
4 May 2013
26 June 2013
27 July 2014

Exp 1 - Results

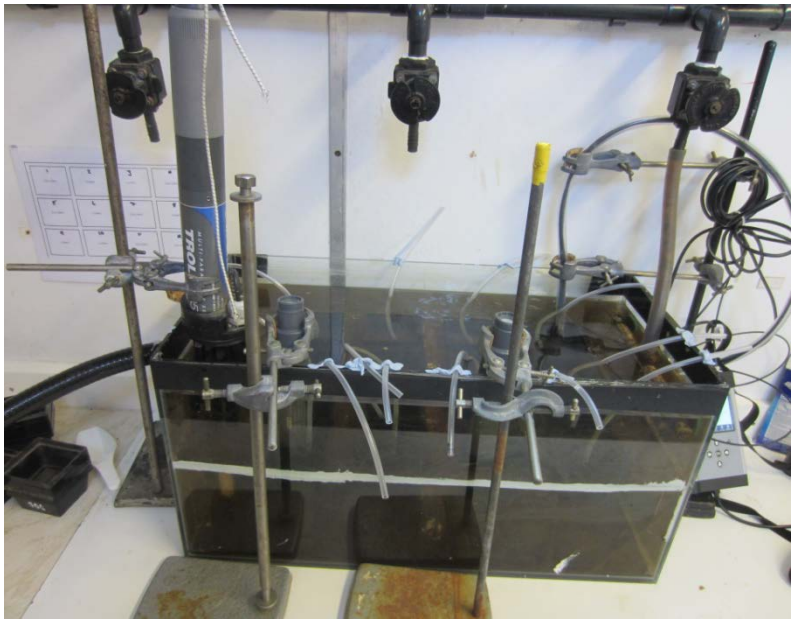
- Size
  - Significantly different ($P < 0.001$) on all sampling occasions
  - Weekly treatments were statistically the same on day 362 ($P = 0.058$) but all treatments were significantly different by day 758 ($P < 0.001$) and size ranges increase
  - 1-2 mm weekly > 0.25-1 mm weekly > 1-2 mm monthly > 0.25-1 mm monthly

Exp 1 – Conclusions

- Higher survival in larger substrates
- Higher growth in substrates cleaned weekly
- Near cessation of growth < 10 °C concurring with previous findings e.g. Ziuganov *et al.*, 1994; Buddensiek, 1995; Hruška, 1999
- No size-dependent over-winter survival observed in any treatment
- *…but why these differences???*

Additional experiment – Exp 2

- Investigating factors which may affect growth and survival:
  - Flow rate through substrate & interstitial space
  - Dissolved oxygen
  - Ammonia concentration at 4 weeks



Exp 2 - Methods

- Same experimental set up but only 3 replicates
- Took place over 8 weeks starting 21/07/2015

1. Flow rate:
   - Measured time to clear 1L of water
   - Repeated after substrate had been cleaned
2. Interstitial space:
   - Emptied substrate into measuring cylinder and topped up with water until meniscus touched top of substrate

Exp 2 - Methods

3. DO – logged every 15 minutes over weeks 5-8
   - **Water column** - multi-parameter sonde
   - **Monthly treatments** – Onset DO loggers
   - **Weekly treatments** – PreSens DO dipping probe
4. Ammonia concentration of interstitial water taken after 4 weeks by siphoning 2 ml out of sieves using plastic tubing (spectrophotometer)

Exp 2 - Results

- Survival (%)

Treatment	2012	2015
0.25-1 mm weekly	40 ±7	72 ±2
0.25-1 mm monthly	75 ±4	68 ±4
1-2 mm weekly	76 ±6	80 ±4
1-2 mm monthly	85 ±4	81 ±8

Exp 2 - Results

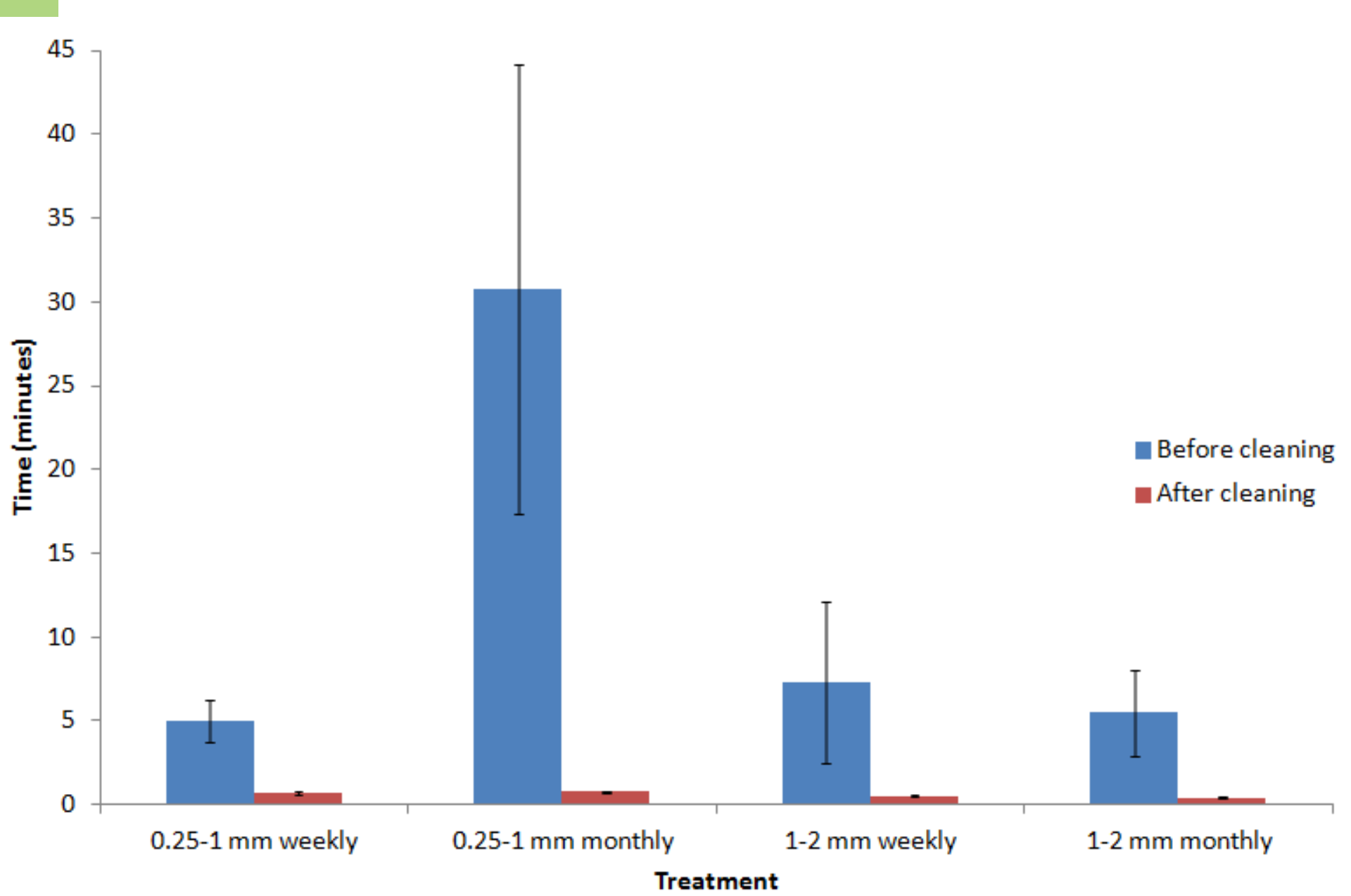
- Juvenile length (mm) was not significantly different in 2015 (P = 0.53)

Treatment	2012	2015
0.25-1 mm weekly	0.72 ±0.07	0.82 ±0.10
0.25-1 mm monthly	0.70 ±0.07	0.83 ±0.08
1-2 mm weekly	0.65 ±0.08	0.84 ±0.10
1-2 mm monthly	0.63 ±0.07	0.81 ±0.09

- More analysis required on size differences & relationship to temperature

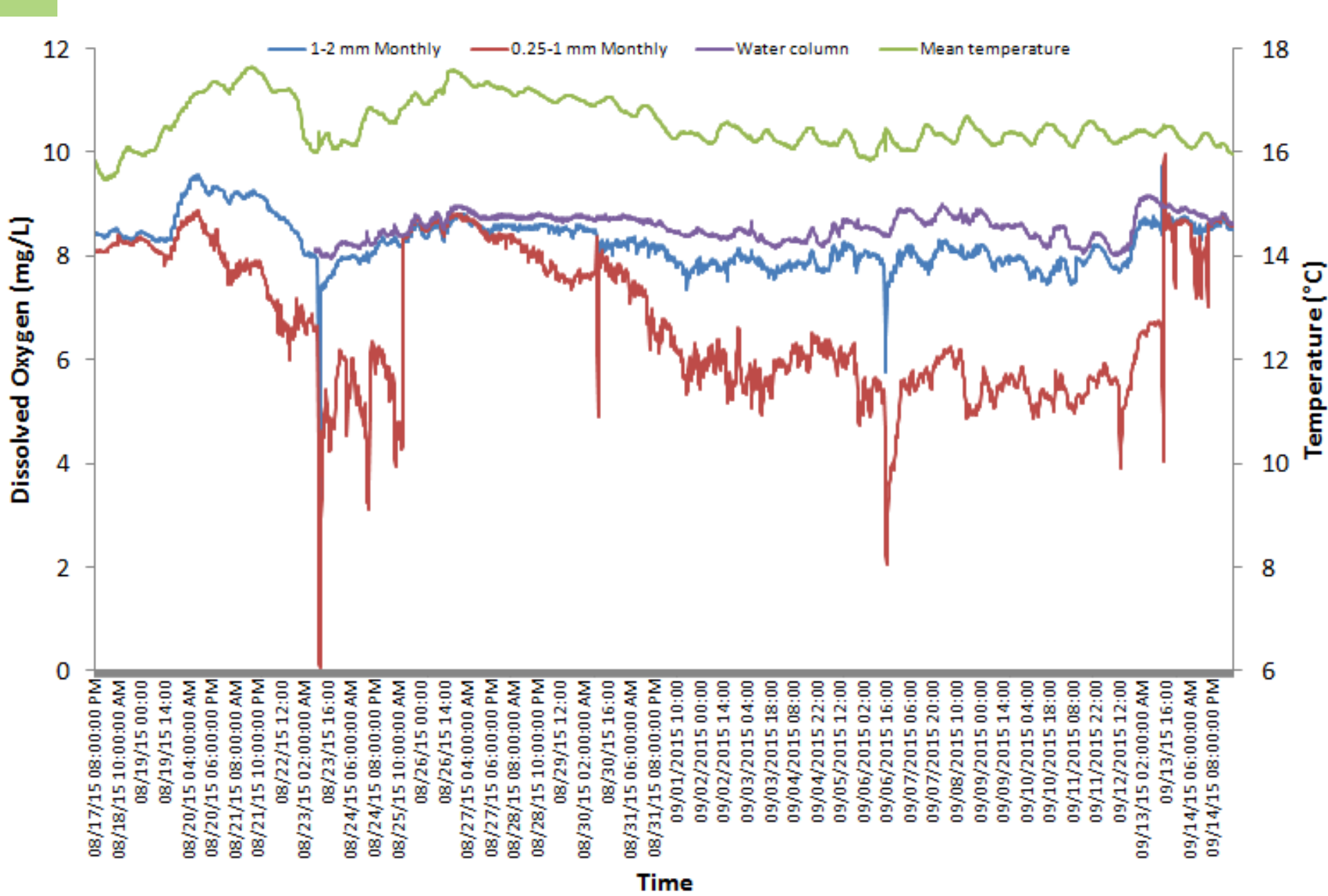
Exp 2 - Results

1. Flow rate:
   - Pre-cleaning, flow through 0.25-1 mm monthly treatment was significantly slower than all other treatments ($F_{(3,8)} = 8.83$, $P = 0.006$)
   - Flow through cleaned substrates was significantly higher ($F_{(3,8)} = 18.80$, $P = 0.001$) in the 1-2 mm compared to the 0.25-1 mm substrates
2. Interstitial space:
   - 0.25-1 mm substrates had significantly less ($t_{(10)} = -4.72$, $P = 0.001$) interstitial space (2.6 ±0.61 ml) compared with 1-2 mm substrates (4.3 ±0.25 ml)


45
40
35
30
25
20
15
10
5
0
Time (minutes)
Before cleaning
After cleaning
0.25-1 mm weekly
0.25-1 mm monthly
1-2 mm weekly
1-2 mm monthly
Treatment

Exp 2 – Results

3. Dissolved Oxygen


1-2 mm Monthly
0.25-1 mm Monthly
Water column
Mean temperature
Dissolved Oxygen (mg/L)
Temperature (°C)
Time
0
2
4
6
8
10
12
6
8
10
12
14
16
18
08/17/15 08:00:00 PM
08/18/15 10:00:00 AM
08/19/15 00:00
08/19/15 14:00
08/20/15 04:00:00 AM
08/20/15 06:00:00 PM
08/21/15 08:00:00 AM
08/21/15 10:00:00 PM
08/22/15 12:00
08/23/15 02:00:00 AM
08/23/15 16:00
08/24/15 06:00:00 AM
08/24/15 08:00:00 PM
08/25/15 10:00:00 AM
08/26/15 00:00
08/26/15 14:00
08/27/15 04:00:00 AM
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08/30/15 16:00
08/31/15 06:00:00 AM
08/31/15 08:00:00 PM
09/01/2015 10:00
09/02/2015 00:00
09/02/2015 14:00
09/03/2015 04:00
09/03/2015 18:00
09/04/2015 08:00
09/04/2015 22:00
09/05/2015 12:00
09/06/2015 02:00
09/06/2015 16:00
09/07/2015 06:00
09/07/2015 20:00
09/08/2015 10:00
09/09/2015 00:00
09/09/2015 14:00
09/10/2015 04:00
09/10/2015 18:00
09/11/2015 08:00
09/11/2015 22:00
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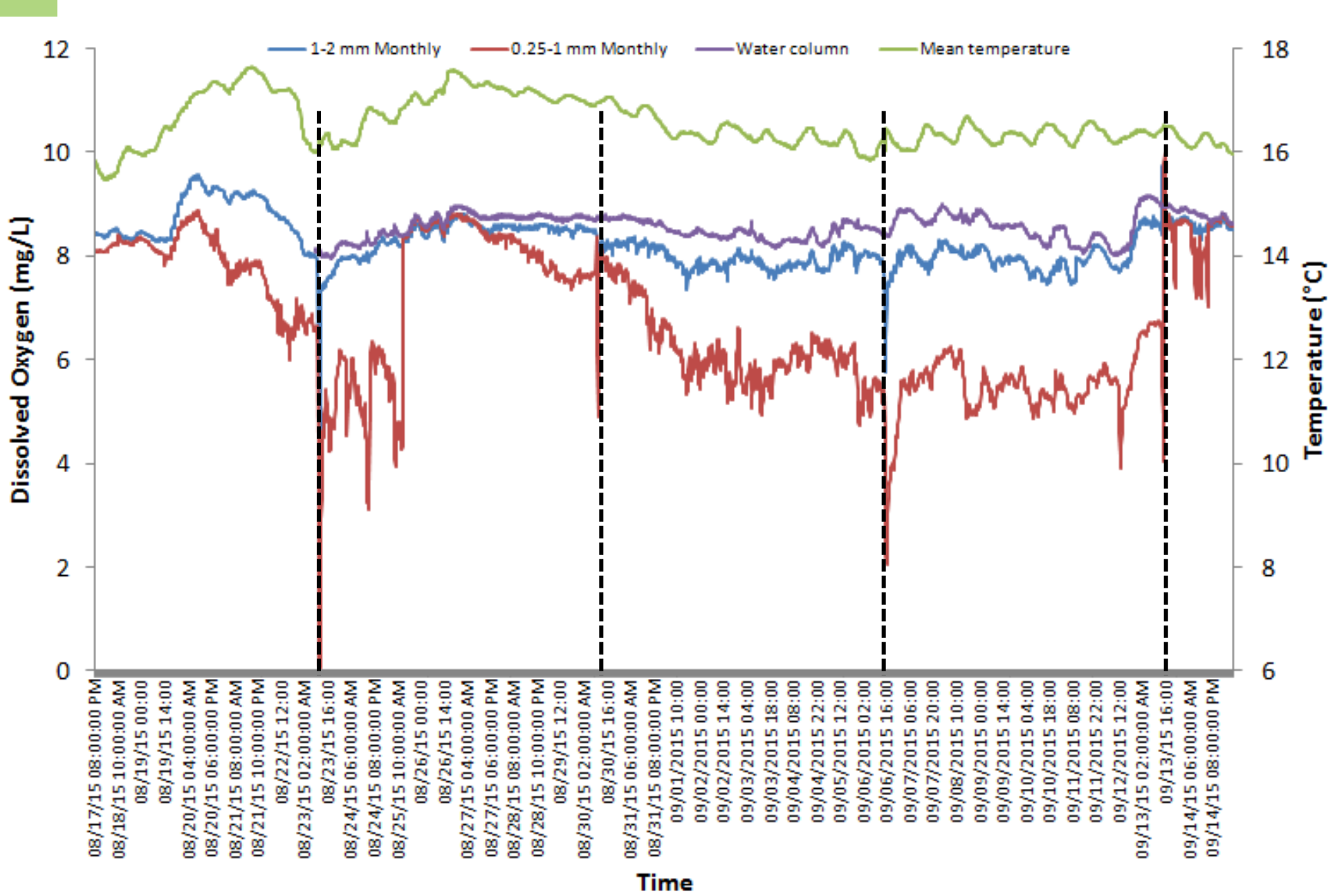
1-2 mm Monthly
0.25-1 mm Monthly
Water column
Mean temperature
Dissolved Oxygen (mg/L)
Temperature (°C)
Time
0
2
4
6
8
10
12
6
8
10
12
14
16
18
08/17/15 08:00:00 PM
08/18/15 10:00:00 AM
08/19/15 00:00
08/19/15 14:00
08/20/15 04:00:00 AM
08/20/15 06:00:00 PM
08/21/15 08:00:00 AM
08/21/15 10:00:00 PM
08/22/15 12:00
08/23/15 02:00:00 AM
08/23/15 16:00
08/24/15 06:00:00 AM
08/24/15 08:00:00 PM
08/25/15 10:00:00 AM
08/26/15 00:00
08/26/15 14:00
08/27/15 04:00:00 AM
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08/28/15 10:00:00 PM
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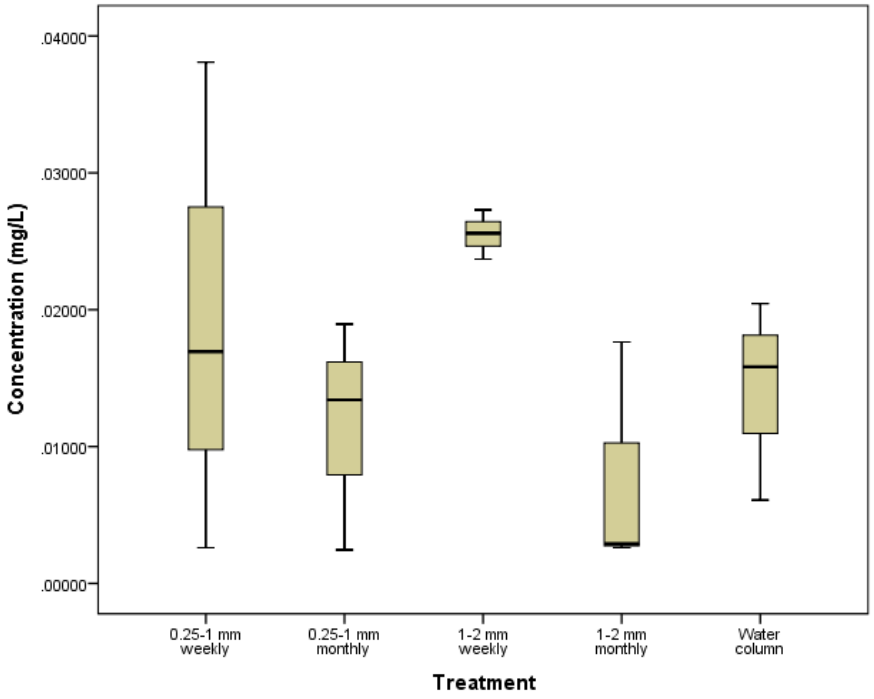
Exp 2 - Results

- Ammonia concentrations were the same between the four treatments ($F_{(4, 14)}$=1.38; *P*=0.307)



Conclusions

- Larger substrate provides better survival rates
- Growth patterns were different between exp 1 & 2
- DO and flow are significantly affected by substrate size and cleaning regime
- Further analysis of DO data will help explain this further

References & acknowledgements

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Hruška, J. (1992) The freshwater pearl mussel in South Bohemia: Evaluation of the effect of temperature on reproduction, growth and age structure of the population. *Archiv für Hydrobiologie* **126:** 181-191.

Ziuganov, V., Zontin, A., Nezlin, L. and Tretiakov, V. (1994) *The freshwater pearl mussels and their relationships with salmonid fish*. VNIRO Publishing House, Moscow.

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