Are oiled rock column (ORC) experiments confounded by particulate oil?

Carls et al. NOAA/NMFS Auke Bay Laboratories

Brannon et al. (2006) published a paper in Environmental Toxicology and Chemistry that repeated an embryo toxicity study (Heintz et al. 1999) where we concluded that dissolved polynuclear aromatic hydrocarbons (PAHs) from oil are toxic at concentrations between 1 and 18 µg/L (parts per billion). In contrast, they argue that toxicity is an artifact caused by contact with PAH-laden oil microdroplets instead of dissolved PAHs and that toxic levels are many orders of magnitude higher. We conclude that flawed analysis logic and the lack of any direct evidence of oil droplets in water and eggs led Brannon et al. (2006) to erroneous conclusions (Heintz et al. 2008; Carls et al. 2008a). In their rebuttal, Brannon et al. (2008) reanalyzed a small portion of our data for the presence of oil droplets using phytane as a surrogate indicator, again reaching the scientifically indefensible conclusion that microdroplets explain embryo toxicity. Brannon et al. have presented no evidence of microdroplets as an agent of embryo mortality, whereas we have done so for PAHs (Marty et al. 1997; Heintz et al. 1999, 2000; Carls et al. 1999, 2005) and we have definitively eliminated microdroplets as directly toxic (Carls et al. 2008b). This web document provides the data in question so readers can evaluate the validities of conflicting conclusions based on identical observations

Yes: ORC experiments are confounded by particulate oil

(Brannon et al. 2006)

- The presence of phytane in water is evidence of oil microdroplets.
- Of the 31 exposure water samples, 29 contained phytane above its method detection limit.
- Hence oil droplets were present in exposure water.
- Embryos were exposed to both dissolved and droplet-associated PAH in effluent water
- Consequently, the conclusion that dissolved PAH concentrations as low as 1 ppb were toxic is incorrect
- Because oil droplets were present in exposure water, embryos received a higher dose of high molecular weight PAH than predicted by dissolved PAH.

No: ORC experiments are not confounded by particulate oil

(Carls et al. 2008a)

- The presence of phytane in water is evidence of oil microdroplets.
- Phytane was not detected in 75% of 32 water samples and analytic methods provide no certainty that the remaining 25% contained phytane, despite the non-zero concentrations estimated for them. In the 8 samples where the presence of phytane could not be discounted, estimated concentrations were small fractions of the concentration of the smallest standard (1.6 to 8.2%, mean 3.6%), precluding reliable quantification. Analytic methods for phytane can produce false positives because standard curves are not forced through zero and false positives cannot be eliminated procedurally because phytane is so insoluble that we have been unable to establish method detection limits for it in water.
- Aqueous phytane concentrations were unrelated to oil treatment, hence particulate oil was either absent or negligible in exposure water.
- Embryo exposure to particulate oil was negligible (*see data*). Phytane was above method detection limits in eggs in 1 intermediate dose at 1 intermediate time.
- The absence of phytane in water and eggs and changes in PAH composition from whole oil to water and eggs in multiple experiments (Marty et al. 1997; Heintz et al. 1999, 2000; Carls et al. 1999, 2005) consistently identify dissolved PAHs as the source of embryo toxicity, not particulate oil.
- Higher molecular weight PAH were never observed in eggs, eliminating whole oil as the source of contamination. **All hydrocarbons present in water samples were measured, regardless of phase (solid or dissolved)** in their experiment and ours. Acceptance or rejection of the particulate oil hypothesis has no influence on measured concentration.
- Damage to embryos in effluent water (without oil contact) was the same as damage to embryos in contact with whole oil (Heintz et al. 1999; Brannon et al. 2006). This indicates direct contact is unimportant, also demonstrated with zebrafish embryos exposed to or isolated from oil droplets (Carls et al. 2008b).

Data

(click to view)

Concluding remarks

Are oiled rock column (ORC) experiments confounded by particulate oil?

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Brannon et al. (2006) published a paper in Environmental Toxicology and Chem… embryo toxicity study (Heintz et al. 1999) where we concluded that dissolved polynuclear aromatic hydrocarbons (PAHs) from oil are toxi… rts per billion). In contrast, they argue that toxicity is an artifact caused by contact with PAH-laden oil microdroplets in… lers of magnitude higher. We conclude that flawed analysis logic and the lack of any direct evidence of oil dr… ns (Heintz et al. 2008; Carls et al. 2008a). In their rebuttal, Brannon et al. (2008) reanalyzed a s… surrogate indicator, again reaching the scientifically indefensible conclusion that microdr… lets as an agent of embryo mortality, whereas we have done so for PAHs (Marty et… d microdroplets as directly toxic (Carls et al. 2008b). This web document pr… dentical observations

Yes: ORC experiments ar… d by particulate oil

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- Consequently, the… …l composition ppb were toxic is i… …ty et al. 1997; …tently identify …e oil.
- Because oil droplet… …ggs, eliminating higher dose of high… **…ons present in …l or dissolved)** in PAH. …the particulate oil

…oil contact) was the …hole oil (Heintz et al. …eates direct contact is …ish embryos exposed to …008b).

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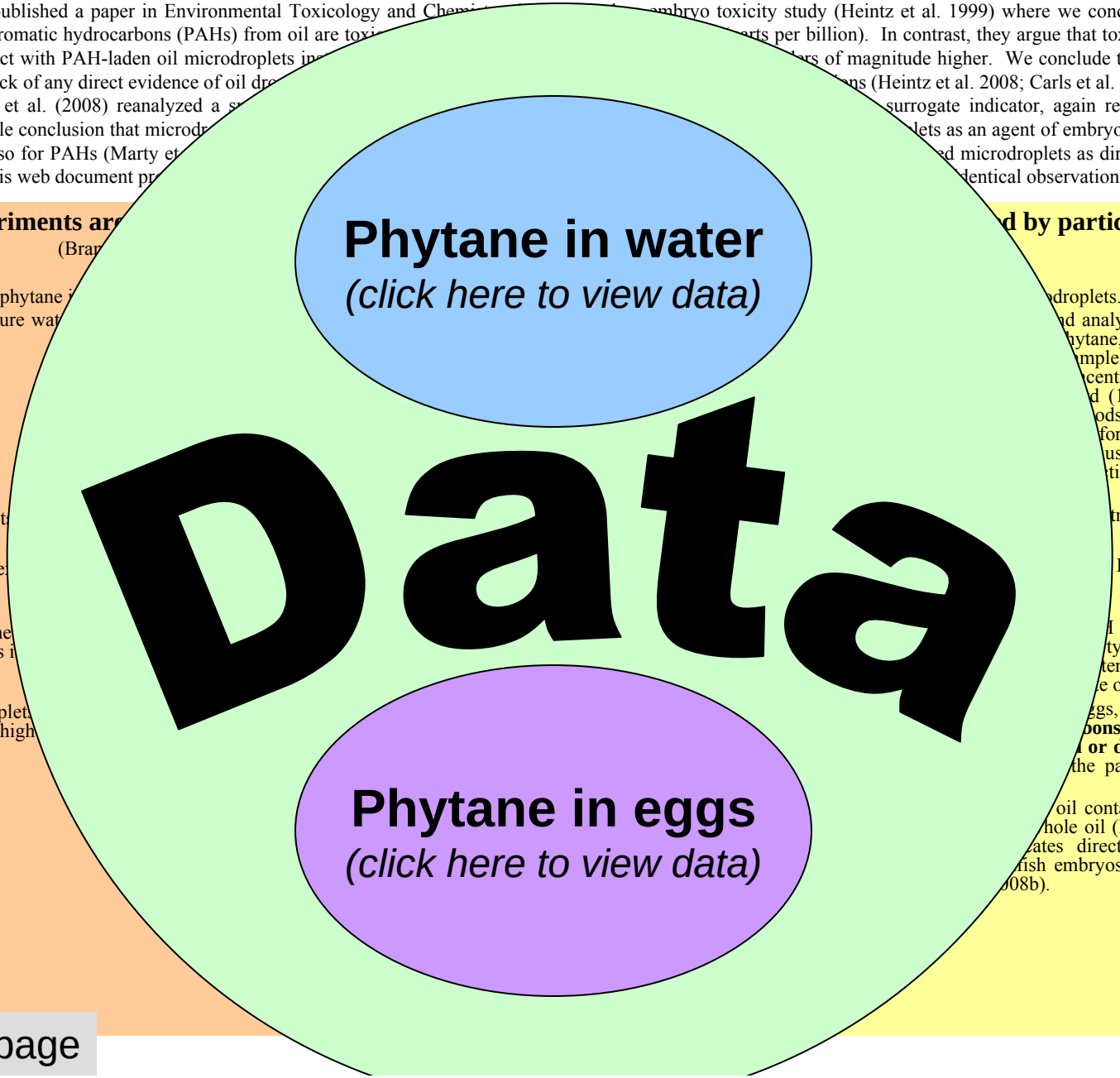

Phytane in water

Phytane × QCbatch
(click for illustration)

Phytane × treatment
(click for illustration)

For comparison, TPAH × treatment
(click for illustration)

treatment	level	day	SIN	QCbatch	phytane	QC
control	0	-1	404316	R08034	0.014	a
control	0	0	404436	R08034	0.014	a
control	0	0	404405	R08124	0.018	a
control	0	35	405219	R08224	0.020	a
control	0	35	405217	R08224	0.020	a
control	0	35	405218	R08224	0.020	a
control	0	63	405448	R08264	0.030	a
control	0	63	405437	R08264	0.030	a
control	0	63	405438	R08264	0.030	a
control	0	93	405701	R08124	0.025	b
oil	1	0	404404	R08124	0.018	a
oil	1	35	405237	R08224	0.020	a
oil	1	63	405503	R08264	0.030	a
oil	2	0	404403	R08124	0.018	a
oil	2	35	405244	R08224	0.020	a
oil	2	63	405506	R08264	0.030	a
oil	3	0	404402	R08124	0.032	b
oil	3	36	405313	R08264	0.030	a
oil	3	63	405510	R08264	0.030	a
oil	3	93	405702	R08124	0.018	a
oil	4	0	404401	R08034	0.040	b
oil	4	36	405325	R08264	0.030	a
oil	4	63	405518	R08304	0.020	a
oil	5	0	404350	R08034	0.014	a
oil	5	36	405330	R08264	0.030	a
oil	5	63	405521	R08304	0.028	b
oil	5	93	405703	R08124	0.023	b
oil	6	0	406001	R02285	0.010	b
oil	6	36	405340	R08264	0.041	b
oil	6	63	405522	R02285	0.000	b
VWO	7	0	404349	R02285	0.000	a
VWO	7	63	405513	R08264	0.030	a

SIN: sample identification number QCbatch: quality control batch
QC: a = below level of detection, b = below level of quantification
VWO: very weathered oil. Phytane concentration is µg/L.
Level: 1 is low oil, ... 6 is high oil

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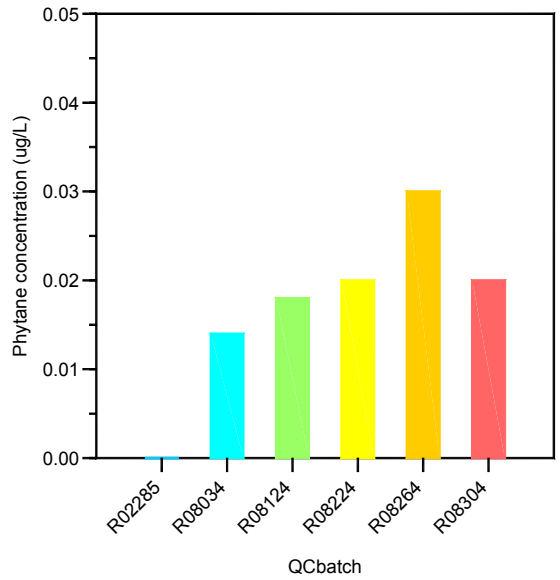
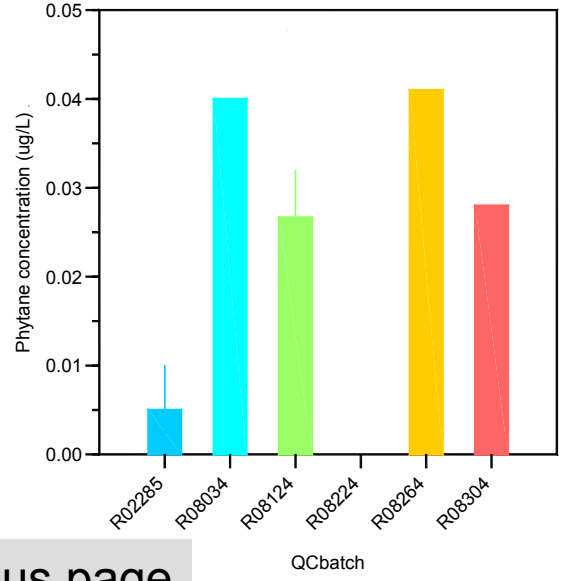
Phytane in water: phytane × QCbatch

Click here for our conclusion

A) Samples where phytane was not detected (n = 24). There was no variance within QCbatch, only among QCbatch.



B) Samples where phytane was below quantification limits (n = 8). Error bars are minimum to maximum range.



treatment	level	day	SIN	QCbatch	phytane (ug/L)	QC
VWO	7	0	404349	R02285	0.000	a
oil	5	0	404350	R08034	0.014	a
control	0	0	404436	R08034	0.014	a
control	0	-1	404316	R08034	0.014	a
control	0	0	404405	R08124	0.018	a
oil	1	0	404404	R08124	0.018	a
oil	2	0	404403	R08124	0.018	a
oil	3	93	405702	R08124	0.018	a
control	0	35	405219	R08224	0.020	a
oil	1	35	405237	R08224	0.020	a
oil	2	35	405244	R08224	0.020	a
control	0	35	405217	R08224	0.020	a
control	0	35	405218	R08224	0.020	a
control	0	63	405448	R08264	0.030	a
oil	1	63	405503	R08264	0.030	a
oil	2	63	405506	R08264	0.030	a
oil	3	36	405313	R08264	0.030	a
oil	3	63	405510	R08264	0.030	a
oil	4	36	405325	R08264	0.030	a
oil	5	36	405330	R08264	0.030	a
VWO	7	63	405513	R08264	0.030	a
control	0	63	405437	R08264	0.030	a
control	0	63	405438	R08264	0.030	a
oil	4	63	405518	R08304	0.020	a
control	0	93	405701	R08124	0.025	b
oil	3	0	404402	R08124	0.032	b
oil	4	0	404401	R08034	0.040	b
oil	5	63	405521	R08304	0.028	b
oil	5	93	405703	R08124	0.023	b
oil	6	0	406001	R02285	0.010	b
oil	6	36	405340	R08264	0.041	b
oil	6	63	405522	R02285	0.000	b

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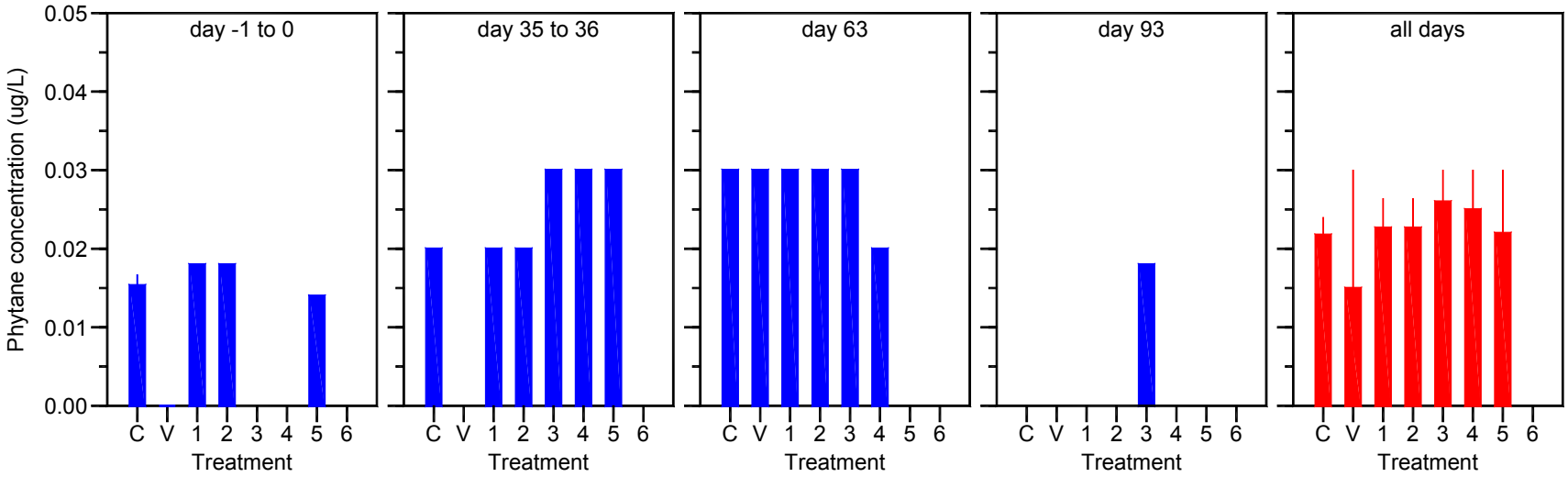
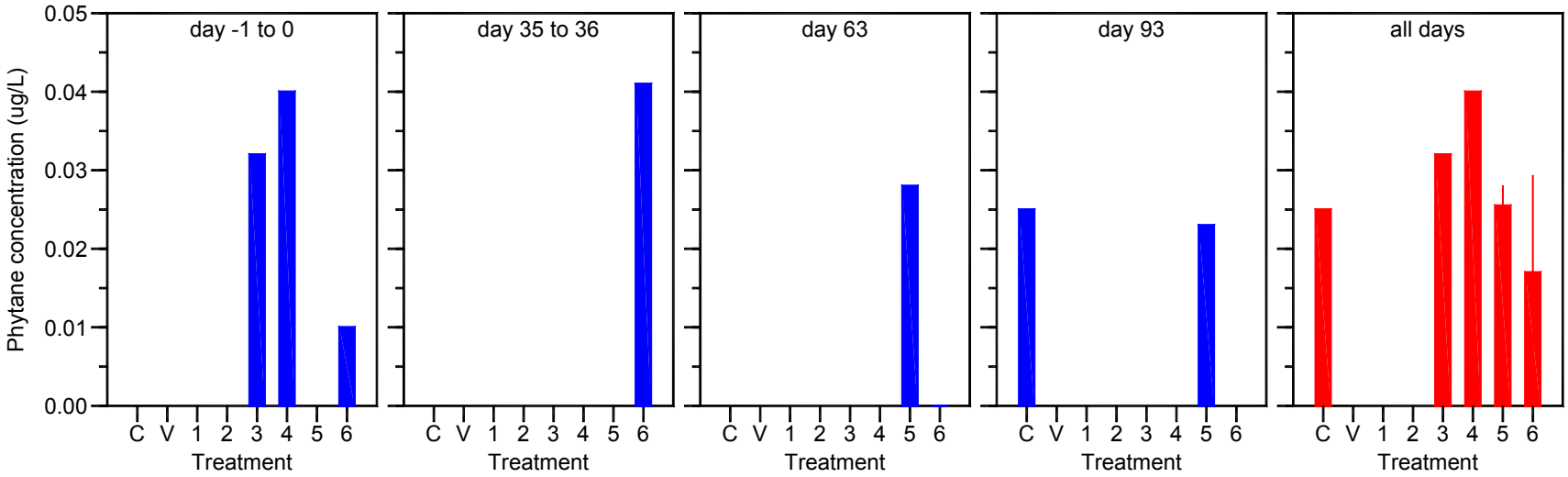
Phytane in water: phytane × treatment

Click here for our conclusion

A) Samples where phytane was not detected (n = 24). Error bars are standard error. Treatment C is control, V is very weathered oil, 1 is low oil, 6 is high oil.



B) Samples where phytane was detected (n = 8). Error bars are standard error.



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Phytane in eggs

Click here for our conclusion

treat		sday	ng/g dry weight phytane
0		36	0
0		65	0
0	*	65	0
0		93	0
0		126	0
0	*	178	0
0		183	0
1		36	0
1		65	0
1		183	0
2		36	0
2		65	0
2		183	0
3		42	0
3	*	42	0
3		65	0
3		65	0
3		93	230
3		126	0
3	*	178	0
3		183	0
4		36	0
4		65	0
4		183	0
5		36	0
5	*	42	0
5		42	0
5		65	0
5	*	65	0
5		93	0
5		126	0
5	*	178	0
5		183	0
6		36	0
6		65	0
6		183	0
7		36	0
7		65	0
7		183	0

Treatment 0 = control, 1=lowest oil dose, ... 6=highest oil dose; 7 = very weathered oil.
*Eggs were suspended in effluent and not in contact with whole oil. All other eggs were in contact or potential contact with oiled gravel.

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Phytane in water: phytane × QCbatch, our conclusion

All reported aqueous phytane concentrations are likely random noise. Phytane was not detected in 75% of 32 water samples. Aqueous phytane concentrations in the remaining samples were far below the level of quantification (1.6 to 8.2% of the concentration of the smallest standard) and were not significantly different from those where phytane was not detected.

$$P_{ANOVA} = 0.387$$

The reason undetected phytane concentration estimates are sometimes greater than zero is because standard curves are not forced through zero; positive intercepts result in small, nonzero positive estimates where no phytane was detected.

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Phytane in water: phytane × treatment, our conclusion

Aqueous phytane concentrations were unrelated to oil treatment, hence particulate oil was either absent or negligible in exposure water. Aqueous phytane concentrations were not dose-dependent and were unrelated to time.

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Phytane in eggs: our conclusion

Embryos did not accumulate phytane, indicating exposure to whole oil was either negligible or inconsequential. Phytane concentrations in tissue were neither dose nor time-dependent. Lipophylic eggs are capable of accumulating hydrocarbons to concentrations approximately 1000 times greater than in water, thus they conveniently amply hydrocarbon levels, improving detection. Despite this amplification (bioaccumulation), phytane was below method detection limits in pink salmon eggs with one exception at an intermediate dose and time (n_{total} = 39). We suspect the exception was caused by sample contamination: this was the only significantly elevated phytane concentration in 147 egg samples collected across five experiments and was more than 5 times greater than any other non-zero phytane concentration in eggs. In contrast, TPAH concentrations in eggs were dose and time dependent.

Click here to compare to TPAH in eggs

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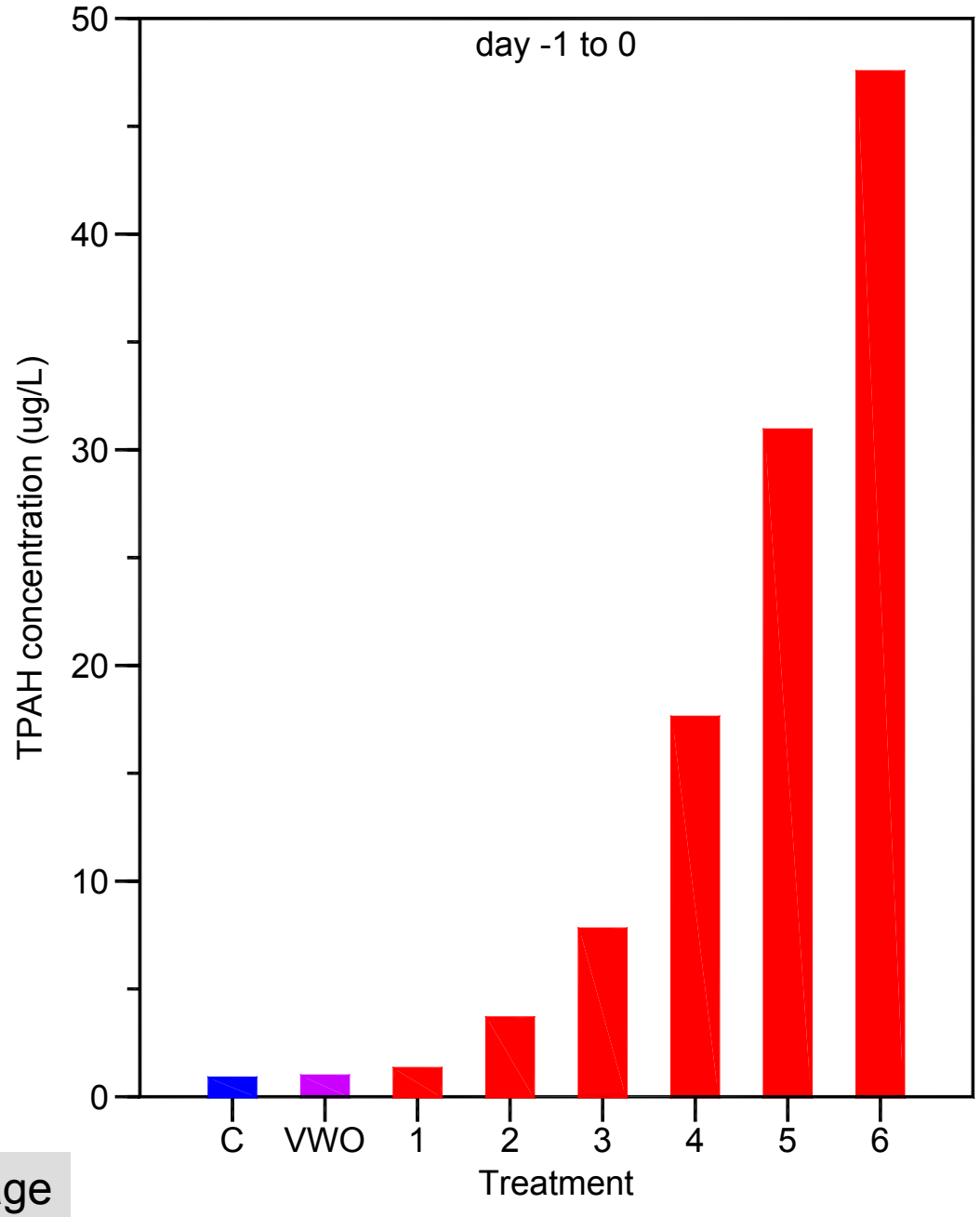
Total polynuclear aromatic hydrocarbons (TPAH) in water

Click here for our conclusion



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Polynuclear aromatic hydrocarbons (PAHs) in eggs

Click here for our conclusion

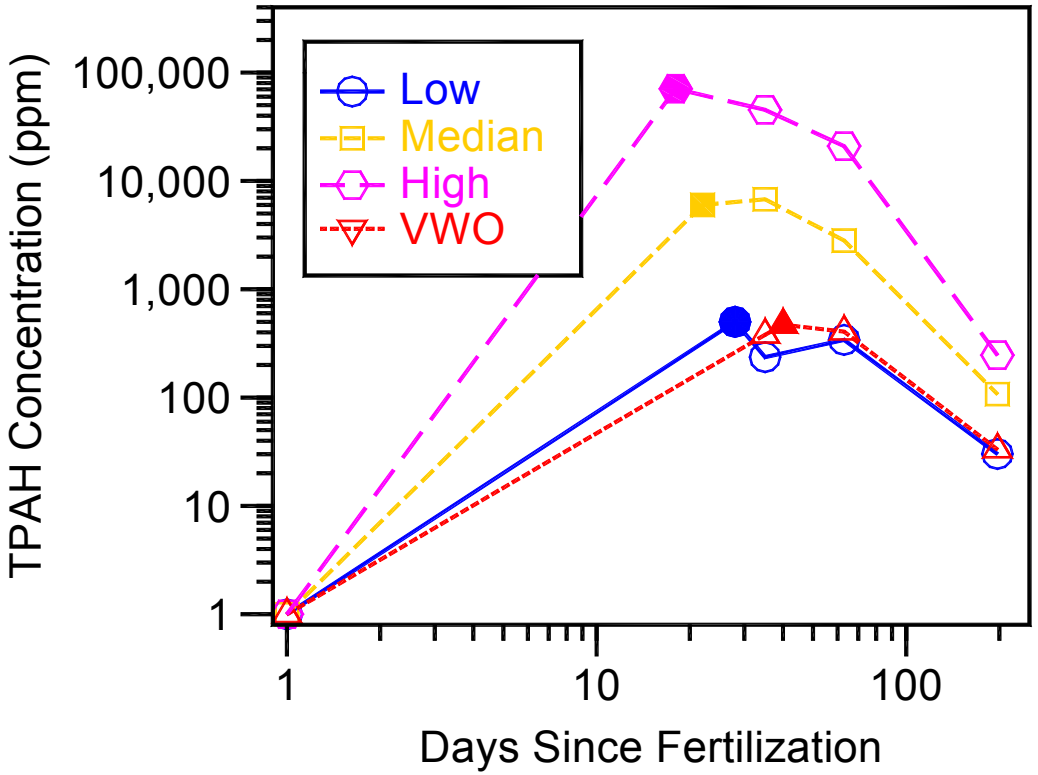

Fig. 3 from Heintz et al. 1999 [Environ Toxicol Chem 18(3):494-503]. Changes in tissue concentrations of total polynuclear aromatic hydrocarbon (TPAH) with time for pink salmon incubated in oil. Lines depict uptake for highest, median, and lowest doses used in the direct exposure experiment and very weathered oil (VWO). Open symbols depict values obtained by gas chromatography and mass spectrometry; filled symbols depict estimates of maximum TPAH concentrations as described in the original paper.

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Total polynuclear aromatic hydrocarbons (TPAH) in water: our conclusion

In contrast to aqueous phytane concentrations, total polynuclear aromatic hydrocarbon (TPAH) concentrations in water were dose (and time) dependent.

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Total polynuclear aromatic hydrocarbons (TPAH) in eggs: our conclusion

In contrast to phytane concentrations in eggs, PAH concentrations were both dose- and time-dependent.

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Concluding remarks

The scientifically indefensible conclusion Brannon et al. (2008) reach, that our phytane data demonstrates our experiments were contaminated by particulate oil, illustrates the interpretive problems we identified in their original paper (Brannon et al. 2006). Their argument that "the multiple ABL studies … have incorrectly determined the actual PAH dose" because "the dosing columns produce an aqueous effluent containing oil droplets" is illogical and invalid. All hydrocarbons present in water samples were measured, regardless of phase (solid or dissolved) in their experiment and ours. Acceptance or rejection of the particulate oil hypothesis has no influence on these measured concentrations. Measured aqueous TPAH concentrations were < 100 ppb in all treatments across all our experiments (n = 167) and biologically damaging aqueous TPAH concentrations were < 20 ppb in all experiments.

Whether researchers agree or disagree about the toxicological importance of particulate oil, comparison of nominal oil concentrations and aqueous TPAH concentrations as though they were equal measures is invalid and that is how Brannon et al. (2006) began their paper. This invalid comparison is doubly pernicious because measured aqueous TPAH doses in their experiment corroborated our measures. Apparently Brannon et al. believe that hypothetical oil droplets in water explain the orders of magnitude difference between nominal oil application and measured dose in opposition to consistent chemical evidence to the contrary. Apparently they believe that visual observation of rare oil slicks provides a more rigorous basis for interpretation than the wide variety of chemical evidence that repeatedly documents negligible quantities of particulate oil in water (including their own results) and in embryo tissue.

Evidence across multiple independent studies indicates dissolved PAHs are toxic to fish embryos at low concentrations without the presence of particulate oil (Kiparissis et al. 2003; Rhodes et al. 2005; Farwell et al. 2006; Carls et al. 2008b). The reliance on nominal dose for interpretation by Brannon et al. (2006) has resulted in multiple errors as detailed in our original review, including toxicity estimates about five orders of magnitude too low and inconsistent with all other literature (Birtwell and McAllister 2002; Rhodes et al. 2005; Farwell et al. 2006). If reliance on nominal dose were acceptable, then no one would need bother with difficult and costly hydrocarbon measurements; crude measures of applied oil would be sufficient. Most toxicologists rejected this approach decades ago.

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