Effects on development, sex differentiation and reproduction of Atlantic cod exposed to produced water during early life stages.

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Abstract:

Two long term studies to assess the effects of produced water (0.01-1 %) on the early life stages of cod were performed. Experiment 1 examined the egg to early fry stage (90 days), and experiment 2 examined the early fry stage to juvenile (78 days). Following exposure, the fish were transferred to clean seawater and monitored for two years till sexual maturation. One group of fish from each exposure regimen was used in spawning experiments

No effects on hatching success, growth, survival or sexual differentiation were detected for cod (eggs, larvae and fry) exposed to low concentrations of produced water (dilution 1:1000 or 1:10000). Yolk sack larvae were found to be the most sensitive to produced water exposure. Larvae exposed to 1 % produced water showed an inability to start feed, resulting in 100 % mortality. At the fry stage a significant up-regulation of VTG was shown in groups exposed to 1% produced water and estradiol. CYP1A were significantly up-regulated in fry exposed to 1% produced water, and down-regulated after exposure to 10 ppb estradiol.

Exposure to 10 ppb estradiol resulted in severe abnormalities of male testis development and many different morphological signs of intersex were observed.

Introduction

The discharge of produced water has increased in the last decade. In 2005, the discharge of produced water in the Norwegian sector of the North Sea amounted to 150 mill m3, containing 3000 m3 of oil, in addition to many chemicals. There has been a dramatic reduction in recruitment of cod in the North Sea. The aim of this project was to study the long-term effects of produced water exposure on cod. The work focused on endocrine disruption and abnormalities in sex differentiation.

Material and methods:

Produced water (PW): 4000 l PW was transported from the oil platform, Oseberg C (Hydro) to IMR, Bergen. The PW was bubbled with air for 10 minutes to remove toxic H_2S (g) and then frozen at -30 °C until use.

Table 1. Groups and nominal doses.

Groups	Dilution factor	Estimated distance from platform (m)
High (H)	1:100 (1 %)	0-50
Medium (M)	1:1000 (0.1 %)	50-1000
Low (L)	1:10000 (0.01 %)	> 2000
Positive control (E)	10 µg/l 17 β-estradiol	-
Control (C)	-	-

PW contain a very complex mixture of oil compounds and production chemicals. Alkylated phenols and PAHs are the main components of concern regarding long-term effects.

Table 2. GC-MS measurement of the alkylphenol concentration (µg/l) in the produced water and exposure tanks. The results shows the average of 10 measurements taken throughout the experiment.

	PW	H	M	L
Phenol	4563	19	6,4	4,4
ΣCresol	6969	27	4,3	1,3
Σ C2-Phenol	638	5,37	0,57	0,11
Σ C3-Phenol	206	2,33	0,25	0,05
Σ C4-Phenol	57	0,39	0,05	0,02
Σ ≥C5-Phenol	6	0,09	0,04	0,01

Fish:

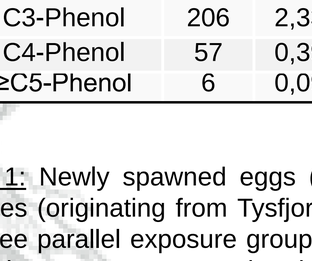
Exp. 1: Newly spawned eggs (5 x 12000) from 5 broodstock families (originating from Tysfjord, Northern Norway) were used in three parallel exposure groups. After hatching, the number of living larvae was measured and the exposure continued on 6000 larvae from each parallel group. The larvae and early juveniles were fed with natural zooplankton and later marine fish pellets. The exposure time in this experiment was 90 days.

Exp. 2: Fish from the same 5 families were raised in clean seawater and transferred to the exposure tanks (2 parallels) at day 86 post-hatching. They were then exposured to PW for 78 days. The exposure was stopped when dissection showed that sex differentiation was completed.

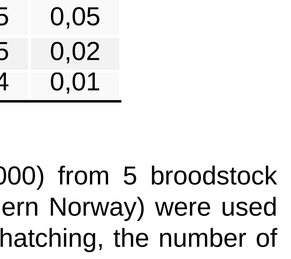
Following PW exposure, the fish were transferred to clean seawater and monitored for two years till sexual maturation.

ELISA was carried out using cod anti-CYP1A and cod anti-VTG antibodies (Biosense AS, Norway).

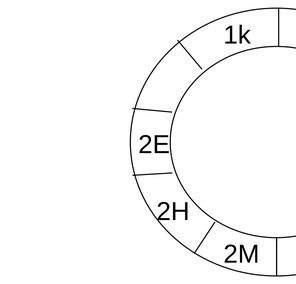
In the spawning experiment 6 females and 6 males from each treatment were kept together in a 10 m^3 tank. Eggs were collected every day through the whole spawning season (60 days). (Cod are batch spawners, and each female normally spawn 15-20 batches).



Control male, GSI = 18

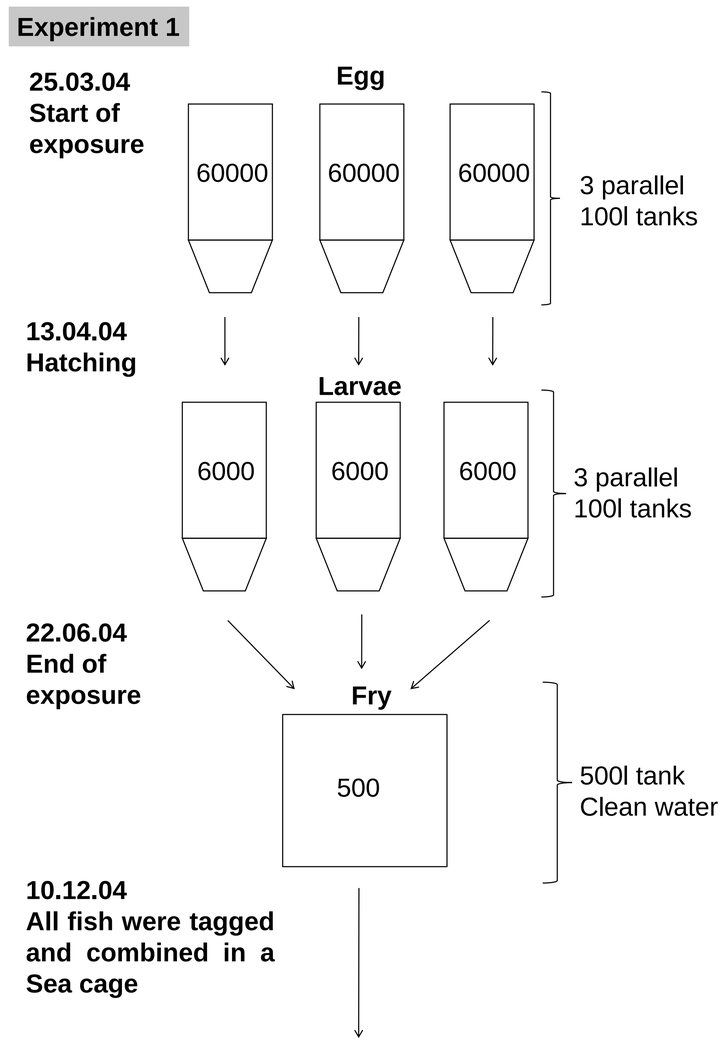


Hermaphrodite, GSI = 1



Intersex, GSI =0.5

Experimental design for each group



Conclusion:

No effects on survival, growth or reproduction were observed in cod after early life long-term exposure to realistic concentrations of PW (0.1 % and 0.01 %).

However, larvae exposed to 1 % PW showed an inability to start-feed, resulting in ≈100 % mortality. A significant up-regulation of VTG was observed in juvenile stages exposed to 1 % PW, showing that PW contains estrogenic compounds. No intersex or other disturbances in gonad development were observed for the 1 % PW group, but the spawning experiment showed a 16 % reduction in the amount of eggs and there was a higher proportion of malformations in the resultant embryos.

Estrogen exposure resulted in a severe and irreversible disturbance of sex differentiation and testis development. The effects were most serious when the exposure occurred at the juvenile stage (100 % intersex). In Exp. 1, where the cod were exposed in early-life stages, we observed both functional males and intersex males.

Results

Experiment 1

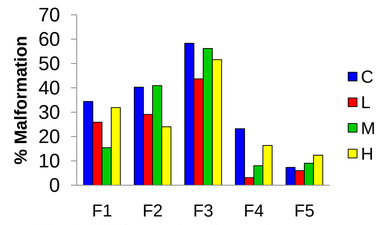


Fig.1. Malformation through embryo stages. Exposure of 100 individual eggs from 5 broodstock families (F1-F5). No increase in malformations was observed after PW exposure.

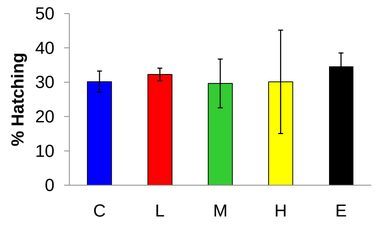


Fig.2. No difference in hatching success was observed in any of the treatments.

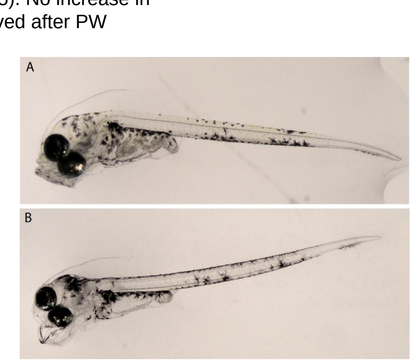


Fig .3. Startfeeding larvae (14 days post-hatching) from A. control group (C); B. high PW doses (H). The majority of the larvae exposed to 1 % PW did not have food in the intestine. A high mortality due of starvation was observed.

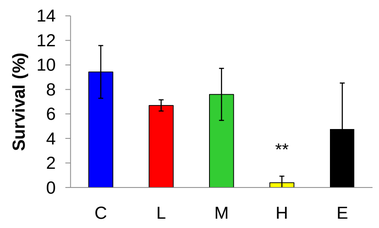


Fig.4. Survival after 90 days exposure. Due to the high mortality in H (high dose PW), no fry from this group were transferred to long-term studies.

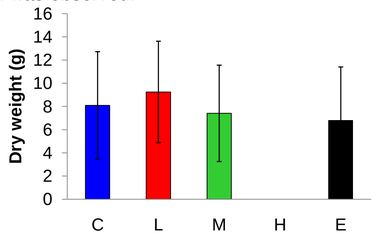


Fig.5. No difference between treatments were seen for growth. Too few fry survived from group H to be measured.

Experiment 2

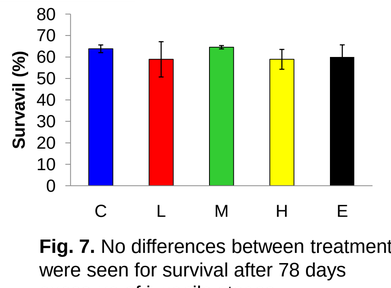


Fig. 7. No differences between treatments were seen for survival after 78 days exposure of juvenile stages.

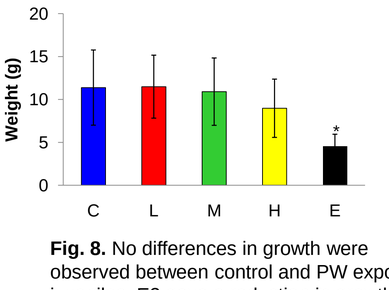


Fig. 8. No differences in growth were observed between control and PW exposed juveniles. E2 gave a reduction in growth.

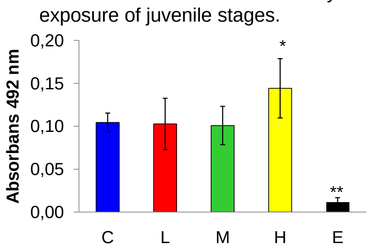


Fig.9. This ELISA shows that exposure to high doses of PW gave an up-regulation of CYP1A in the liver, while E2 exposure gave a down-regulation.

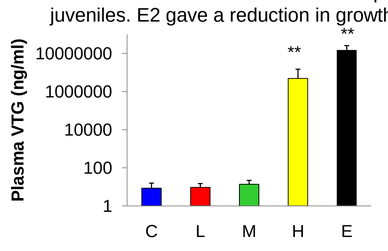
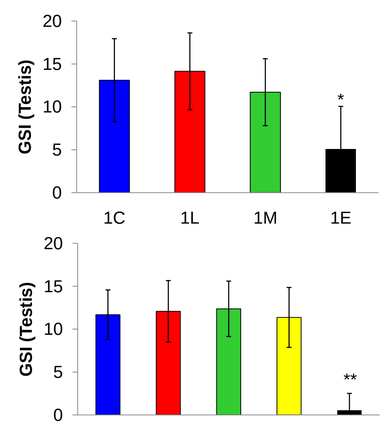


Fig.10. This ELISA shows that high doses of either PW or E2 produced an up-regulation of vitellogenin (VTG) expression levels.

Spawning experiment



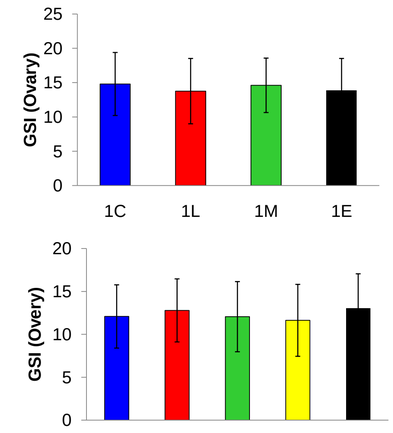


Fig. 11. Gonadosomatic index (GSI) for testis and ovary from Exp.1 and Exp.2. No differences were found between the control group and the PW exposed fish. The E2 treatment resulted in a severe disturbance of the testis development, many different kinds of intersex and deformations were observed.

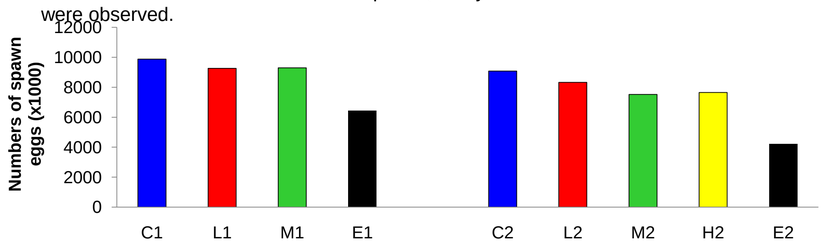


Fig.12. Sum of all egg spawned from 6 female and 6 males from each treatment group.

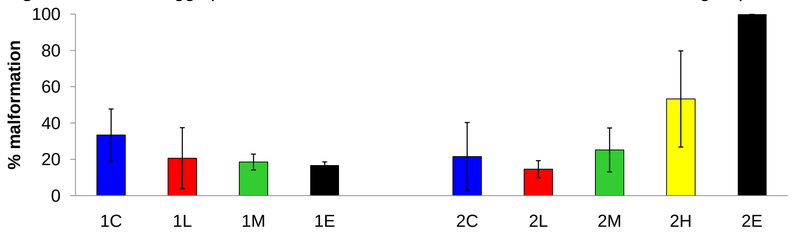


Fig.13. Malformation through embryo stages. Measurement of 100 individual egg from three different spawning dates. There were a higher proportion of malformations in the embryos from the high dose PW group (2H). None of the eggs from the 2E group were fertilized.

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