# Effect of Water-Accommodated Fraction of Kuwait Crude Oil on Developmental Stages of Orange-Spotted Grouper Hamoor (*Epinephelus coicoides*)

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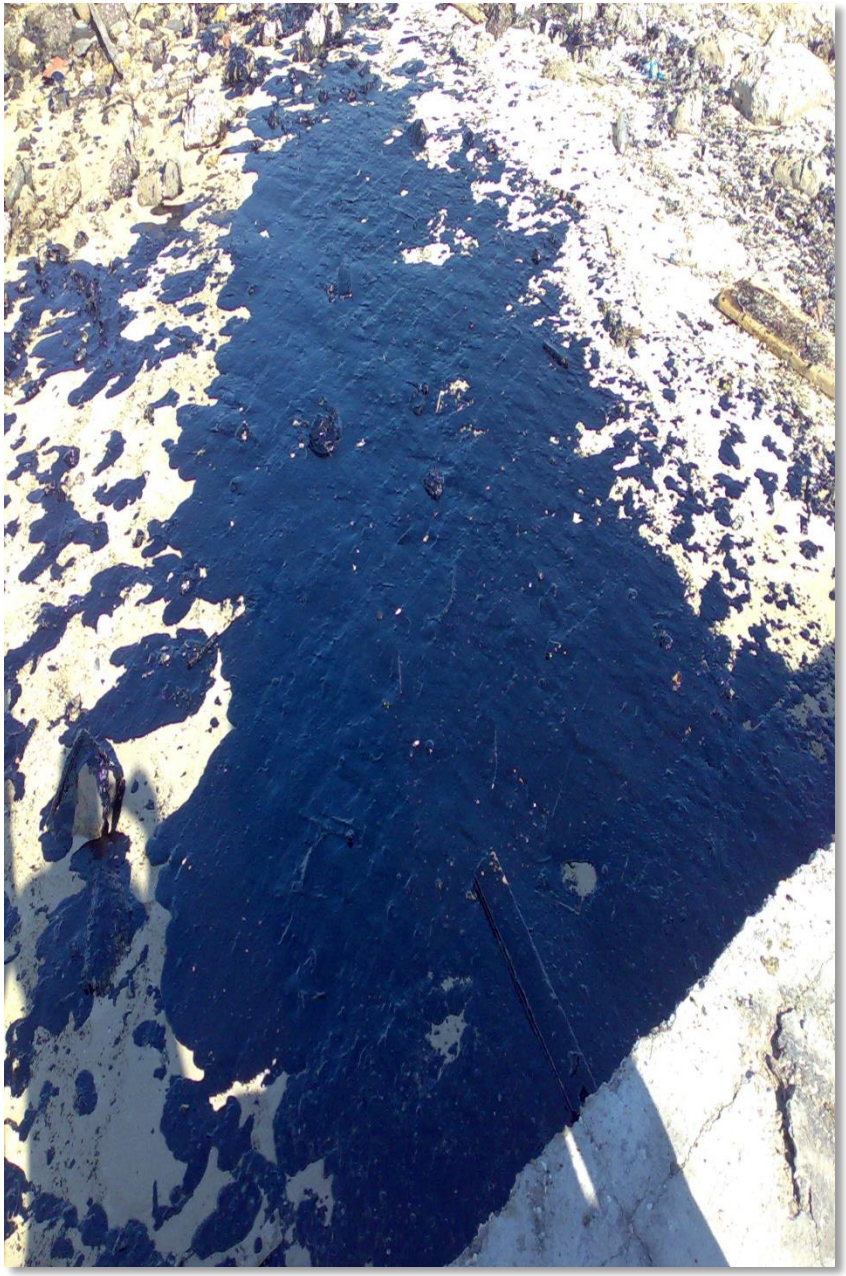


# Presentation Overview

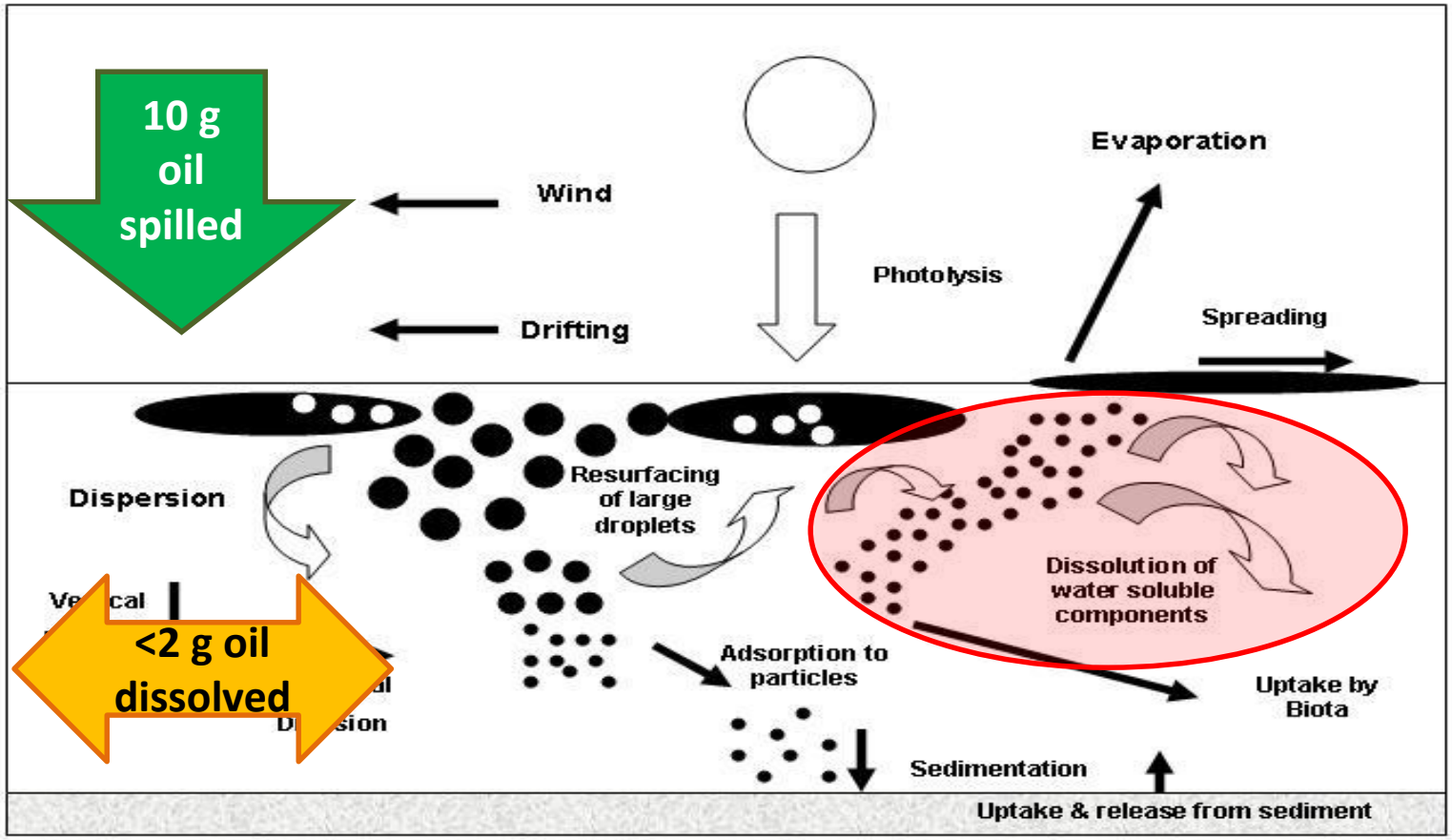
1. Oil Spill Process.
2. WAF Preparation Methodology.
3. Chemical Characterization of KCO WAF.
4. Toxicity Testing of Kuwait Crude Oil.
5. Morphological Deformities of Fish Larvae.

# Objectives

1) To assess the effects of Kuwait crude oil water-accommodated fraction **"KCO WAF"** on embryonated egg hatching and larval survival of marine fish.

2) To gather a detailed visual evidence of the types of morphological deformities in eggs and larvae upon exposure to **KCO WAF**.

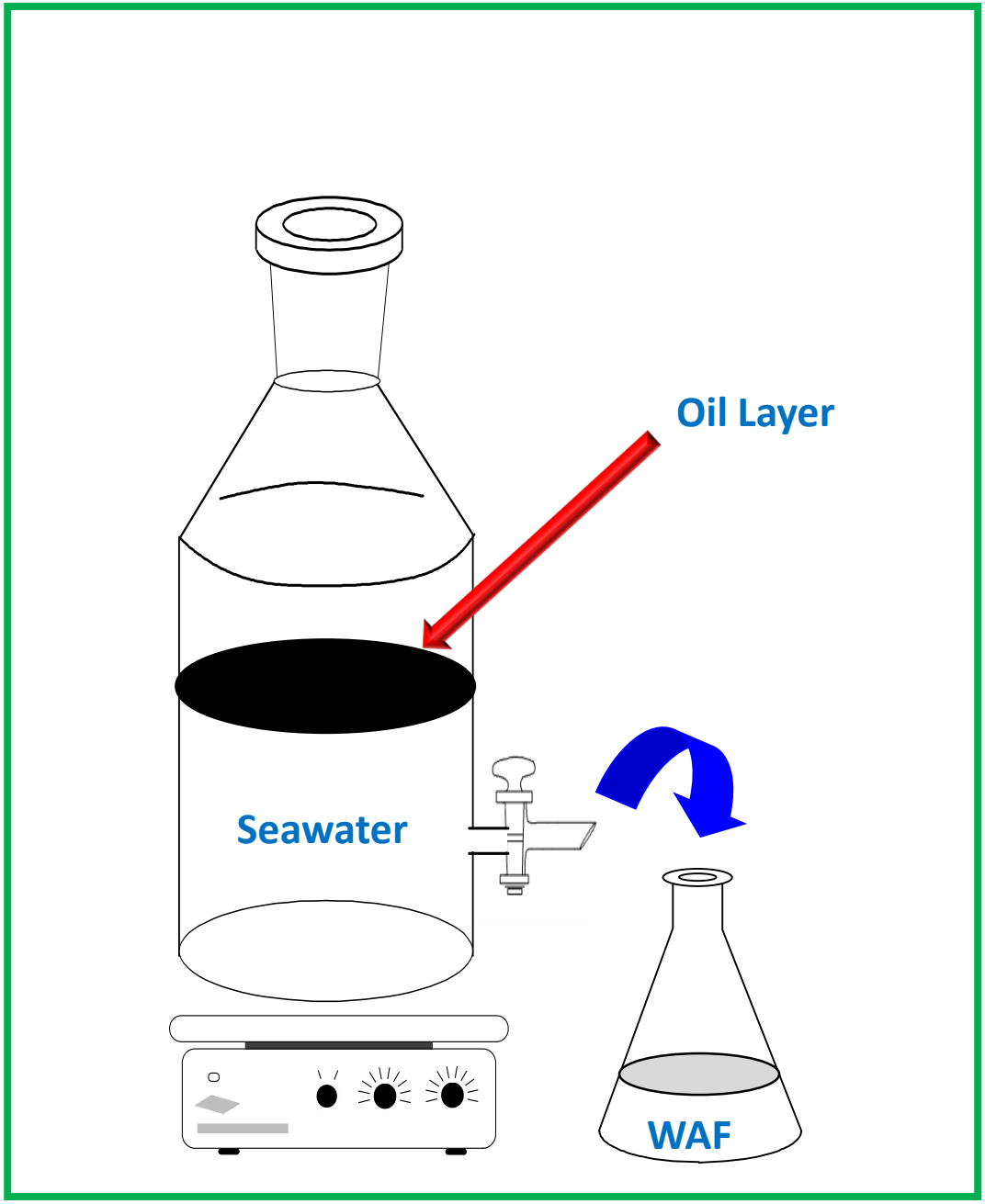
# Oil Spill Process




(from Daling et al., 1990)

# Preparation of Kuwait Crude Oil Water-Accommodated Fraction (KCO WAF ) for Toxicity Testing

| | |
|---|---|
| **Oil to Water Ratio** | 1:10 , with 1.0 g KCO/L seawater serially diluted. |
| **Mixing Duration** | 24 h |
| **Mixing Energy** | 200 rpm |
| **Settling Period** | 3h |
| **Method** | UNEP, 1989 |


Oil Layer
Seawater
WAF

# Chemical Characterization of KCO WAF (1.0 g KCO)

**BTEX –**

**Volatile Fraction**

**Benzene: 0.04 mg/L**

**Toluene: 0.10 mg/L**

**Ethylbenzene: 0.10 mg/L**

**Xylene: 0.06 mg/L**

**TPH -**

**Total Petroleum Hydrocarbons**

**2.22 mg/L**

**PAH -**

**Polycyclic Aromatic Hydrocarbons**

**22.0 ng/L**

# Main Findings

1) The levels of BTEX, TPH and PAHs estimated in KCO WAF which are known to exert toxic effects on the early life stages of fish.
2) Fish can quickly absorb a part of the volatile fraction (BTEX) and PAHs, which can induce adverse consequences on fish growth.
3) TPH is a better representative of KCO WAF with respect to the selected oil loading (1.0 g KCO/L seawater).

# Toxicity Testing

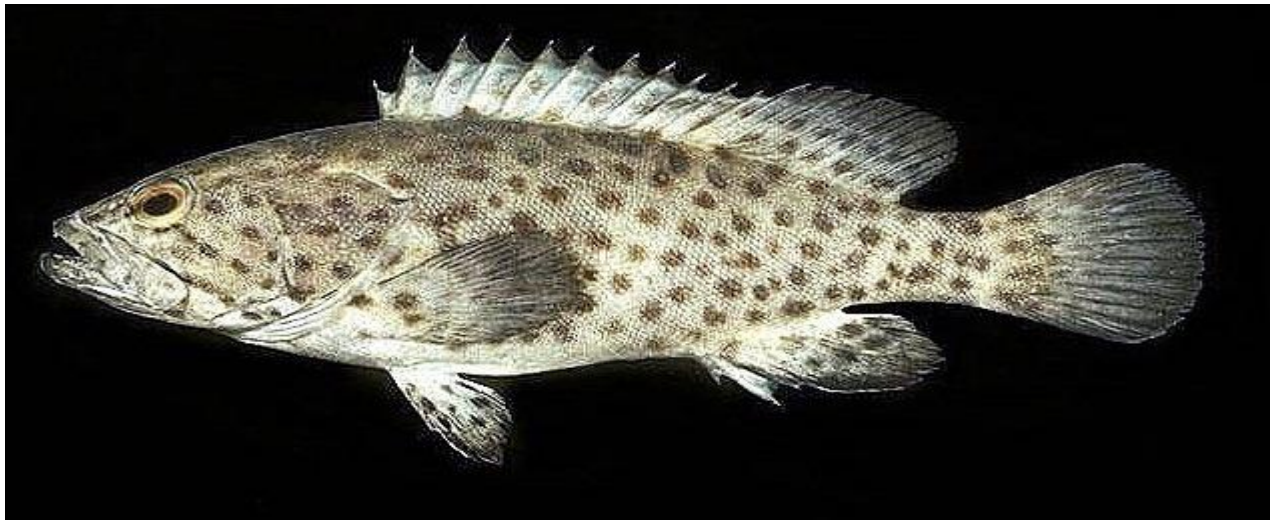
# of

# KCO WAF

# Against Orange-Spotted Grouper

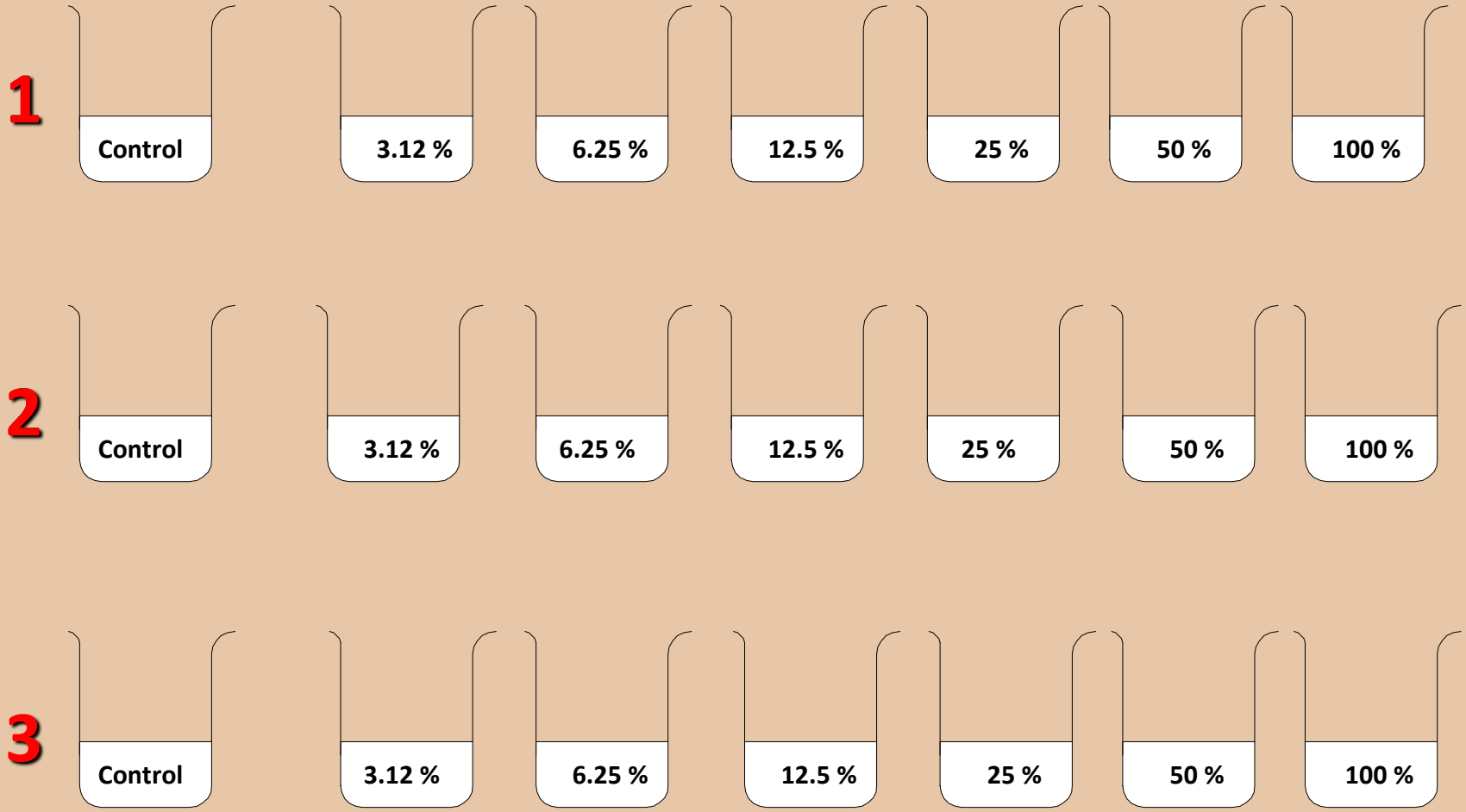
# (*Epinephelus coicoides*)

# Test Species



| | |
|---|---|
| **Common Name** | Orange-Spotted Grouper |
| **Scientific Name** | *Epinephelus coicoides* |
| **Local Name** | Hamoor |
| **Age** | 48 h embryonated eggs |
| **Method** | ASTM –E729-96 “Standard guide for conducting acute toxicity tests on test materials with fishes, microinvertebrates, and amphibians” 2002. |

# Serial Dilutions & Controls

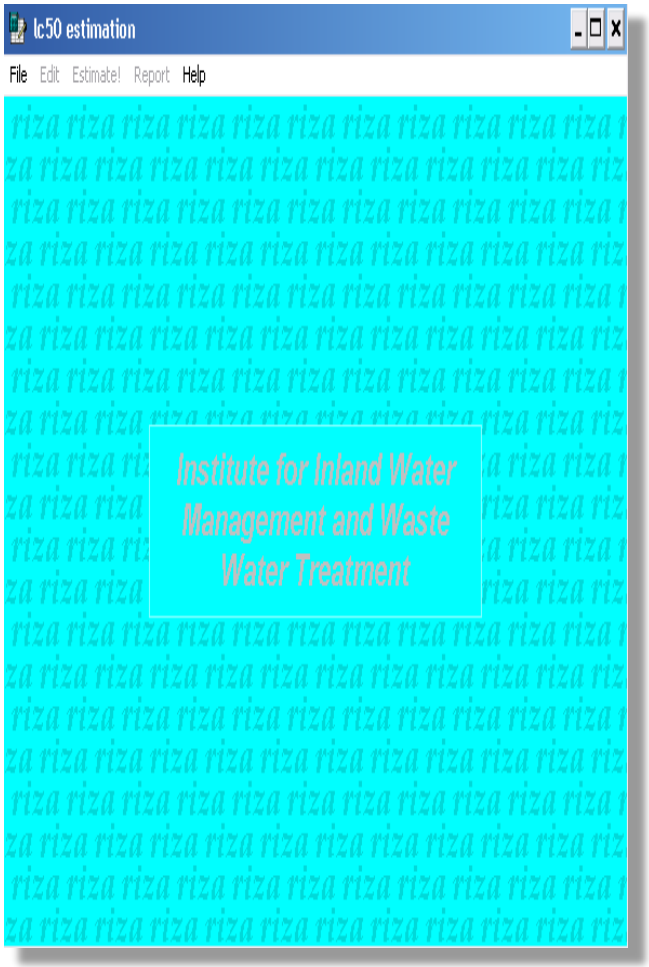


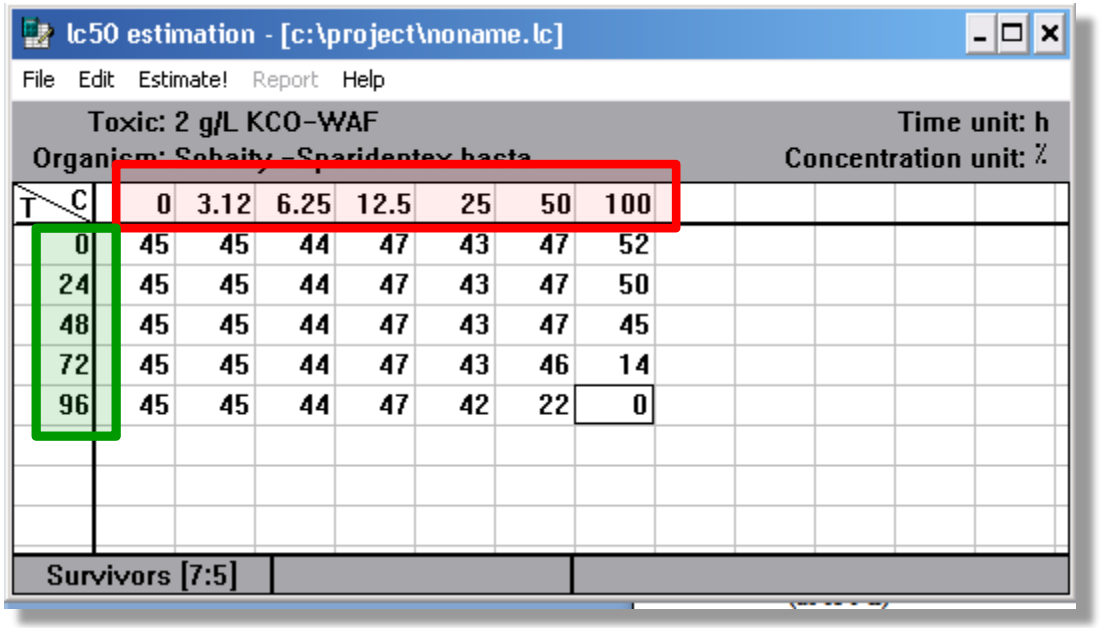
# Control Seawater

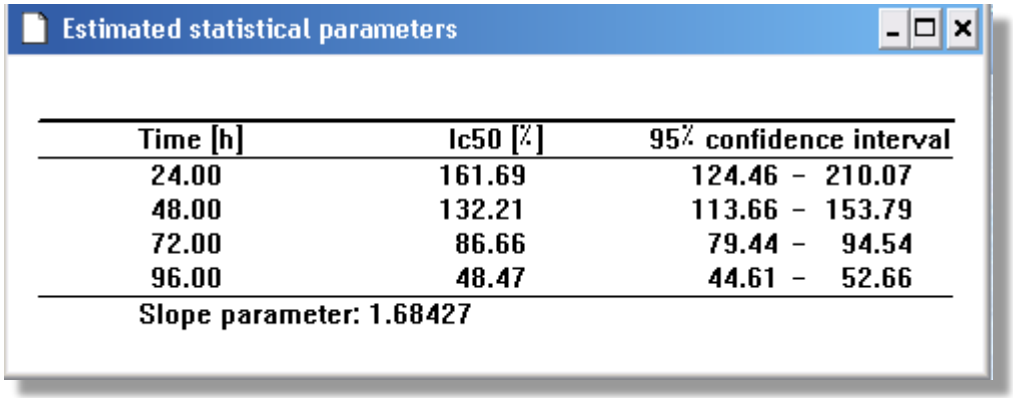
PERCENTAGES and AVERAGES OF SOBAITY 48-h LARVAL HATCHING and 96-h SURVIVAL SUCCESS for CONTROL SEAWATER.

| | No. of Controls | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Exposure Time (h) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| 0 h | 16 | 20 | 16 | 16 | 15 | 15 | 19 | 18 | 16 | 17 | 10 | 10 | 10 | 10 | 11 |
| 24 h | 16 | 18 | 16 | 15 | 14 | 15 | 19 | 18 | 16 | 16 | 8 | 10 | 10 | 9 | 11 |
| 24-h Hatching Success(%) | 100 | 90 | 100 | 94 | 93 | 100 | 100 | 100 | 100 | 94 | 80 | 100 | 100 | 90 | 100 |
| 48 h | 16 | 20 | 16 | 16 | 15 | 15 | 19 | 18 | 16 | 17 | 10 | 10 | 10 | 10 | 11 |
| 48-h Hatching Success (%) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 72 h | 16 | 20 | 16 | 16 | 15 | 14 | 19 | 18 | 16 | 17 | 10 | 10 | 10 | 10 | 11 |
| 72-h Survival Success (%) | 100 | 100 | 100 | 100 | 100 | 93 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 96 h | 16 | 20 | 16 | 16 | 15 | 14 | 19 | 18 | 16 | 17 | 10 | 9 | 9 | 9 | 10 |
| 96-h Survival Success (%) | 100 | 100 | 100 | 100 | 100 | 93 | 100 | 100 | 100 | 100 | 100 | 90 | 90 | 90 | 91 |

# Lethal Concentration ($LC_{50}$) Determination



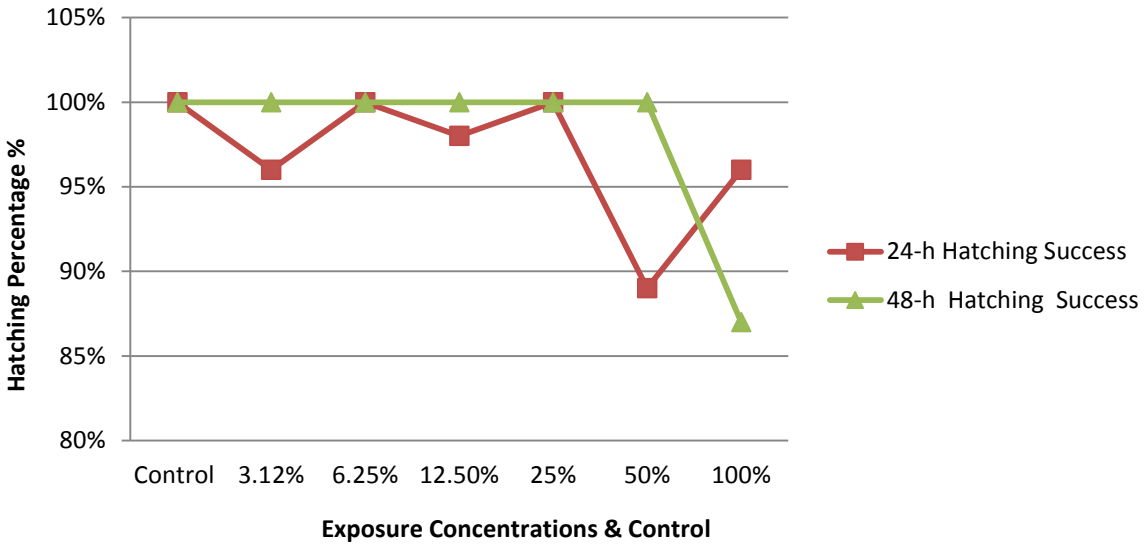



## KCO WAF Exposure

1.0 g KCO/l seawater

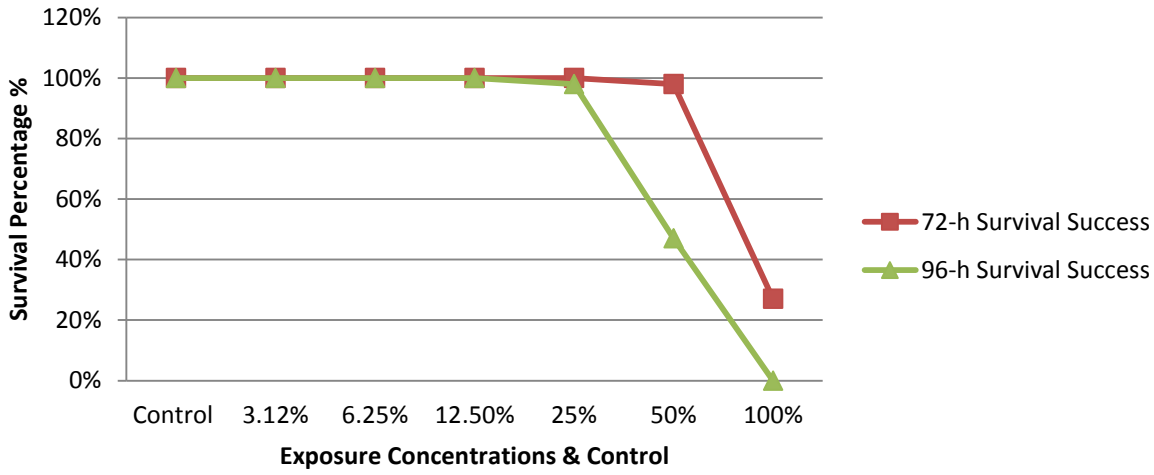
## Egg Hatching Success

48-h $LC_{50}$ 1.13 g KCO/l seawater (95% CI 0.23 to 5.51).



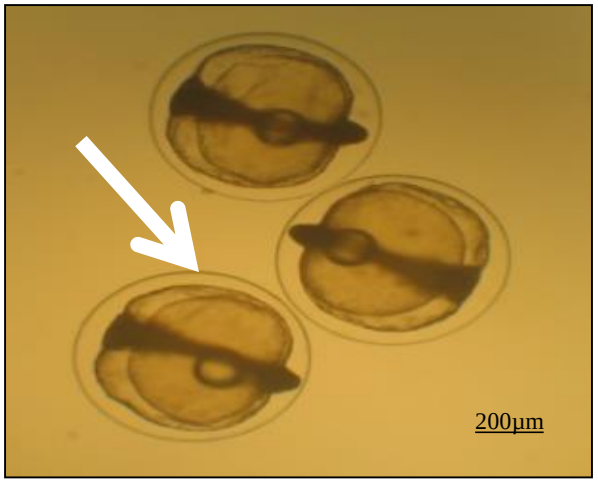

## Larval Survival Success

96-h $LC_{50}$ was 0.48 g KCO/l seawater (95% CI 0.45 to 0.53) ($p<0.05$)

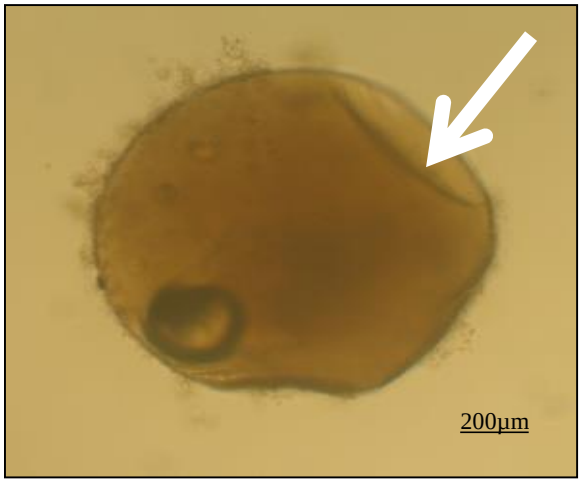


# Morphological Abnormalities in Fish Larvae

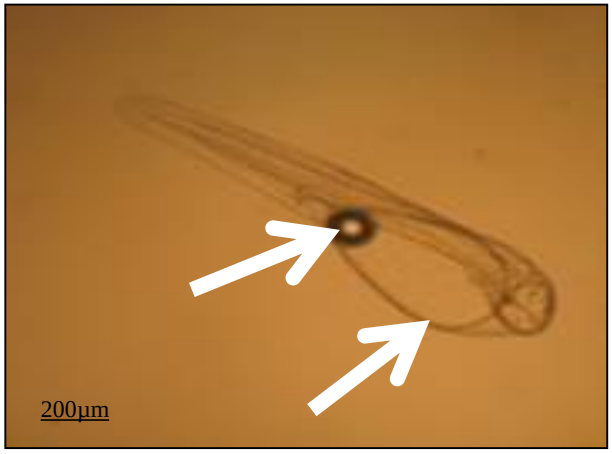
# Control Seawater




Healthy Hamoor Egg




Dead Hamoor Egg




Healthy Hamoor Larvae


Dead Hamoor Larvae

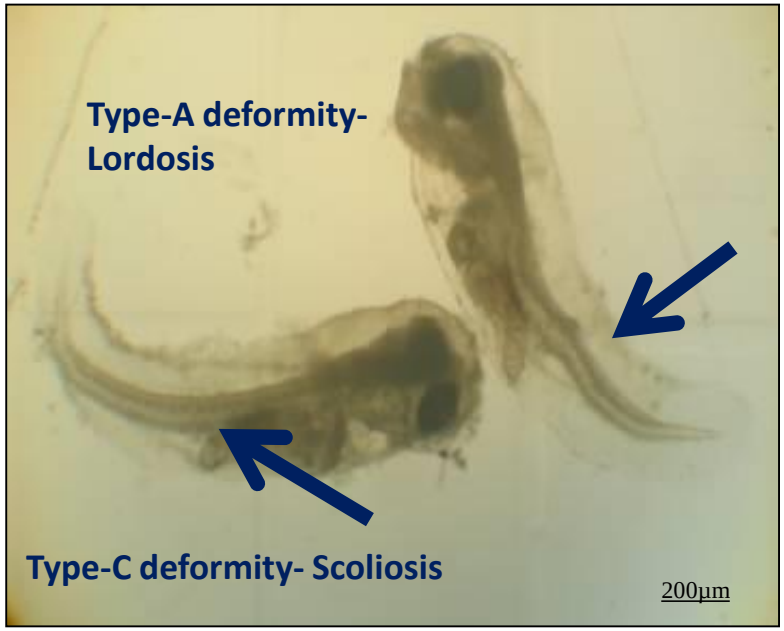
# Hamoor Deformity Classification

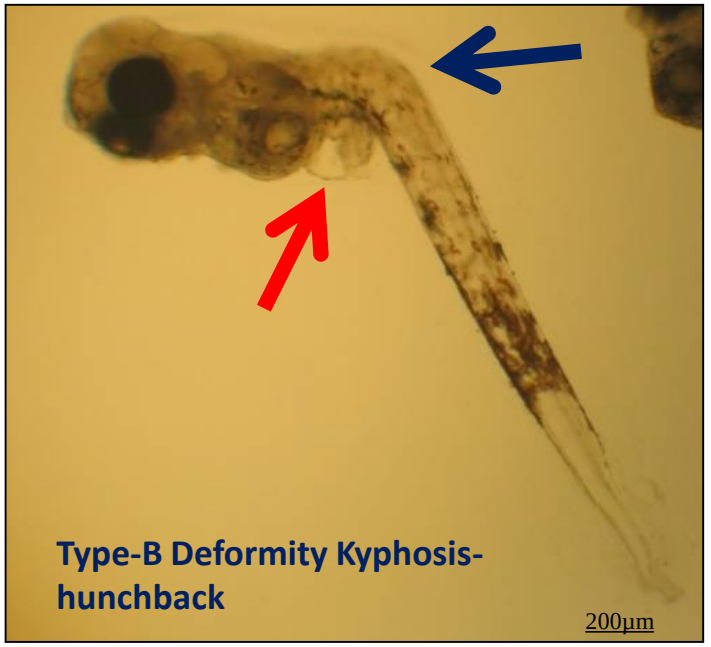
1) **Lordosis / Type–A** : Inward curvature of spine or V-Shaped.

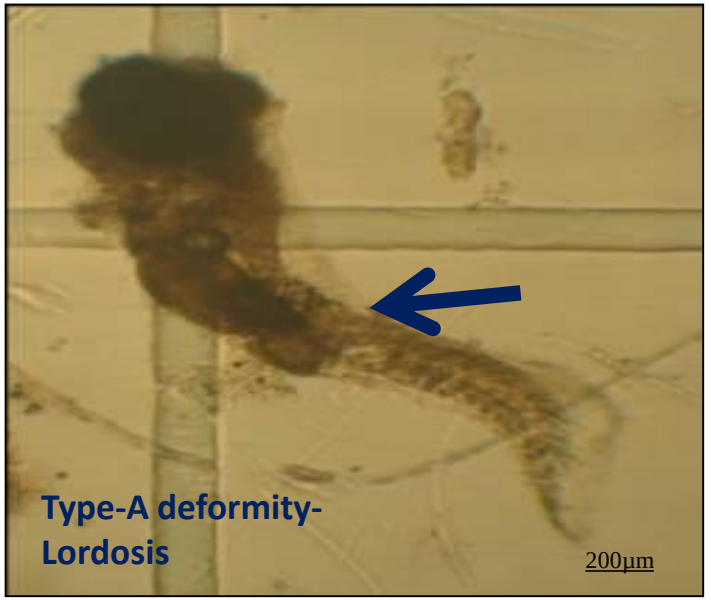
2) **Kyphosis / Type–B** : Hunchback.

3) **Scoliosis / Type–C** : Lateral bending of spine.

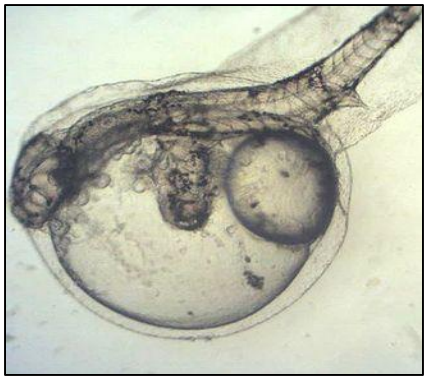
# Developmental Deformities Types



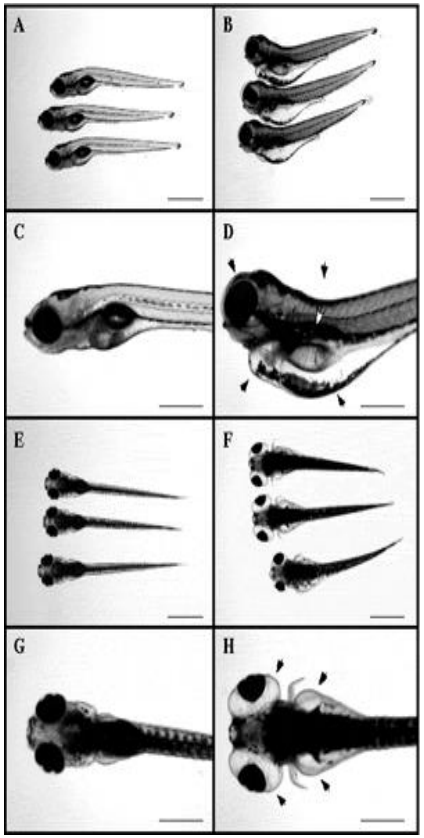



# Fish Morphological Abnormalities in literature

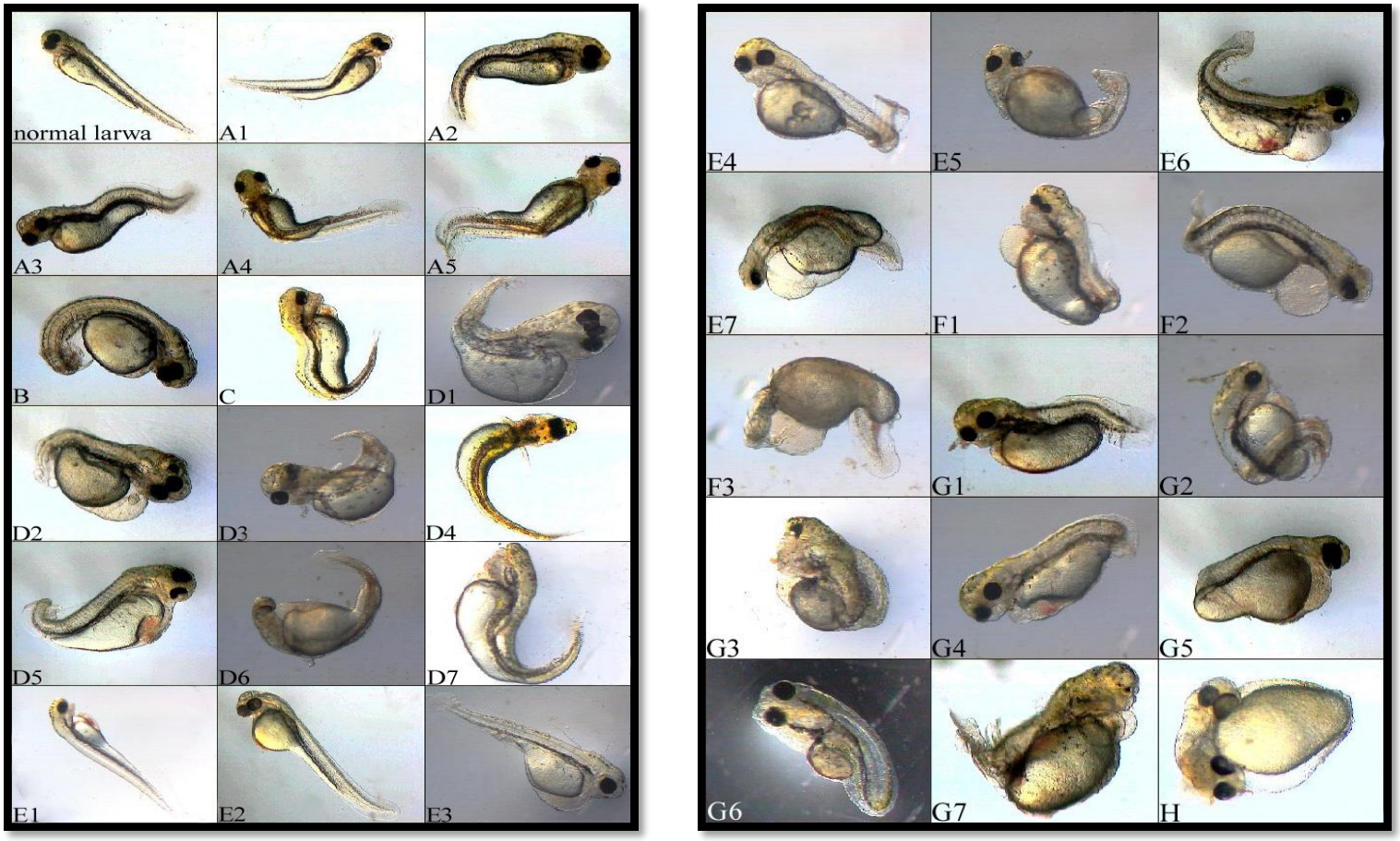


- Yolk sac edema
- Pericardium hemorrhaging (double-walled sac that contains the heart)
- Disruption of cardiac function
- Mutations
- Craniofacial and spinal deformities
- Impaired swimming
- Neuronal cell death
- Reduced growth




- White *et al*., 1999; Barron *et al*., 2003; Billiard *et al*., 1999; 2002; Brinkworth *et al*., 2003; Marty *et al*., 1995; Incardona *et al*., 2004.

# Fish Morphological Abnormalities in literature




**Types of deformed larvae of common carp (Source: Jezierska et al., 2000).**

# Deformity Type Percentages of Hamoor Larvae after Exposure 1.0g KCO WAF

| Exposure Time (h) | WAF Concentration (%) 3.12 | 6.25 | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|---|
| **Exposure No. 1** | | | | | | |
| 0 | 14 [s] | 15 [s] | 16 [s] | 14 [s] | 15 [s] | 26 [s] |
| 24 | nd | nd | nd | nd | nd | nd |
| 48 | nd | nd | nd | d.A-7% | d.A-27% | d.A-73%, d.C-4% |
| 72 | nd | nd | nd | d.A-7% | d.A-73% | All Dead |
| 96 | nd | nd | nd | 13* | d.A-7% | All Dead |
| **Exposure No. 2** | | | | | | |
| 0 | 17 [s] | 14 [s] | 15 [s] | 16 [s] | 15 [s] | 12 [s] |
| 24 | nd | nd | nd | nd | nd | nd |
| 48 | nd | nd | nd | nd | d.A-53% | d.A-33%, d.B-8%, C-8% |
| 72 | nd | nd | nd | nd | d.A-33% | d.A-42%, d.C-8% |
| 96 | nd | nd | nd | nd | d.A-7% | All Dead |
| **Exposure No. 3** | | | | | | |
| 0 | 17 [s] | 14 [s] | 15 [s] | 16 [s] | 15 [s] | 12 [s] |
| 24 | nd | nd | nd | nd | nd | nd |
| 48 | nd | nd | nd | nd | nd | nd |
| 72 | nd | nd | nd | nd | d.A-29% | d.A-34% |
| 96 | nd | nd | nd | nd | d.A-29% | All Dead |

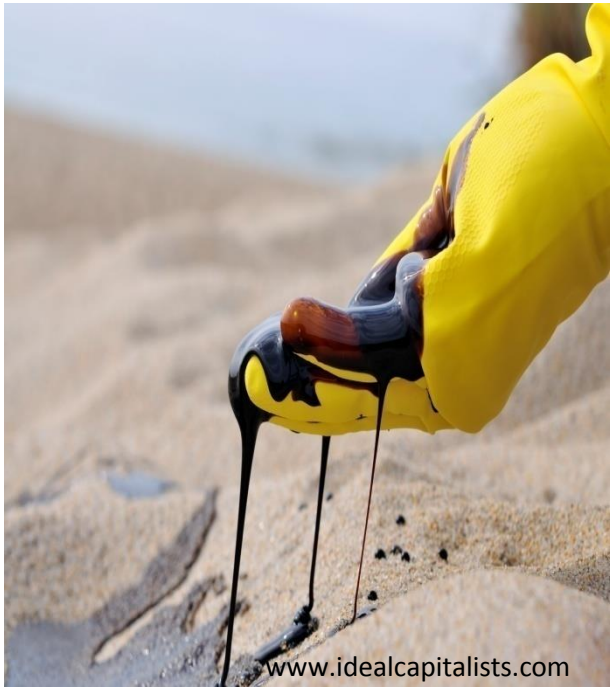
nd: no deformity recorded and all of the larvae survived.
d. A% denotes % of Type-A deformity, d. B %: denotes % of Type-B deformity, and d. C %: denotes % of Type-C deformity.
[s]: denotes number of larvae at start of exposure (0 h).
*: denotes that 13 out of 14 larvae survived.
[f]: denotes WAF prepared by using 1.0g KCO/l seawater.

# Main Findings

1) Larvae hatched during exposure were vulnerable to KCO WAF toxicity.

2) As a result, larvae demonstrated specific deformity types which reflected the stress induced by the toxic effect of crude oil on the overall health status of each larva, compared to the healthy control larvae which did not exhibit any deformity.

3) In the exposed larvae, mainly:
- Type-A (Lordosis-inward curvature of spine or V-shaped).
- Type-B (Kyphosis-hunchback).
-Type-C (Scoliosis-Lateral bending of spine) deformities were observed.



4) TPH of 2.22 mg/l caused fish larval deformity at ≤1.0g KCO/l loading which was higher that what was observed in literature where hatchability was decreased at ≥0.5-mg TPH.

5) WAF prepared at 1-g KCO/l showed a linear response in percentage abnormality of larvae expose to dilution series.

6) Vertebral abnormalities which amounted to more than 90% of skeletal defects recorded in various KCO treatments.

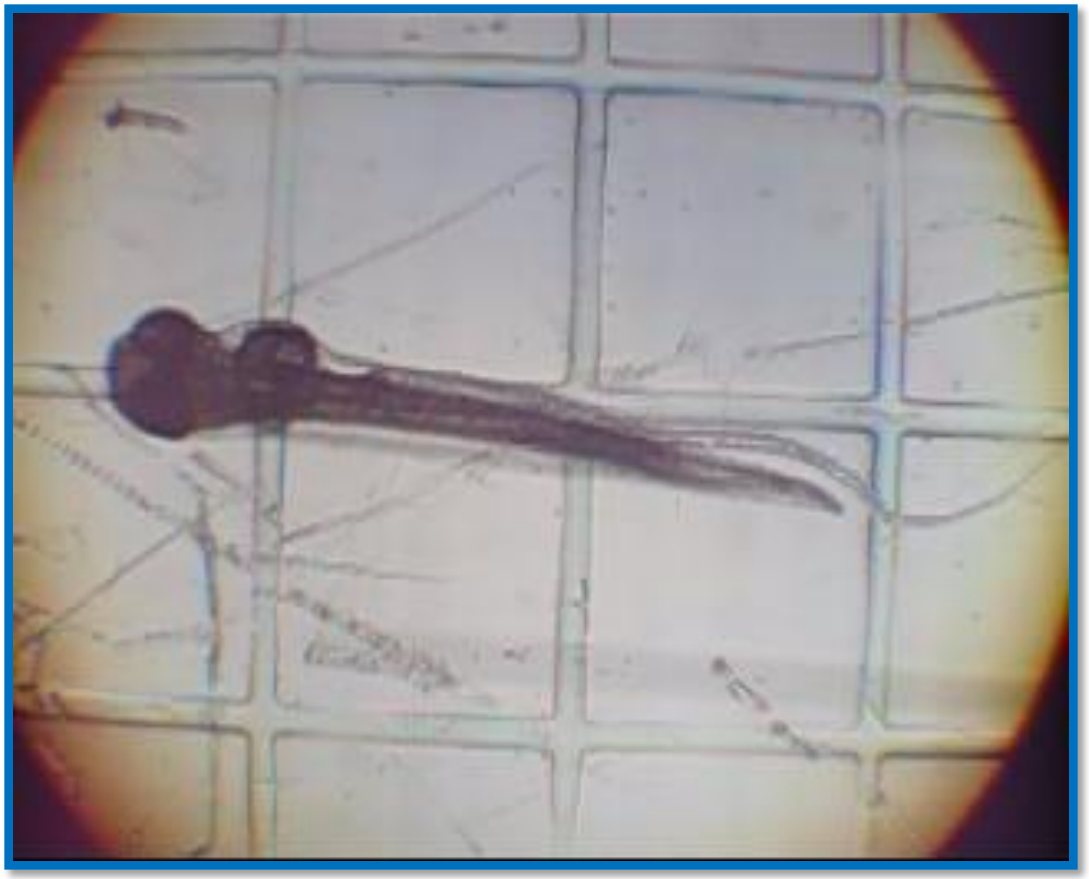
7) PAHs detected in WAF of KCO may possibly be the causative agents as PAHs are known to exert distinct type of abnormalities as observed in the present exposure experiments.

8) In short-term toxicity tests (24, 48, 72, 96 h), the toxicity of crude oil was due to dissolved hydrocarbon.

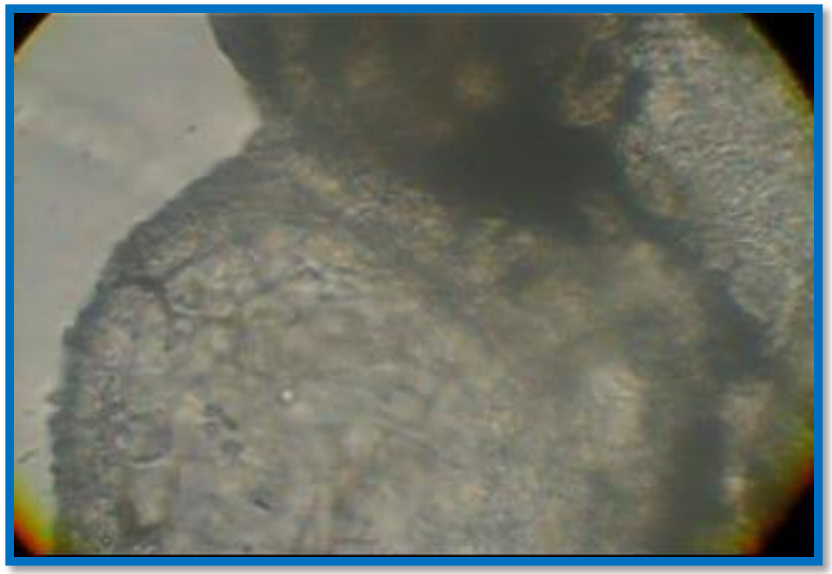
9) The concentration of TPH in marine water of Kuwait ranges from 0.0015 to 0.026 mg TPH/l seawater with occasional values touching more than 0.02 mg TPH/l seawater (EPA Kuwait, 2008).

10) Therefore, although normally, the concentration of TPH in marine water of Kuwait remains far below the concentration that can induce morphological deformity nonetheless, risk of exposure does exist.

# Control Fish Larvae



# Deformed Fish Larvae
# Reduced Heart Beat



# THANK YOU


I WAS WONDERING WHEN THIS WAS GOING TO WASH UP ON SHORE.. -DO YOU THINK ANYONE WILL EVER NOTICE?!
OIL ADDICTION