



# Effects of Copper on Survival and Growth of Marine Finfish

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# Copper release and regulation in Japan

- 101,272 kg of copper were released into public water bodies in 2006 (Ministry of Economy, Trade and Industry)
- Waste Water Standard for industrial effluent: 3mg-Cu/L
- No Environmental Quality Standard

# Environmental Quality Standard to protect aquatic organisms

- Ministry of the Environment started to decide new Environmental Quality Standard in 2,000 to protect aquatic organisms
- Copper: one of the candidates for the Standard
- The Standard value = acceptable concentration with long-term exposure to aquatic organisms living in and around Japan

# Copper toxic data to finfish

- Ecotox database: <http://cfpub.epa.gov/ecotox/>
  - <Fish> <Copper> <Laboratory> <Mortality, Growth>  
<Flow-through, Renewal, Static>
  - Publication years: 1915-2007

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Data in total: 3,708

Freshwater, 3,312

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Saltwater, 390

Unknown, 6

Species along the coast of Japan, 48

Others, 342

$\leq 4$  days, 37

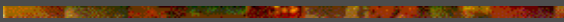
4 days <  $\leq 7$  days, 9

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> 7 days, 2

# Experimental study



- Acute toxicity test for juvenile
    - Testing water
    - Fish size
    - Water temperature
  - Long-term toxicity test for juvenile
  - Toxic data and Environmental Quality Standard
  - Long-term toxicity test for larval stage (trial)
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# Testing fish used in this study

red sea bream  
(*Pagrus major*)



Japanese flounder  
(*Paralichthys olivaceus*)



- Important species for fishery and aquaculture in Japan
- Available of juvenile throughout the year
- Test method: OECD TG 203

# Acute toxicity test procedure

## ■ Fish size

- Red sea bream: 0.5 to 13 g (7 sizes)
- Japanese flounder: 1.3 to 17 g (5 sizes)
- $20.0 \pm 0.5$  C

## ■ Water temperature

- Red sea bream (0.5, 1.1 g): 12, 15, 20, 25 C
- Japanese flounder (0.3, 0.4 g): 10, 15, 20, 25 C

## ■ Testing water

- Low pH artificial seawater

## ■ Range of concentration

- 0.04 to 41 mg-Cu/L

## Copper precipitation in natural seawater (pH 8.1)



Copper added into natural  
seawater at 10 mg-Cu/L

Natural seawater

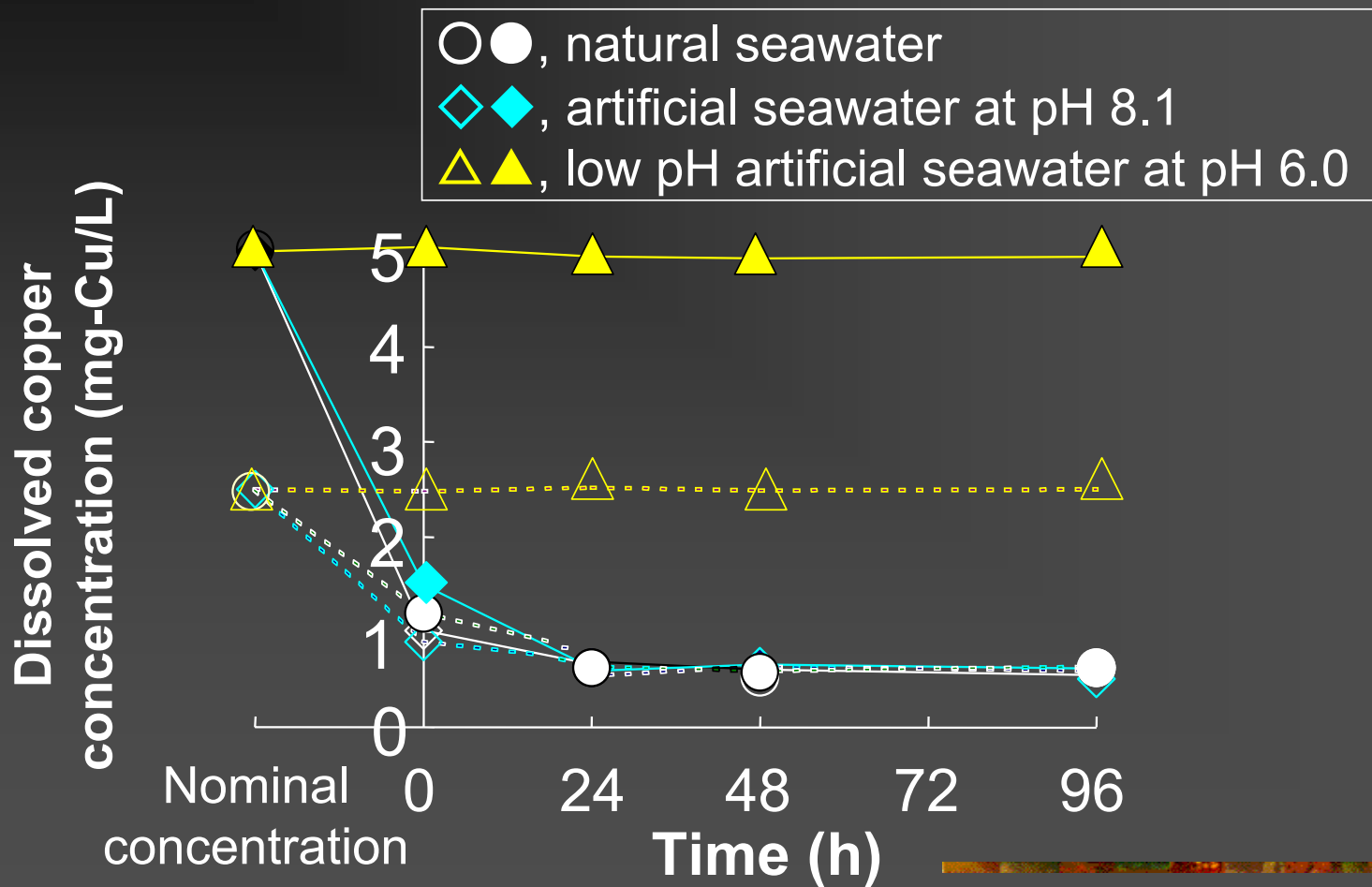


# Composition of low pH artificial seawater used in this study

Reagent	Composition (mg/L)	
NaHCO <sub>3</sub>	192	artificial seawater by Lyman and Fleming (1940)
KCl	660	
CaCl <sub>2</sub> · 2H <sub>2</sub> O	1,459	
Na <sub>2</sub> SO <sub>4</sub>	3,912	
MgCl <sub>2</sub> · 6H <sub>2</sub> O	10,631	
NaCl	23,477	

pH5.4~6.7

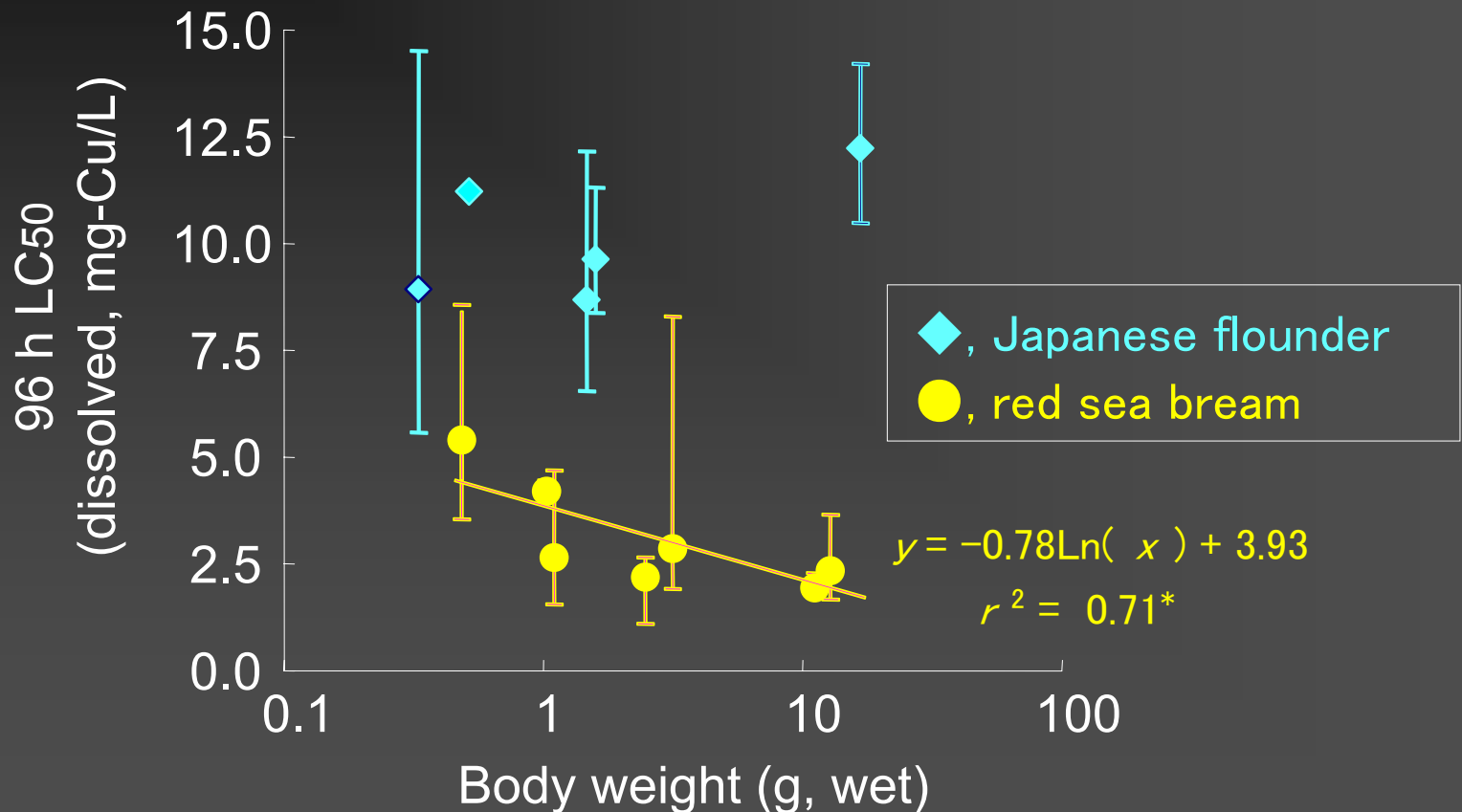
# Copper solubility in natural and low pH artificial seawater



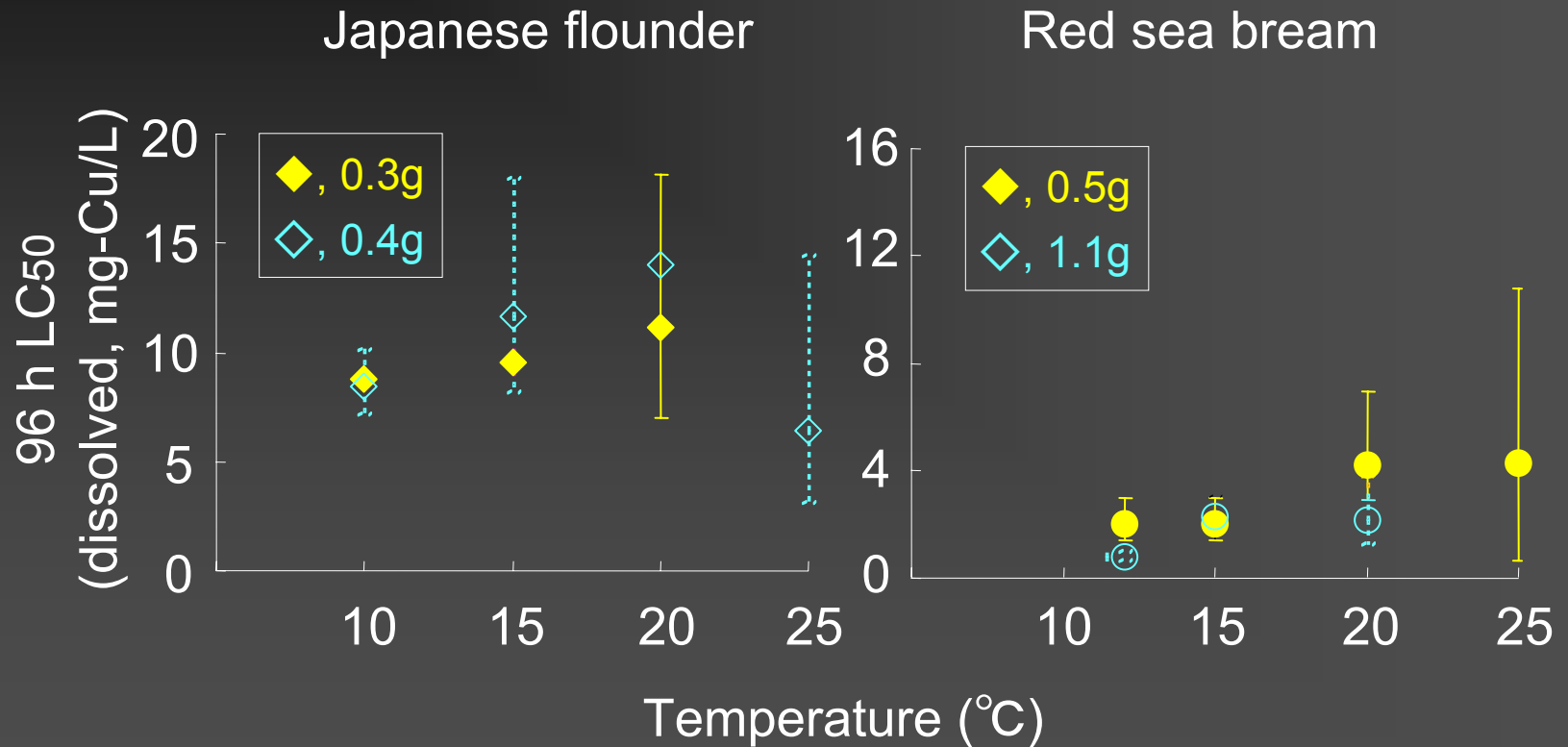
## Mortality and growth of Japanese flounder in natural and low pH artificial seawater

Rearing water	Survival rate (%)	Body weight (wet, g)	
		Initial	Final
Natural seawater	$98 \pm 4$	$0.3 \pm 0.0$	$1.6 \pm 0.2$
Low pH artificial seawater	$95 \pm 3$	$0.3 \pm 0.0$	$1.5 \pm 0.2$

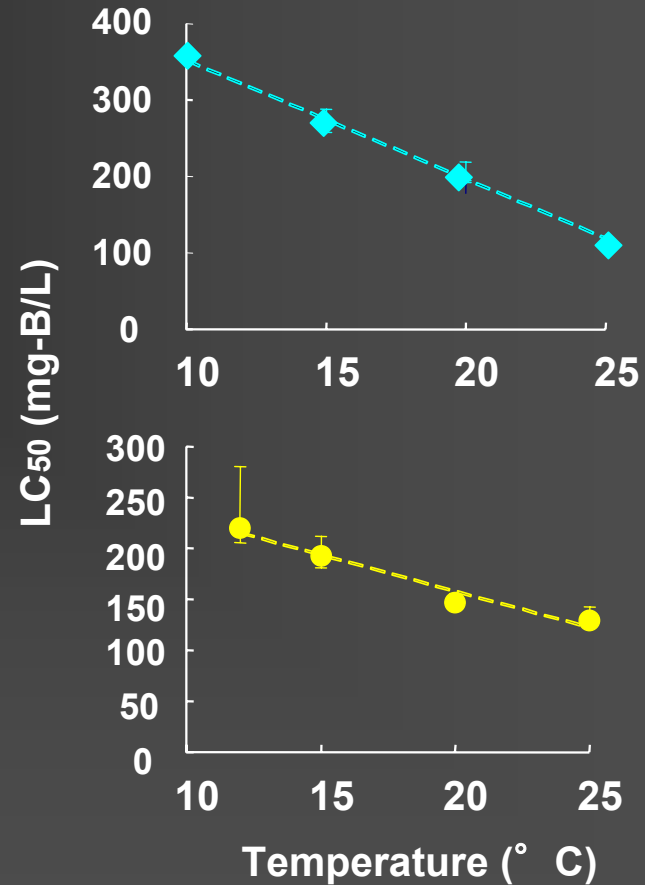
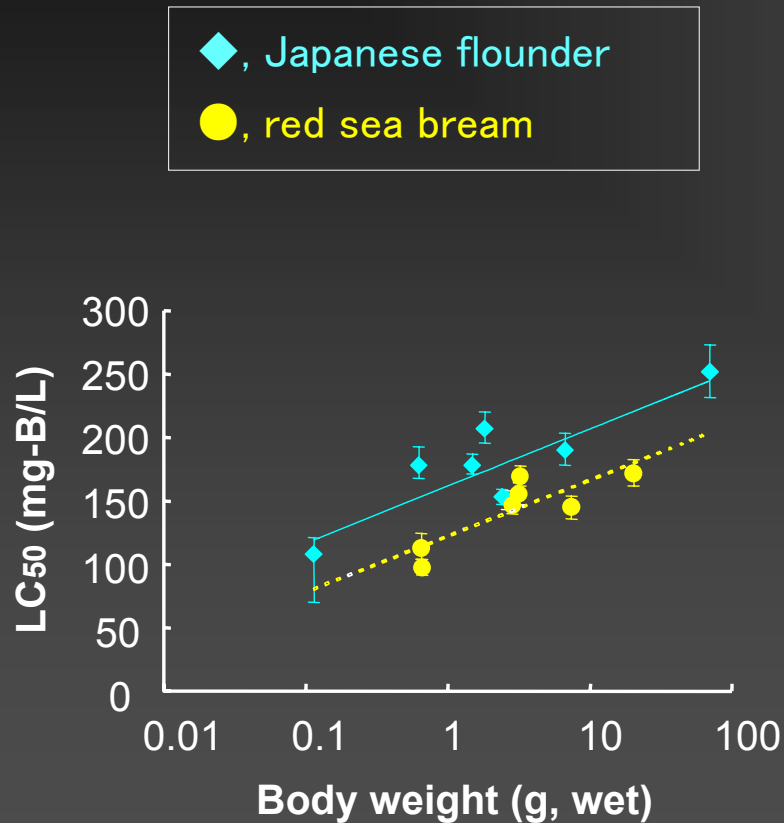
# Effect of fish size on copper acute toxicity



# Effect of water temperature on copper acute toxicity



# Effects of fish size and water temperature on acute toxicity of boron

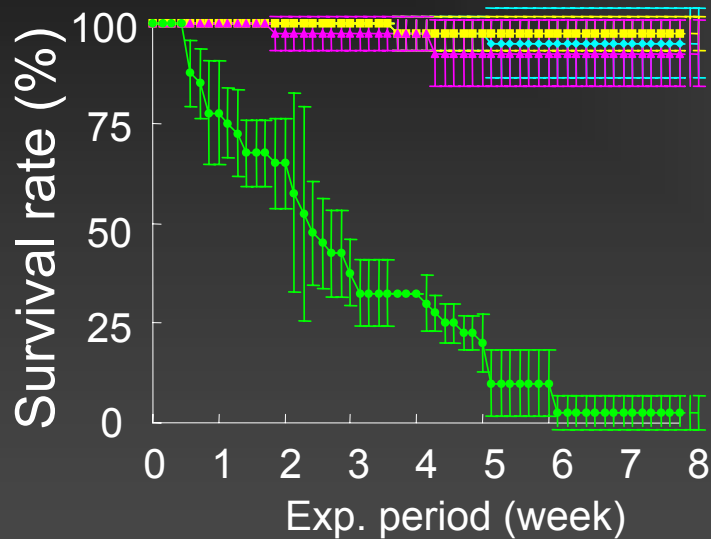


# Long-term toxicity test for juvenile

- OECD TG 215
- Fish: juvenile of 0.5 g
- Water temperature: 20 C
- Duration: 56 days
- Exposure type: flow-through
- Concentrations: 4 including control, 4 replicates
- Testing water: natural seawater (salinity 35, pH 8.1)
- Feeding: commercial pellet diet, twice daily, 6 days a week
- Effect measurement: mortality and growth
- Endpoint: LOEC

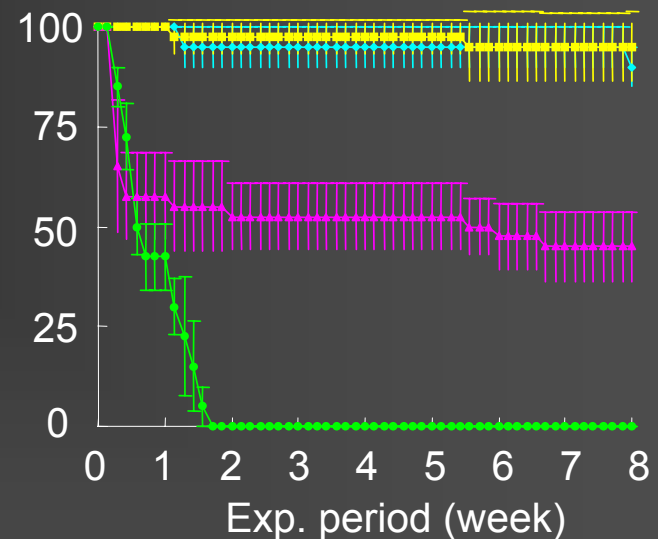
# Mortality in long-term toxicity test

Japanese flounder



Control  
0.1 mg-Cu/L  
0.3 mg-Cu/L  
0.9 mg-Cu/L

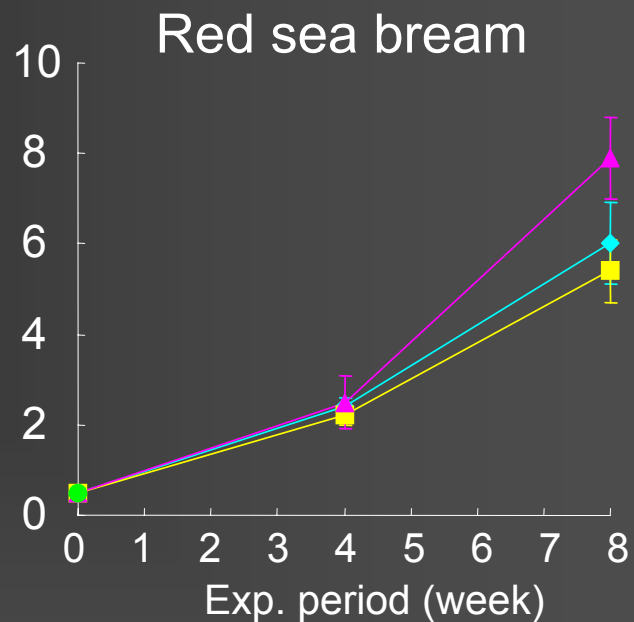
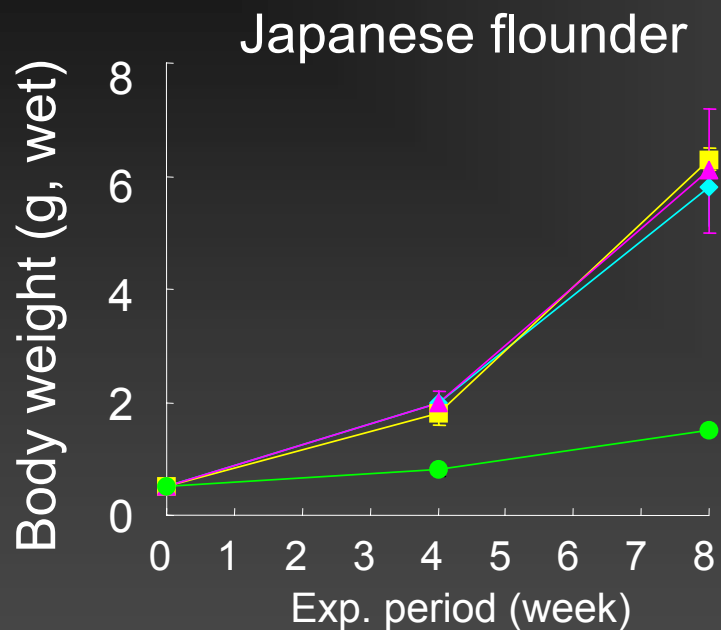
Red sea bream



Control  
0.04 mg-Cu/L  
0.12 mg-Cu/L  
0.36 mg-Cu/L



# Growth of survived fish in long-term toxicity test



# LOEC of copper in long-term toxicity test

Fish	LOEC	
	Mortality	Growth
Red sea bream	0.12	ND
Japanese flounder	0.9	0.9

# Summary of the results in acute and long-term toxicity tests for juvenile

- **Acute toxicity test**
  - Precipitation and testing water
  - Effect of fish size and water temperature on copper toxicity: not clear
- **Long-term toxicity test**
  - Acceptable concentration for mortality and growth: under 0.1 mg-Cu/L
  - Effect concentration: at least one-order smaller of acute LC50
- **Sensitivity: red sea bream > Japanese flounder**
- **Different physiological response between 2 fish species**

# Acceptable concentration of copper for marine finfish

Acute toxic level to juvenile  
> 1 mg-Cu/L

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Long-term toxic level  
to juvenile = 0.1 mg-Cu/L

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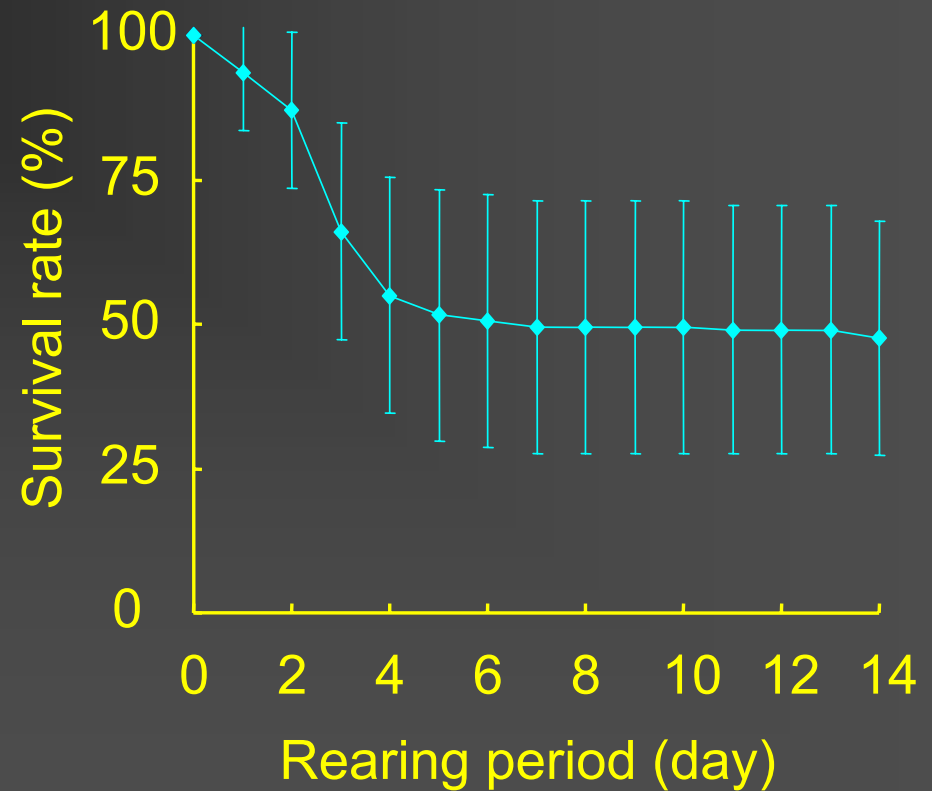
Acceptable copper  
level for grow-out phase:  
under 0.1 mg-Cu/L

Long-term toxic level  
to embryo to larva = ???

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Acceptable copper  
level for reproduction: ???

# Larval survival of false clown anemonefish



# Long-term toxicity test for larva

- Fish: larva just after hatching
- Concentrations: 0, 0.04 – 0.64 mg-Cu/L
- Density: 15 ind./500mL
- Replication: 4
- Testing water: natural seawater
- Water temperature: 26.5 C
- Exposure type: static-renewal
- Feeding: newly hatched *Artemia* nauplius
- Duration: 30 days
- Effect measurement: mortality and growth
- Endpoint: LOEC

# Effect of copper on mortality and growth of larval false clown anemonefish

- NOEC and LOEC for mortality: ND
- NOEC and LOEC for growth: 0.04 and 0.08 mg-Cu/L
- Positive effect of copper at 0.04 to 0.16 mg-Cu/L