larvi 2013

6th fish & shellfish larviculture symposium

Improving visual environment in cod larval rearing by factorial designs

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UNIVERSITY OF NORDLAND

IMPROVING VISUAL ENVIRONMENT IN COD LARVAL REARING BY FACTORIAL DESIGNS

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**Science Insight

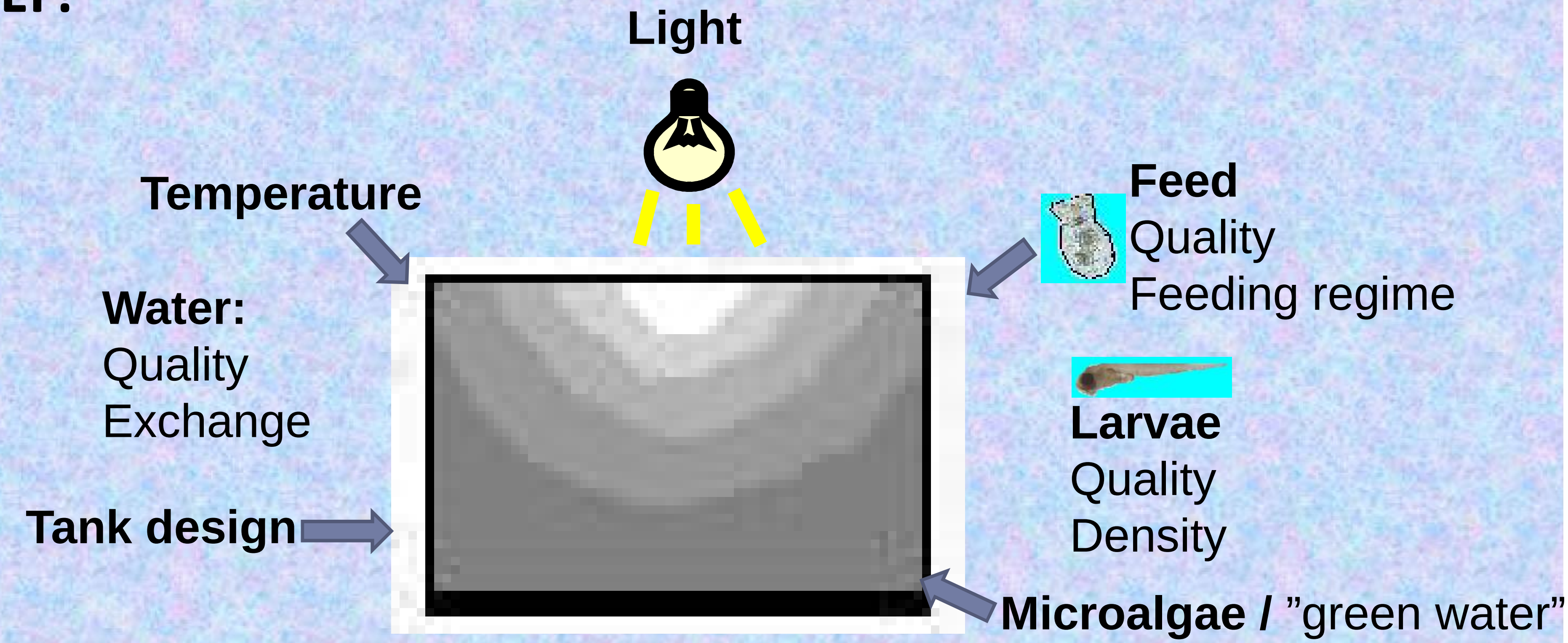
BACKGROUND

- **Hatchery production: Bottleneck when establishing new marine fish species for aquaculture:**
    - Sea bream / sea bass (early 80s – 1990s) Chatain, 1997
    - Atlantic cod (1999 – recent) Rosenlund and Skretting, 2006

- **Still needs for further optimization of Atlantic cod hatcheries**
    - Unstable outcome / crashes
    - Quality/ deformation

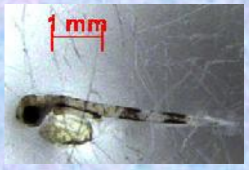
BACKGROUND

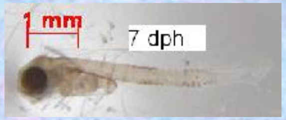
•**COMPLEXITY – multiple factors affect larval performance – JOINTLY!**

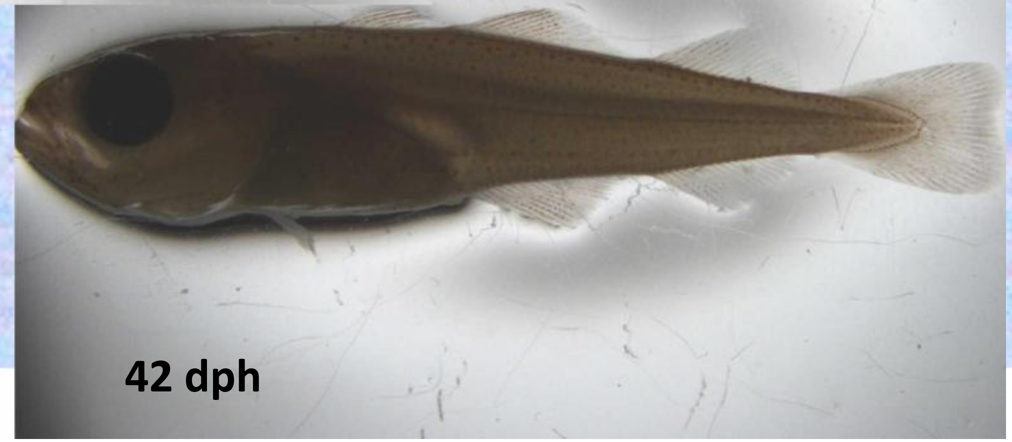


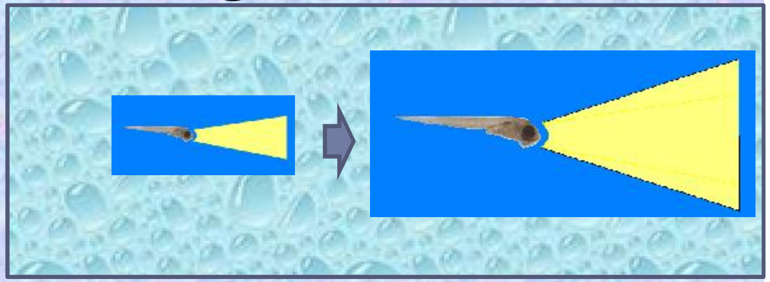
BACKGROUND

- **DEVELOPMENT – environmental demands change**





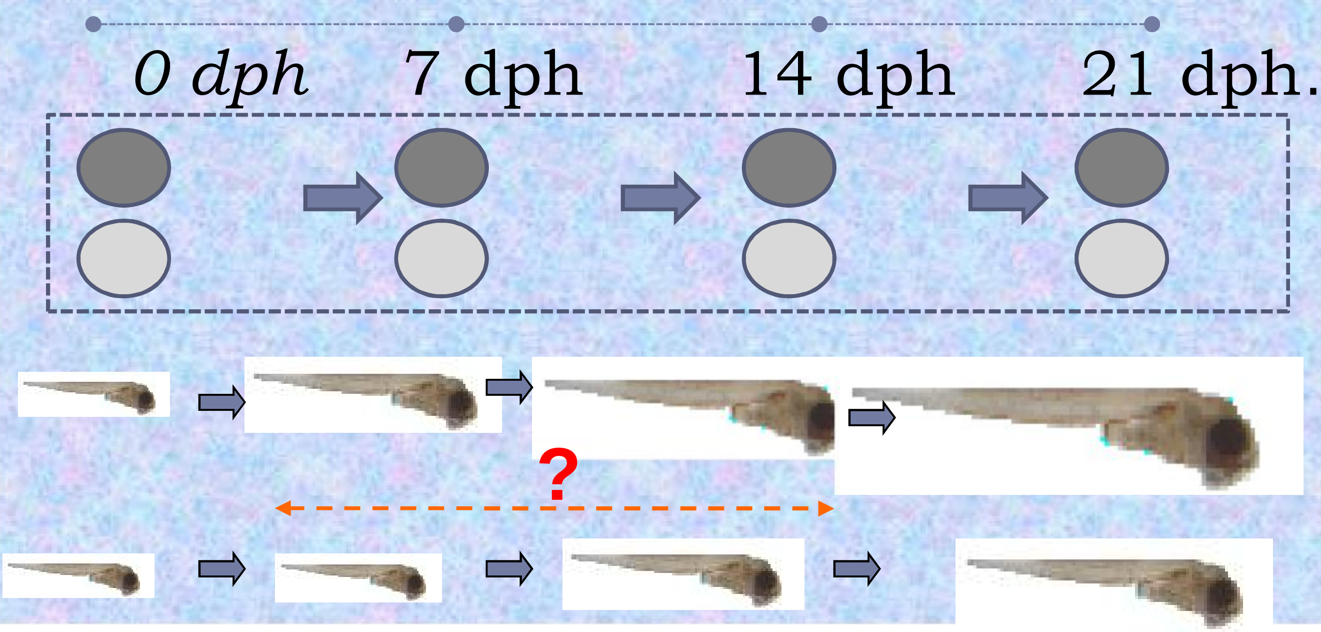




BACKGROUND

•Despite COMPLEXITY and DEVELOPMENTAL issues:

•Most studies of tank environment apply a one-variable-at-a-time approach (OVAT) and "classical" long-term designs:



- Interactions between factors not accessible
- Difficult to assess treatment effects at specific time
- (Relatively large scale – heterogenous environment)

OUR AIM

Through small scale short term factorial approaches study:

- How factors that shape **visual environment** JOINTLY affect larval performance

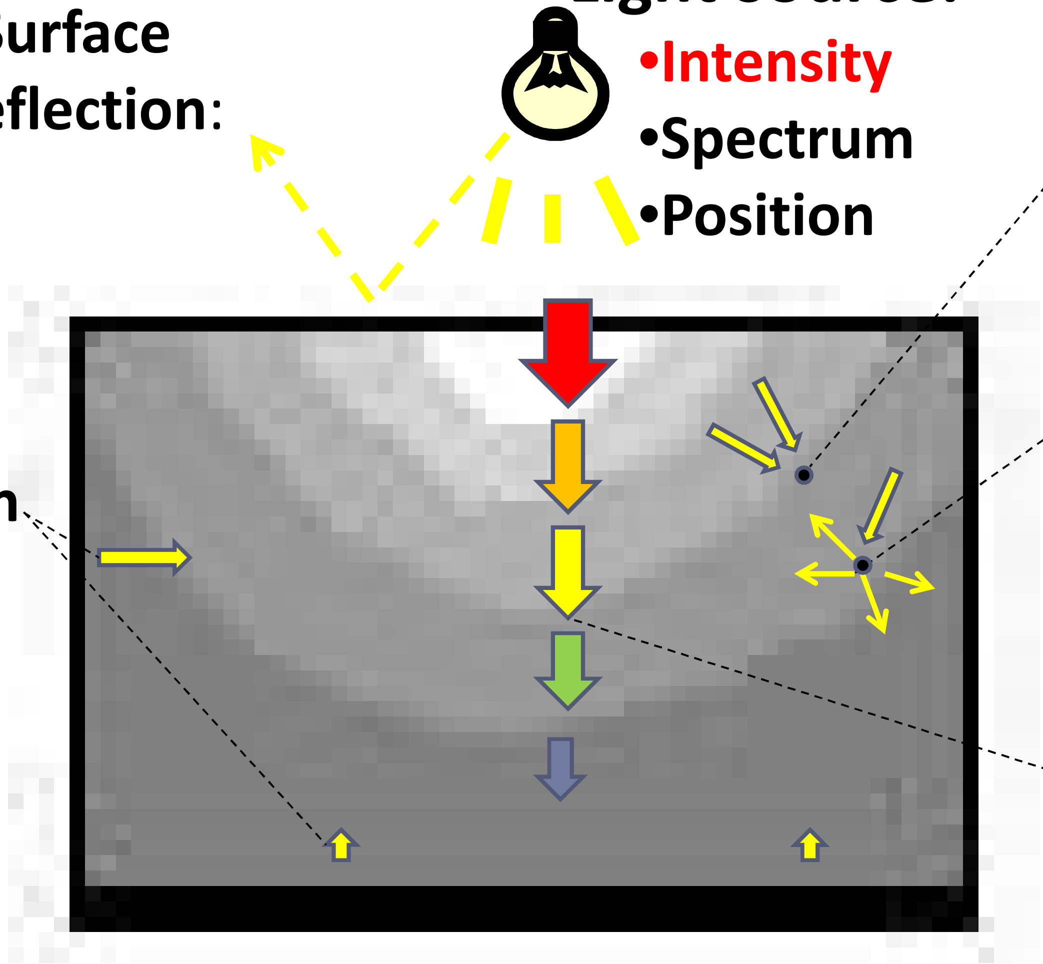
- How larval response to visual environment **changes** with age/ size

in order to guide further fine-tuning of rearing conditions

FACTORS THAT SHAPE VISUAL ENVIRONMENT OF TANKS

- **Light source:**
  - **Intensity**
  - **Spectrum**
  - **Position**

- **Surface reflection:**



- **Particle absorption:**
  - Dissolved particles
  - **Algae**

- **Scattering**
  - Particles
  - Bubbles
  - **Algae**

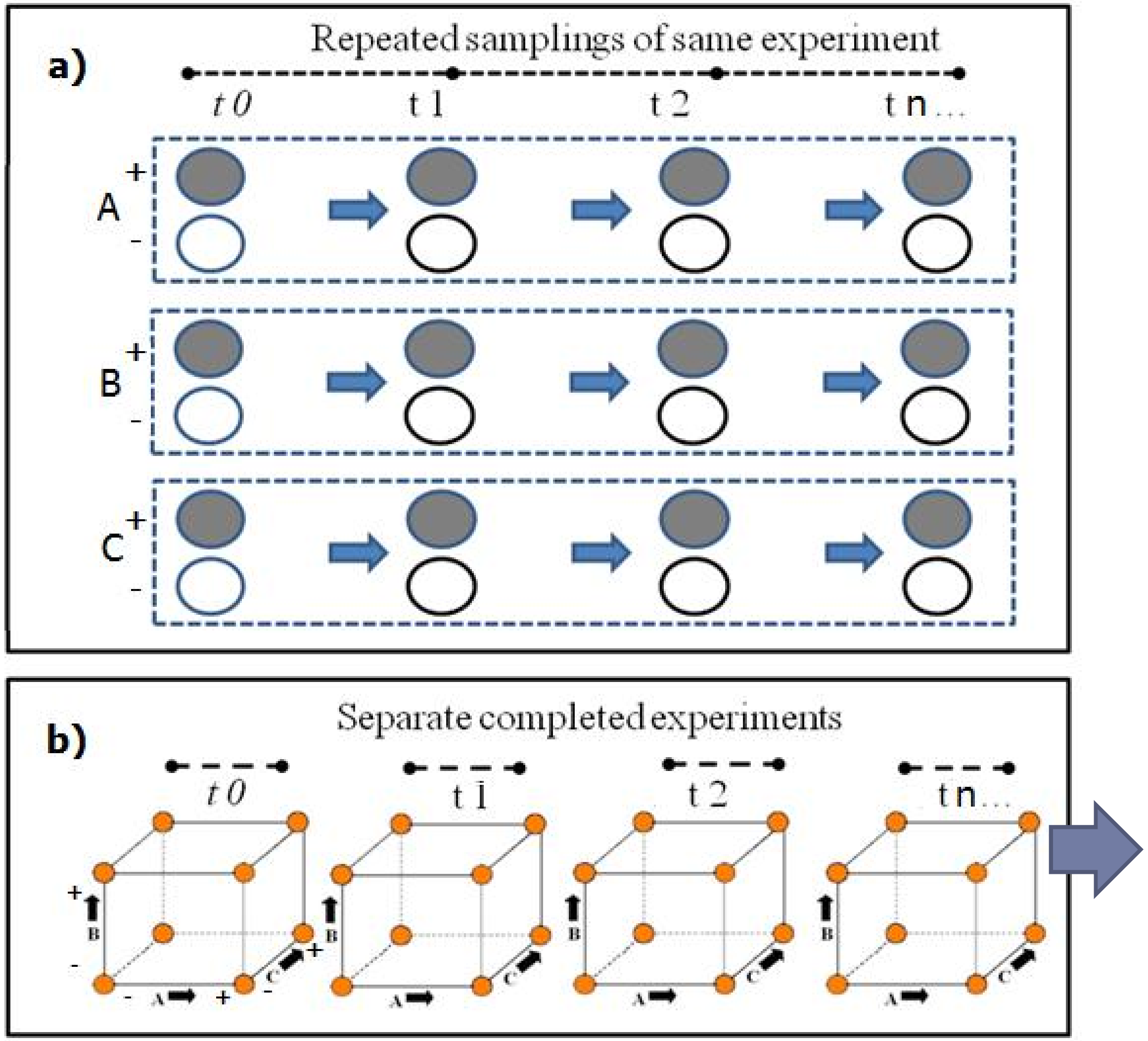
- **Tank reflection**
  - **Tank colour**
  - Materials

- **Seawater absorption:**
  - Selective
  - Tank size/ depth

DESIGN PRINCIPLES

TRADITIONAL:
OVAT
Long-term

HERE:
Factorial design
Short-term
Small scale



A	B	C
-	-	-
-	-	+
-	+	-
-	+	+
+	-	-
+	-	+
+	+	-
+	+	+

OVERVIEW OF EXPERIMENTS

1. **Foraging success:** Examine combined effects from light intensity, bottom colour, algae and rotifer density on foraging as a proxy for larval performance.

2. **Phototaxis:** Examine combined effects from light source(s)and algae on phototaxis

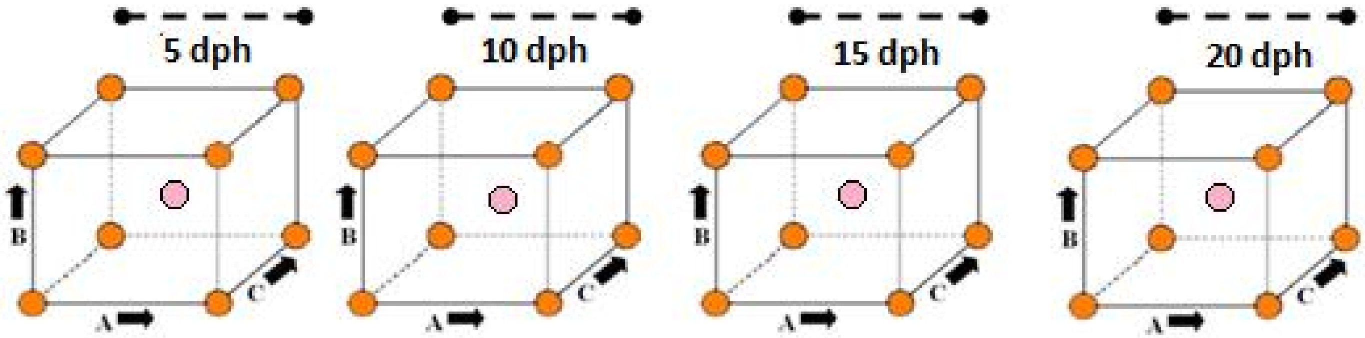
3. **Spatial distribution:** Examine combined effects from wall colour and light intensity on spatial distribution of larvae

EXPERIMENT 1: FORAGING SUCCESS

Nicolaisen, Cuny and Bolla (submitted 2013)

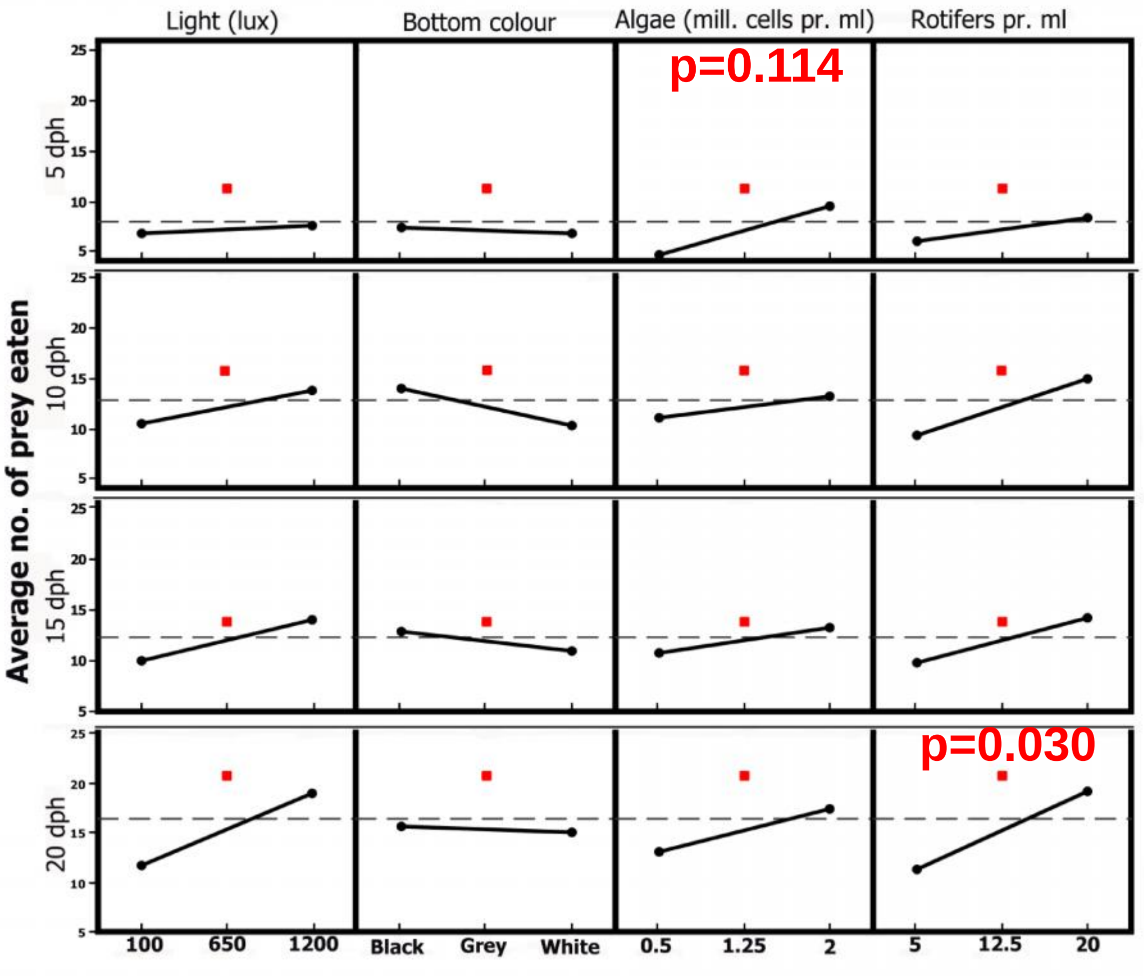
Factors	Low	High
Light(lx)	100	1200
Algae (mill. cells/ml)	0.5	2.0
Feed (rotifers/ml)	5	20
Bottom (black/grey/white)	Black	White

- 2^4 factorial with centre point
- 10 L cylindrical black tanks
- Unfed larvae (+18 hrs)
- 4 hr trials
- Response: average no. of rotifers in larval guts per tank (N=20 units)



● = centerpoint, L=650, A=1.25, F=12.5, B=Grey, n=4

EXPERIMENT 1: FORAGING SUCCESS



MAIN EFFECTS

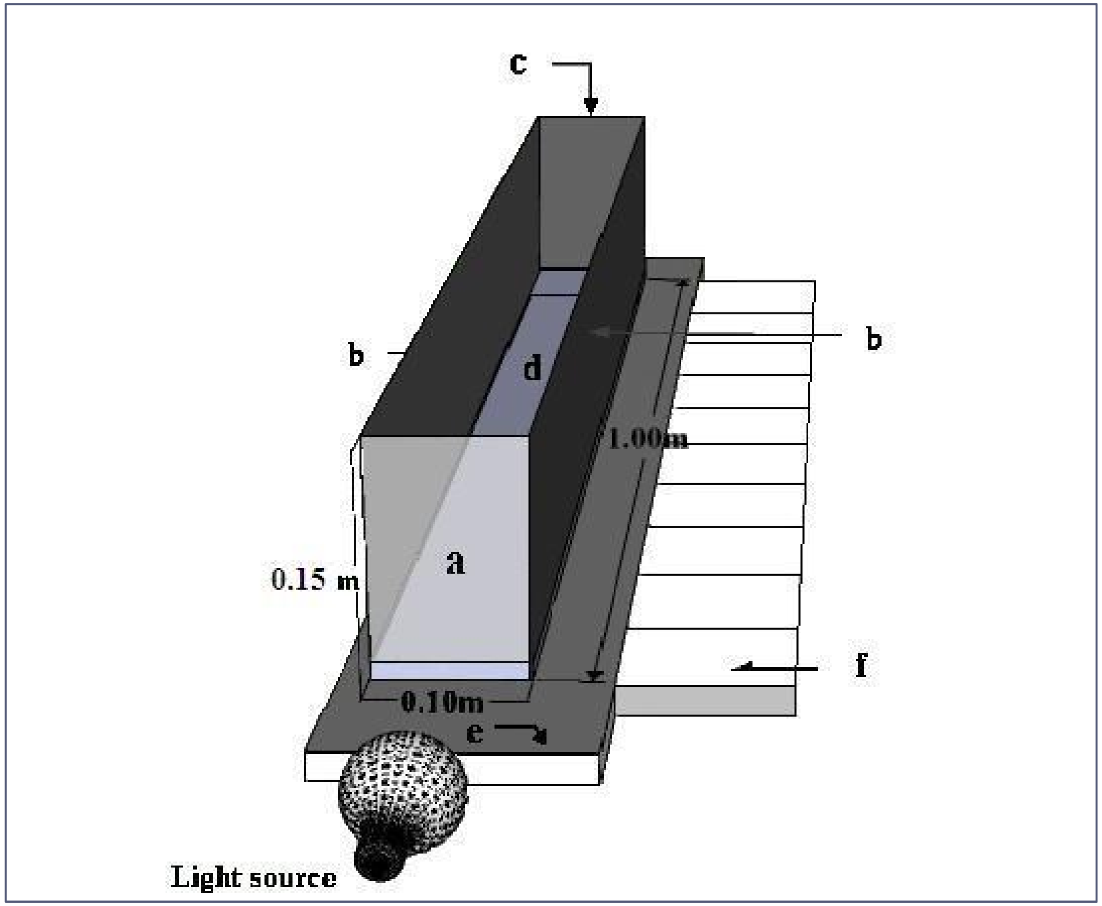
- Grey/ black bottom best all days
- Algae: Strongest effect at 5 dph
- Prey density: Effect at 20 dph

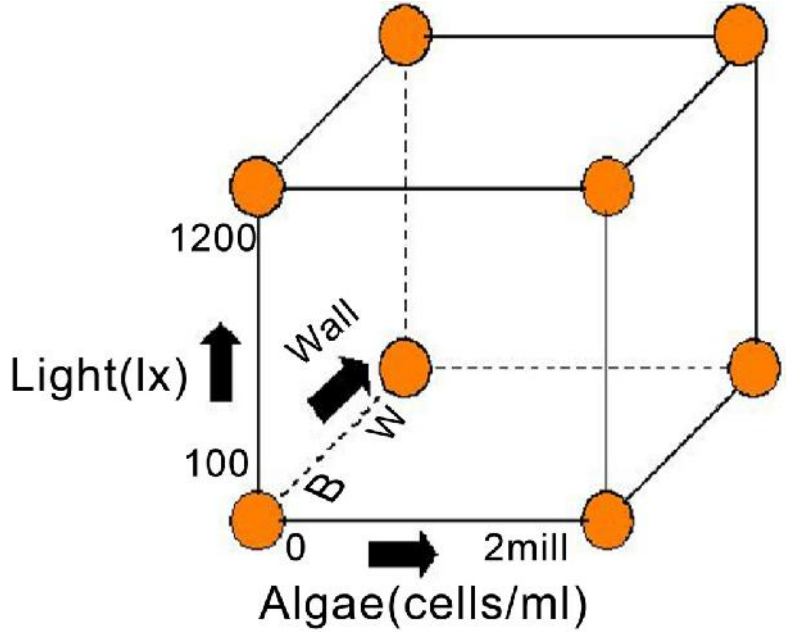
INTERACTIONS

- Light x bottom colour at 15 dph
- Prey x bottom colour at 15 dph
- Light x algae at 20 dph

EXPERIMENT 2: PHOTOTAXIS

Nicolaisen and Bolla (submitted 2013)





- **2^3 factorial experiments, duplicated**
- **At 5, 10, 15, 20, 27 and 35 DPH**
- 40 larvae per run, duration 20 min
- Larval position assessed in duplicate

EXPERIMENT 2: PHOTOTAXIS

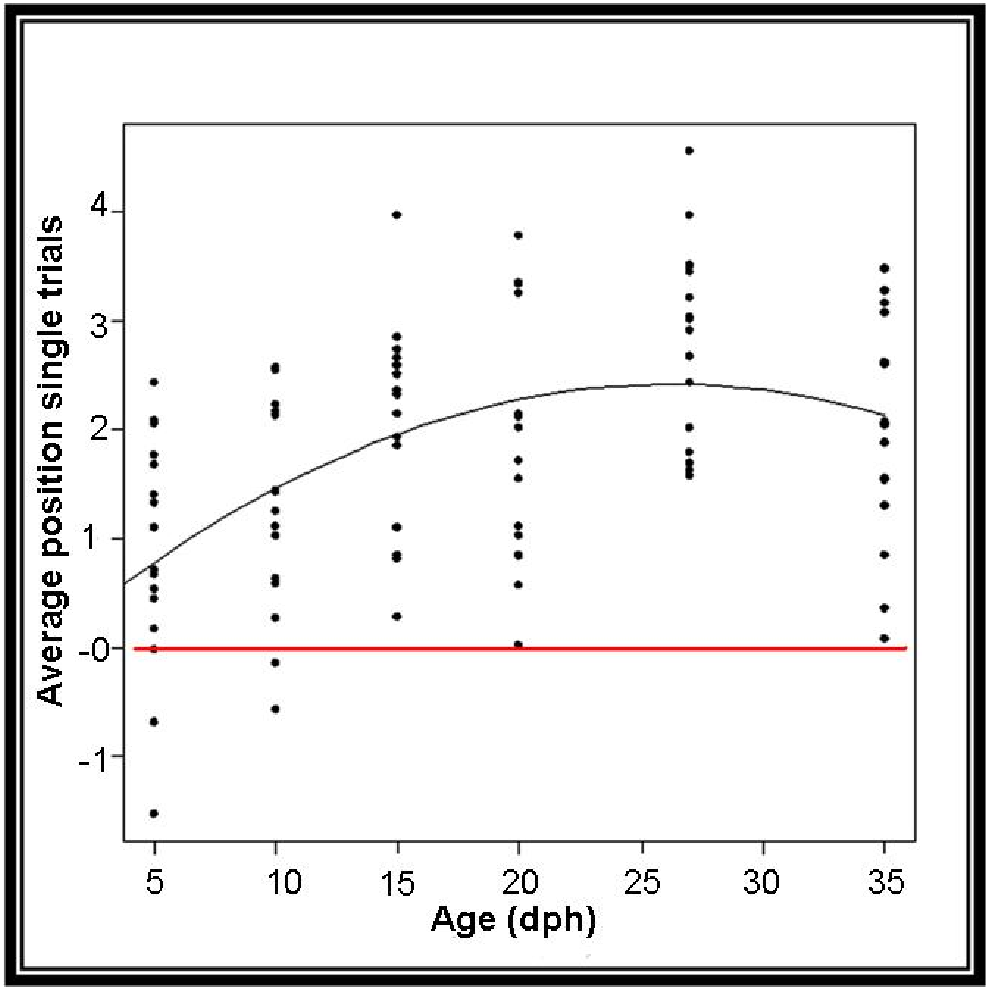


Fig. phototactic response with age

Polynomal regression:

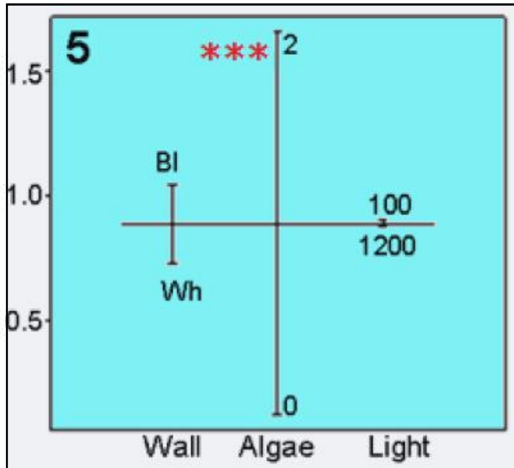
Y=0.08 + 0.19age – 0.004 age^2

	Df	SS	MS	F value	Pr(>F)
Age	1	19.707	19.707	18.6968	3.845e-05 ***
I(Age^2)	1	10.498	10.498	9.9598	0.002156 **
Residuals	93	98.027	1.054		

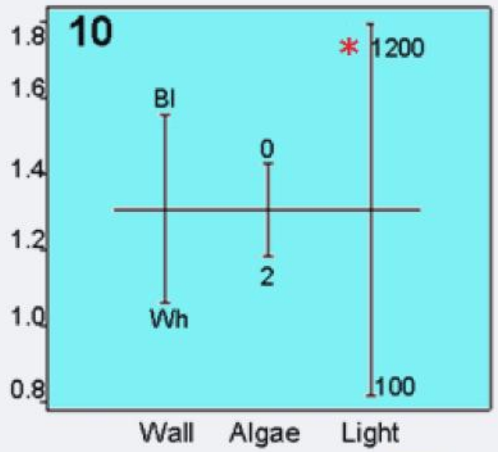
•SIGNIFICANT QUADRATIC TERM INDICATES CURVED RESPONSE

•PREDICTED VALUES INDICATE INCREASED ORIENTATION TOWARDS LIGHT SOURCE FROM 5-27 DPH,
THEN RESPONSE LEVELS OUT / IS REDUCED.

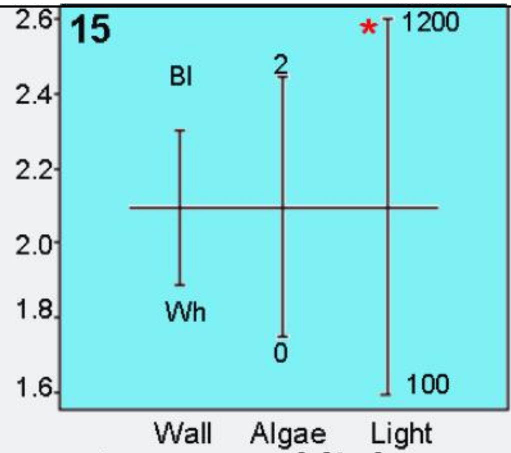
EXPERIMENT 2: PHOTOTAXIS



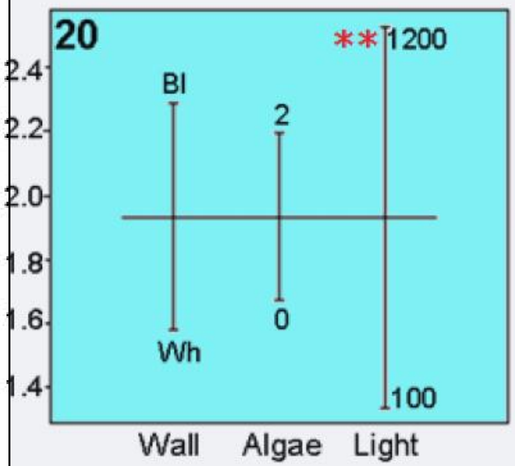
Algae ➡ Towards light source



Increased light ➡ Towards light source

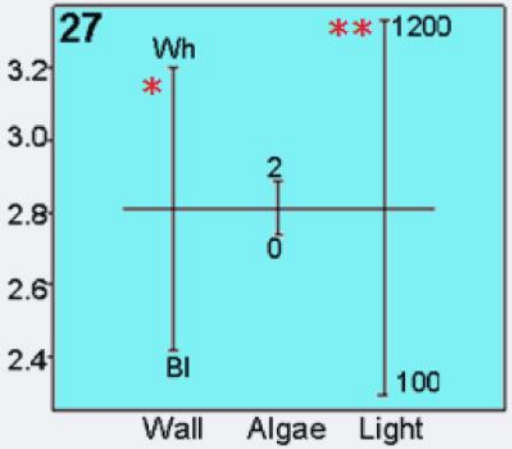


Increased light ➡ Towards light source

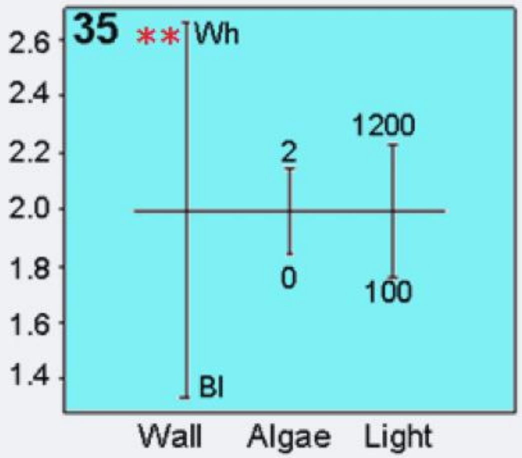


Increased light ➡ Towards light source

3-way interaction **



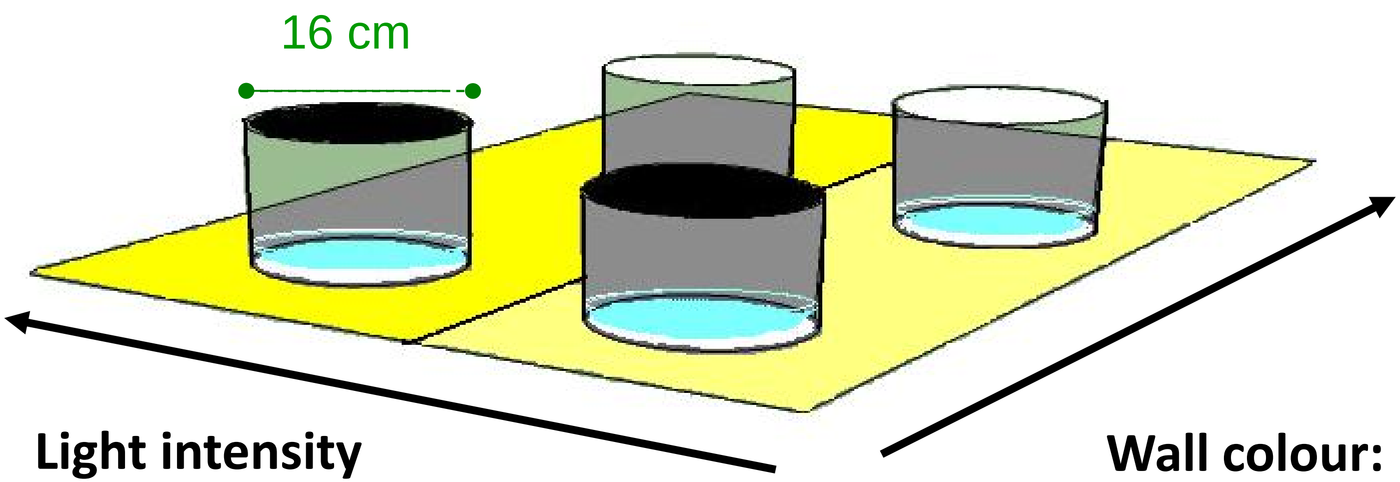
Increased light ➡ Towards light source



White wall ➡ Towards light source

EXPERIMENT 3: SPATIAL DISTRIBUTION

Nicolaisen and Bolla (submitted 2013)



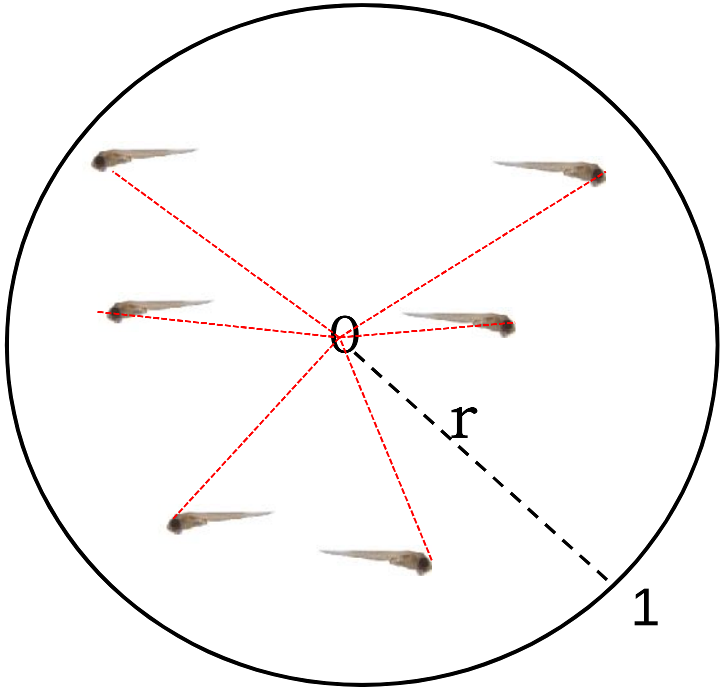
Factor	Low	High
Light(lx)	100	1200
Wall	Black	White

Light intensity
1200 vs 100 lx

Wall colour:
Black vs. white

- **2^2 factorial screening designs, duplicated - totally 8 runs per. day**
- **At 6, 11, 16, 21 and 28 dph**
- **50 larvae, 0.5 liter volume, duration: 20 min**
- **Larvae photographed and the distance from centre measured**

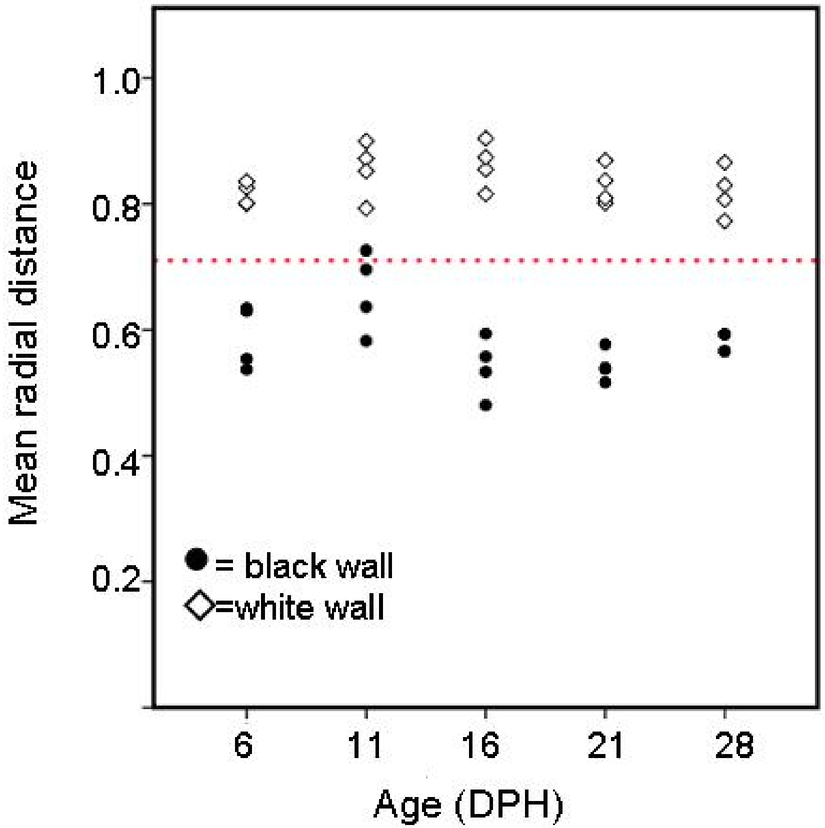
EXPERIMENT 3: SPATIAL DISTRIBUTION

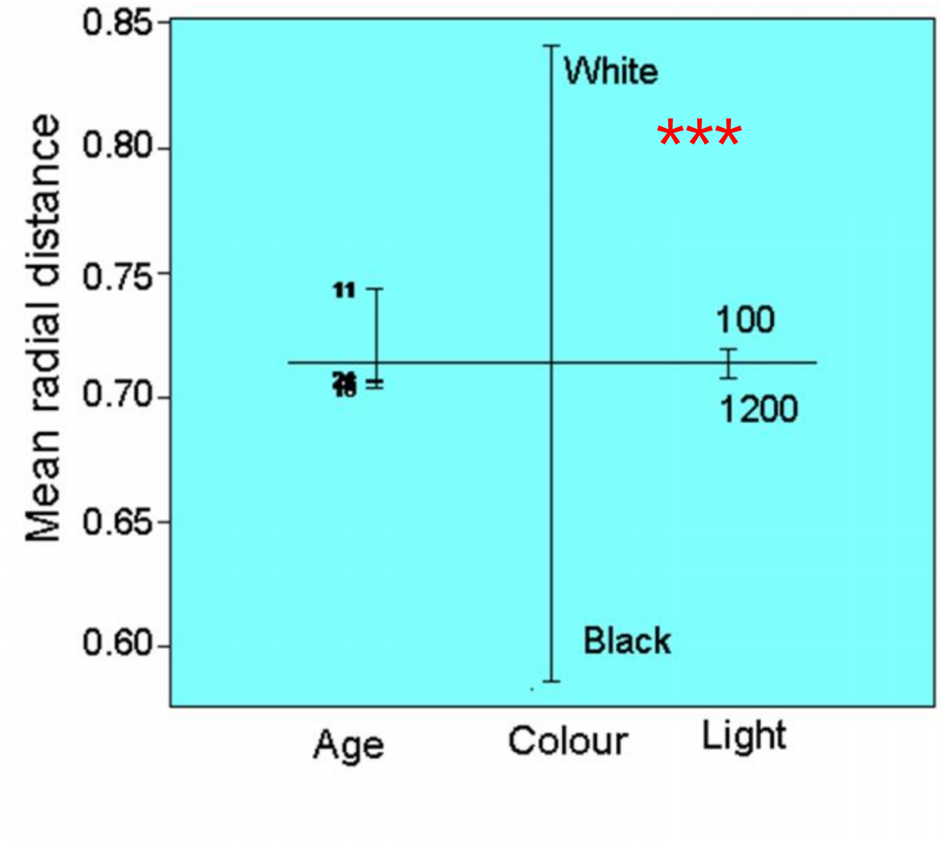


Response= Average relative radial distance at each single run

General linear model:
Covariate=age
Fixed factors= Light intensity and wall colour

EXPERIMENT 3: SPATIAL DISTRIBUTION





•**Wall colour: <u>Very strong</u> effect on larval position** (GLM, $F_{1,38}$=260.7, p<<0.001)

•Average position in black walled tanks 0.58, in white walled tanks 0.84 (43% increase)

•**Minimal effects from age and light intensity within this experimental domain**

SUMMARY OF EXPERIMENTS

FORAGING SUCCESS

- Beneficial effect from algae at 5 DPH
- Darker tank bottoms better than white
- Rotifer density > 5/ml beneficial at increased larval age

PHOTOTAXIS

- Increased positive phototaxis from 5 - 27 DPH
- Effect from algae apparent at 5 DPH

SPATIAL DISTRIBUTION

- White tank walls severely affect larval distribution at all ages - walling

TAKE HOME MESSAGE FACTORIAL APPROACH

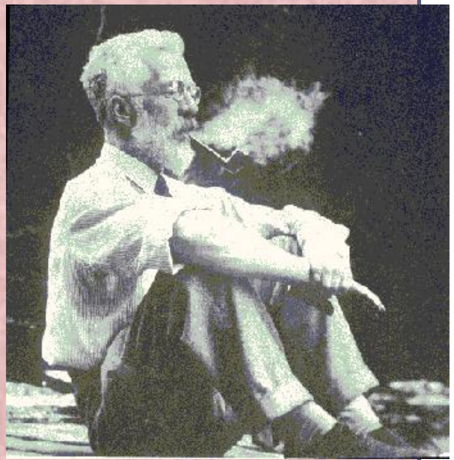
- Capable to reveal both important factors and their respective interactions - suggests future focus

- Reveals stage specific larval responses to multiple factors

- Applicable also in commercial production settings

Widely used in agriculture / industry R&D from the 1920s. Deserves increased use in aquaculture, experimentally as well as in industrial scale R&D.

Superior in search for optimal settings of multiple factors.



Sir Ronald Aylmer Fisher (1890–1962)

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