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# Application of RAS Technology in Hatchery Production

*Spearheading Marine Aquaculture in the Tropics*

# Outline

1. **Background**
2. **Flow-through Systems**
3. **Adoption of RAS Technology**
    - Dynamic Salinity
    - Mass Balance Analysis
    - Microbial Control Strategy
4. **Summary**

# Background

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- **4 of top 10** food fish producers in the world are in South East Asia
- Contributed **15.8%** of global fish culture production
- **31 billion of fish frys required, more expected in future**

**Top 10 Food Fish Aquaculture Producers (2014)**

| Rank | Country | Total (mil tonnes) | % of global share |
|---|---|---|---|
| 1 | China | 27.22 | 54.6% |
| 2 | India | 4.48 | 9.0% |
| 3 | Indonesia | 3.64 | 7.3% |
| 4 | Vietnam | 2.69 | 5.4% |
| 5 | Bangladesh | 1.83 | 3.7% |
| 6 | Norway | 1.33 | 2.7% |
| 7 | Egypt | 1.13 | 2.3% |
| 8 | Chile | 0.97 | 1.9% |
| 9 | Myanmar | 0.90 | 1.8% |
| 10 | Philippines | 0.67 | 1.3% |

Source: SOFIA, 2016

Are we able to produce enough fish fry **sustainably** to meet future demand?

# Background

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## Prevalent Hatchery Method In South East Asia – Outdoor Ponds (aka Mesocosm System)

*Flow-thru' System*

**Advantages**

- Natural Food Chain
- Low density production

→ Low Operating Cost

→ Ease of Management

**Limitations**

- Open environment
- Minimal water treatment

→ Large fluctuations in water quality

→ Risk of entry of pathogens

# Background

## Common Hatchery Method In South East Asia – Indoor Tanks

*Flow-thru' System*



- Mainly for high-value species like groupers
- Production **cost is higher** compared to outdoor pond production
- Better disease control and more intensive production
- **Control** over the physical parameters like lightings and temperature
- **Better water treatment processes**

# Background

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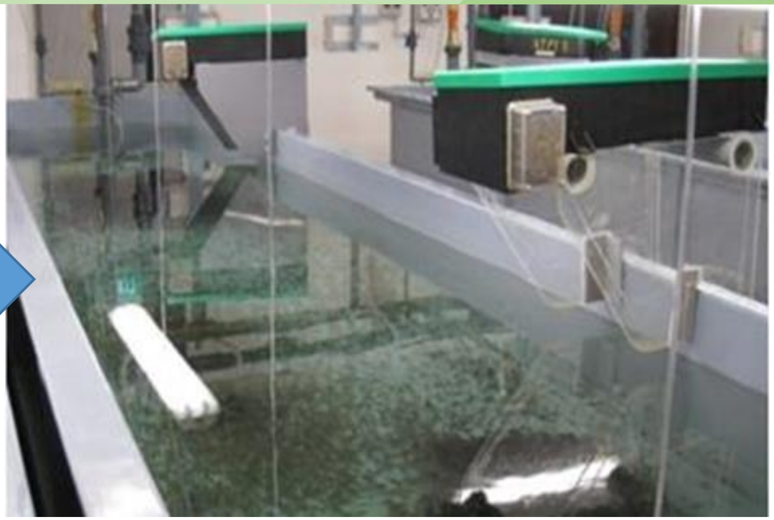
## Our Asian Seabass Hatchery Experience in the Marine Aquaculture Centre (MAC)

- Utilised indoor tank (flow-through) system
- Survival rates @ post-weaning: 10-20% → **40-50%**
- Key to our success is the **early weaning** to reduce cannibalism rate

**Day 0** — Larviculture — **Day 12** — **Weaning** — **Day 21** — Post-weaning — **Day 30**

Larval rearing tanks



Weaning tanks

# Background

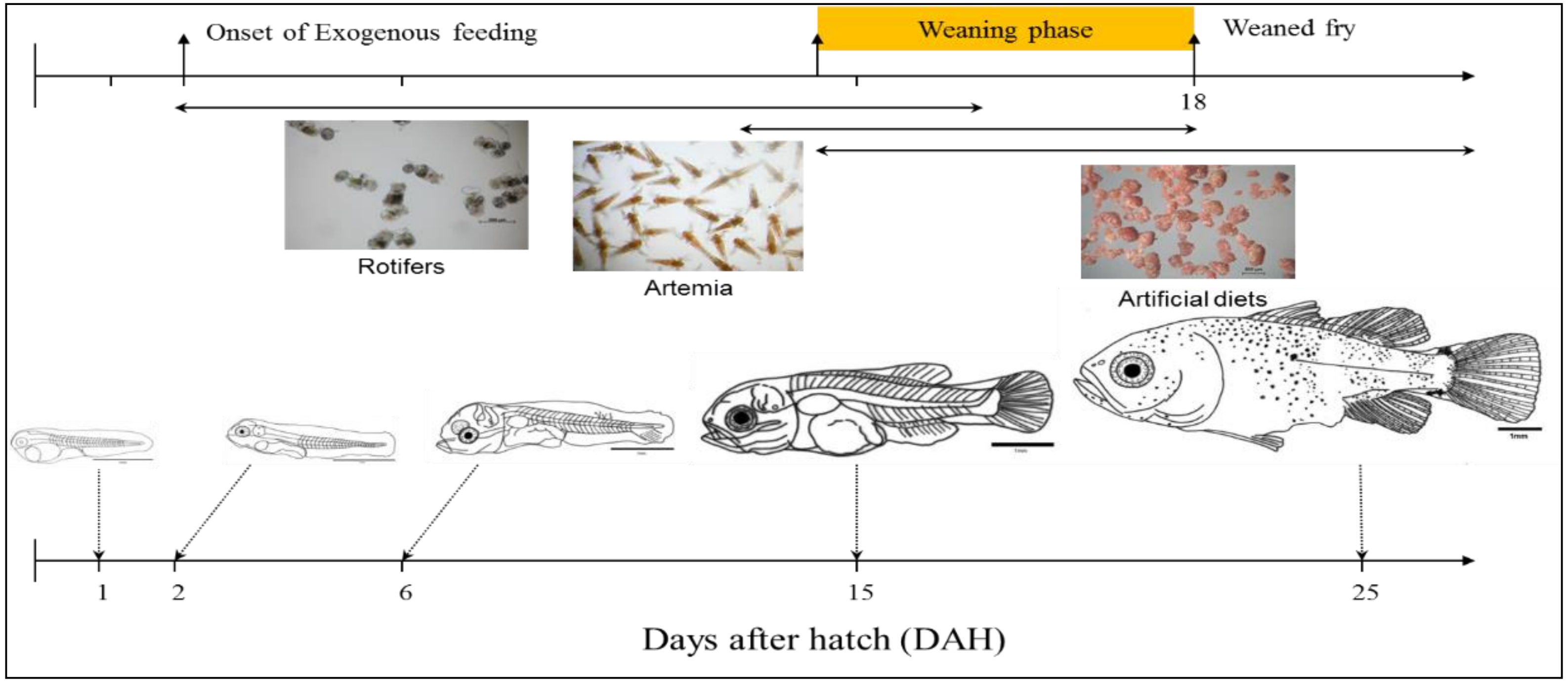
## Larval Development and Feeding Regime of Asian Seabass (*Lates calcarifer*)

During weaning, water quality is maintained through:

i) increasing water flow,

ii) reducing density %

iii) use of purpose-built weaning tanks (for ease of operation)



# Background

## General feeding regime for Seabass Larviculture

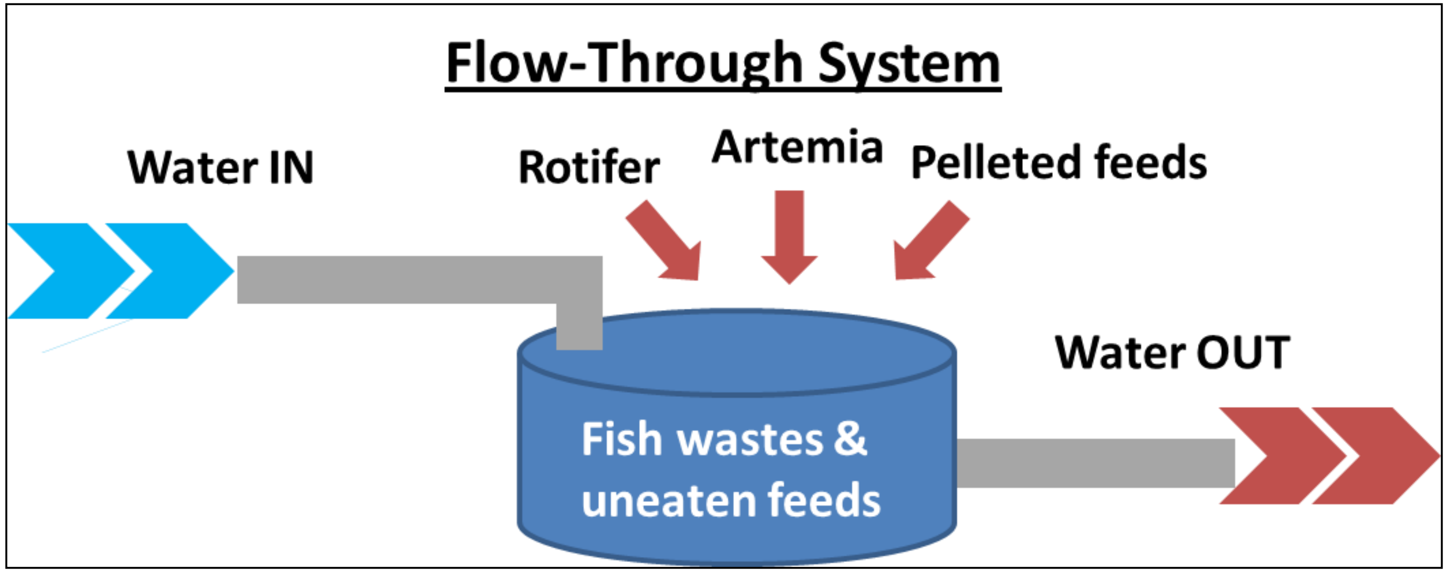
| Day | Algae (mil/ml) | Rotifers (ind/ml) | Artemia (ind/ml) | Artificial feed (g) | Water X-change (%/hr) |
|---|---|---|---|---|---|
| 0-1 | 0.2 | 0 | | | - |
| 2-4 | 0.2 | 2-5 | | | Trickling |
| 5-6 | 0.2 | 5-8 | | | 3-5% |
| 6-12 | 0.2 | 10-15 | | | 5-10% |
| 12-14 | | 15 | 0.5-1 | Sprinkle (2hourly) | 10-20% |
| 15-20 | | | 0.5 | Sprinkle (hourly) | 20-50% |
| 21-27 | | | | 8-10% B.W | 50% |
| 28-35 | | | - | 8-10% B.W | 50% |
| Transfer to Early Nursery | | | | | |

**Water quality management is simply increasing water flow rate to maintain the desired levels**

# Flow-Through Systems (FTS)

**Removal of fish wastes & uneaten feeds from the culture tank:**

- ✓ Toxic ammonia ($NH_3/NH_4$) - <0.5ppm TAN
- ✓ Suspended solids (SS) – linked to gill cover deformity
- ? High bacteria load → disease outbreak




Does higher water exchange (flushing) help to reduce the **bacteria load** in tank?

# Flow-Through Systems (FTS)

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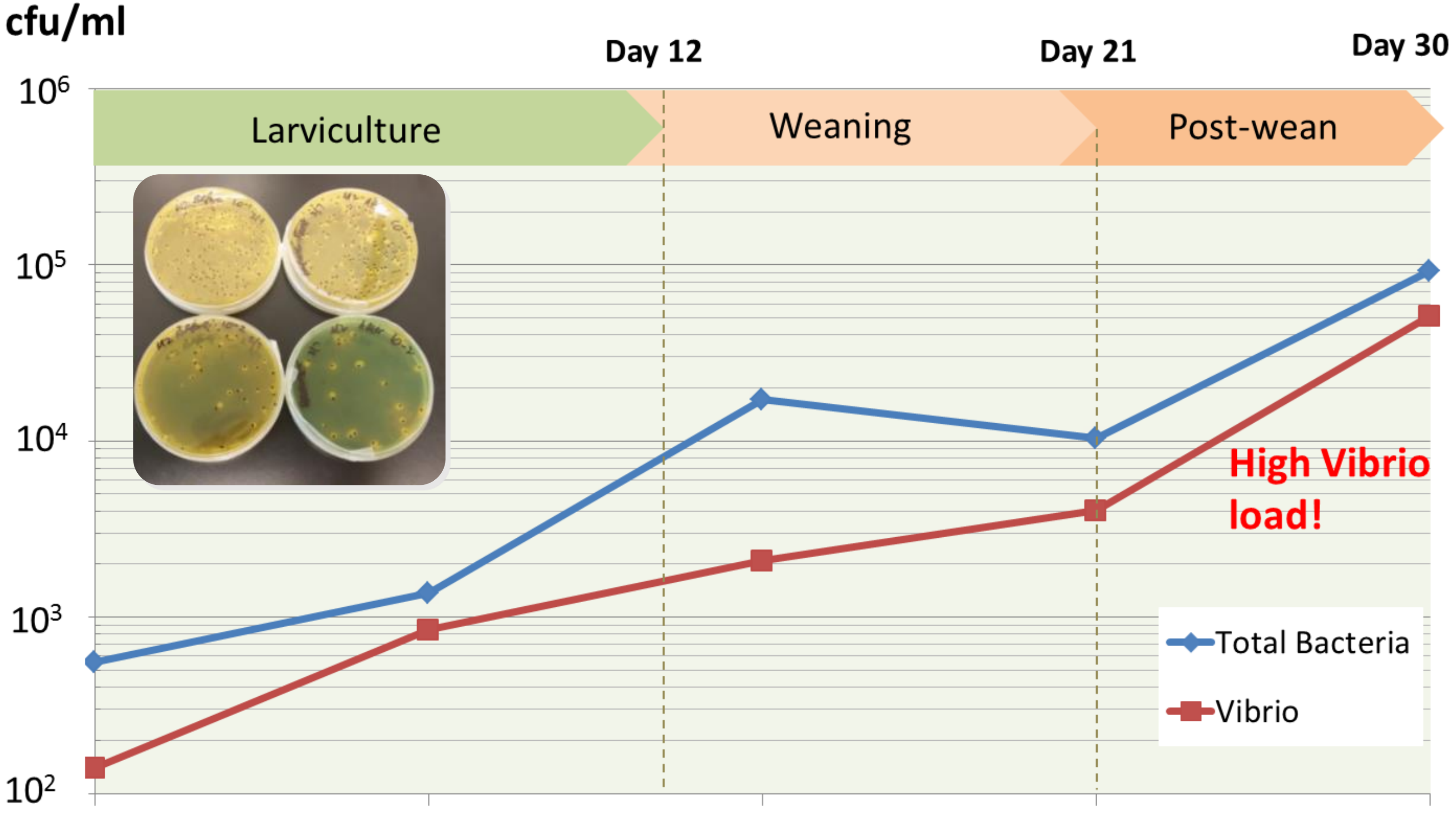
## Bacteria Loads in FTS Larviculture




**Opportunistic (r-selective) bacteria** like ***Vibrio spp.*** can overwhelm the culture system very quickly when there are excess nutrients in water!

# Flow-Through Systems (FTS)

## Disease outbreak! – Big Belly Disease

- With intensifications, productions were severely affected by a **novel disease** called Big-Belly syndrome.
- Pathogen is a intracellular bacteria and <u>very similar to *Vibrio spp.*</u>


* Disease first described by Intervet/MSD

**Big Belly Syndrome***

<u>Size of fish affected:</u>

- Fry (18-30 days) to 20g

<u>Clinical signs:</u>

- Darken and bloated belly
- Very thin tail
- Aggregation of internal organs

<u>Severity:</u>

- Up to **80% cumulative mortality within 1 week**

# Flow-Through Systems (FTS)

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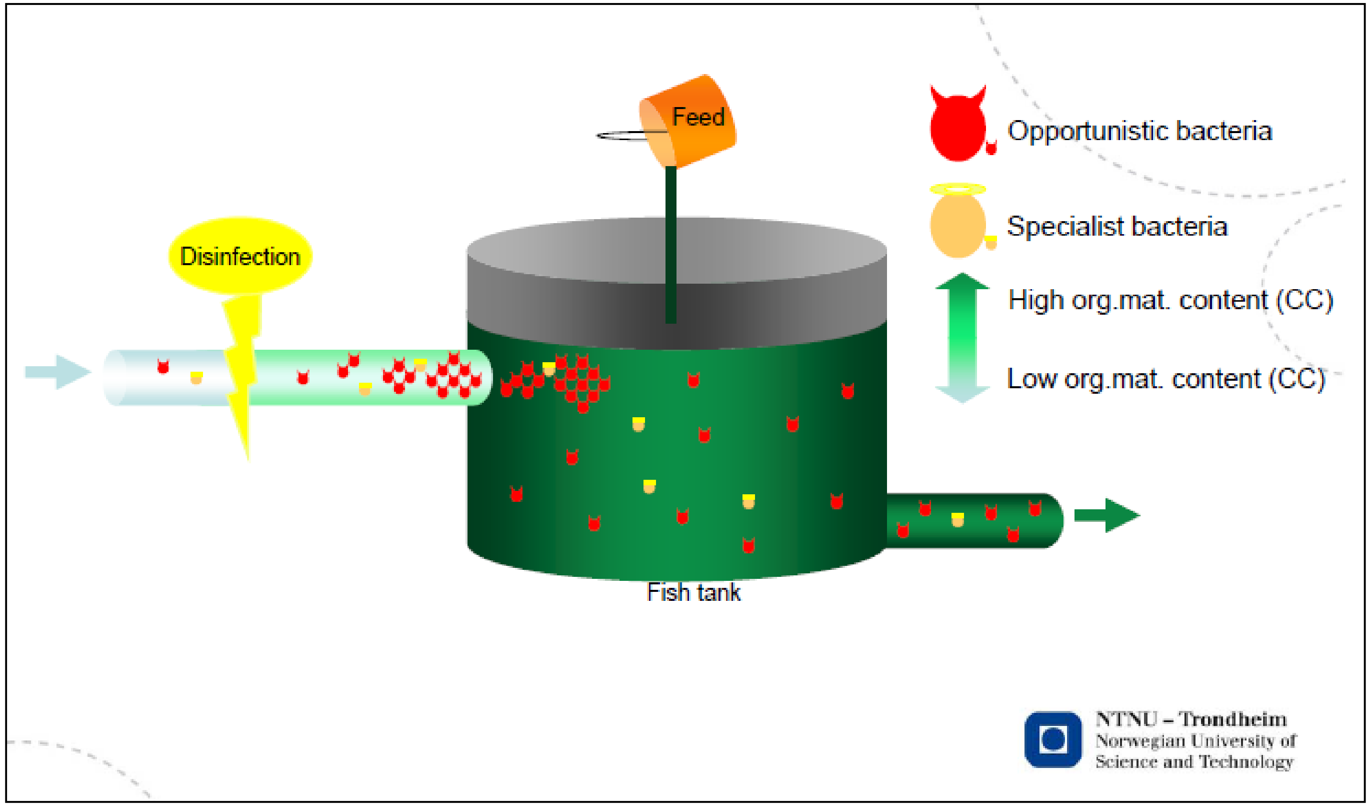
## Possible entry of pathogen in FTS

Bacterial content in water greatly is influenced by inputs like:

1. Live feeds (rotifers/artemia)
2. Intake water

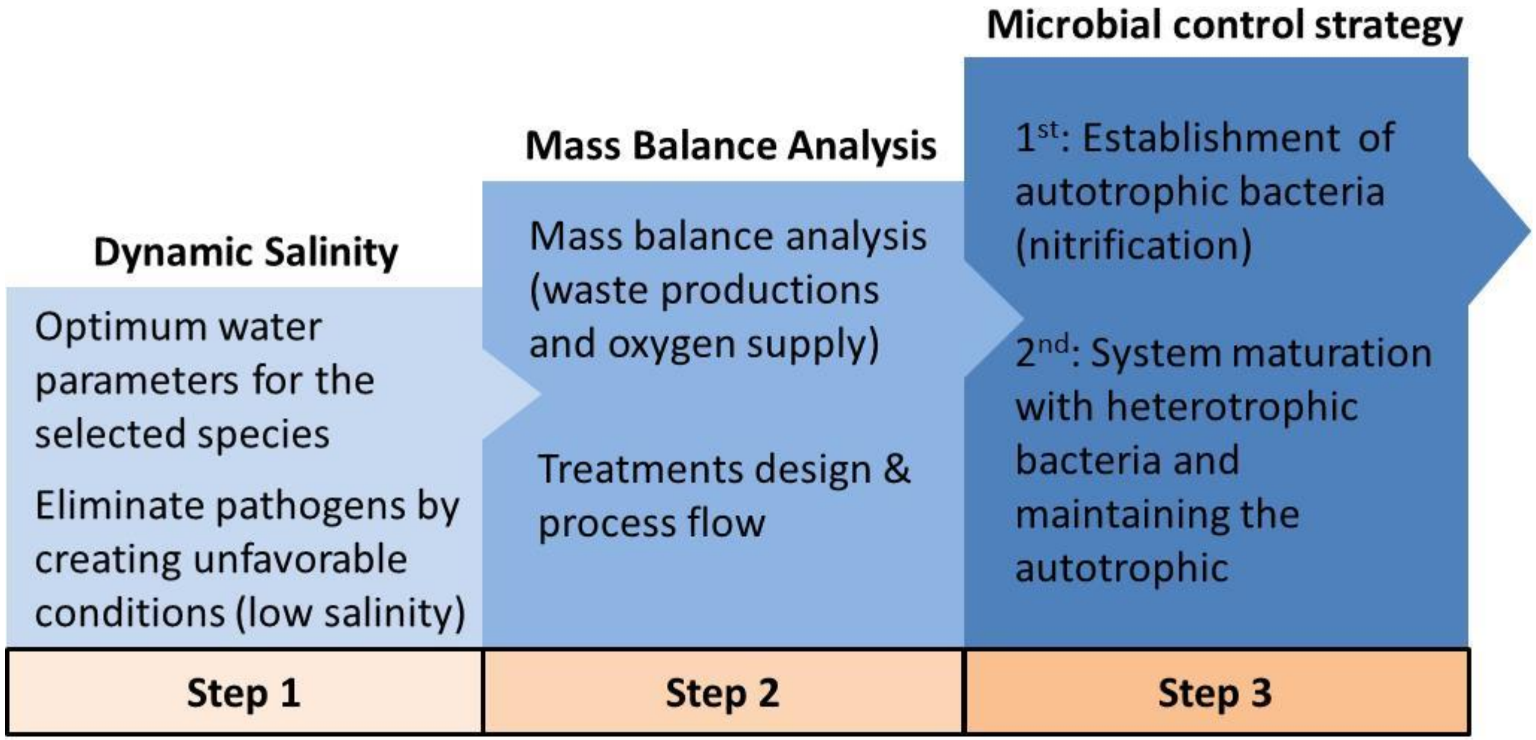



Source: Kari Attramadal *et al.*

# Adoption of RAS Technology

There are **3 key steps** in the our approach at MAC:

1. Creating the “ideal environment” – thru’ dynamic salinity
2. Sizing of RAS - larviculture - using Mass balance Analysis
3. Seeding & maturation of bacteria - Microbial Control Strategy



# Adoption of RAS Technology

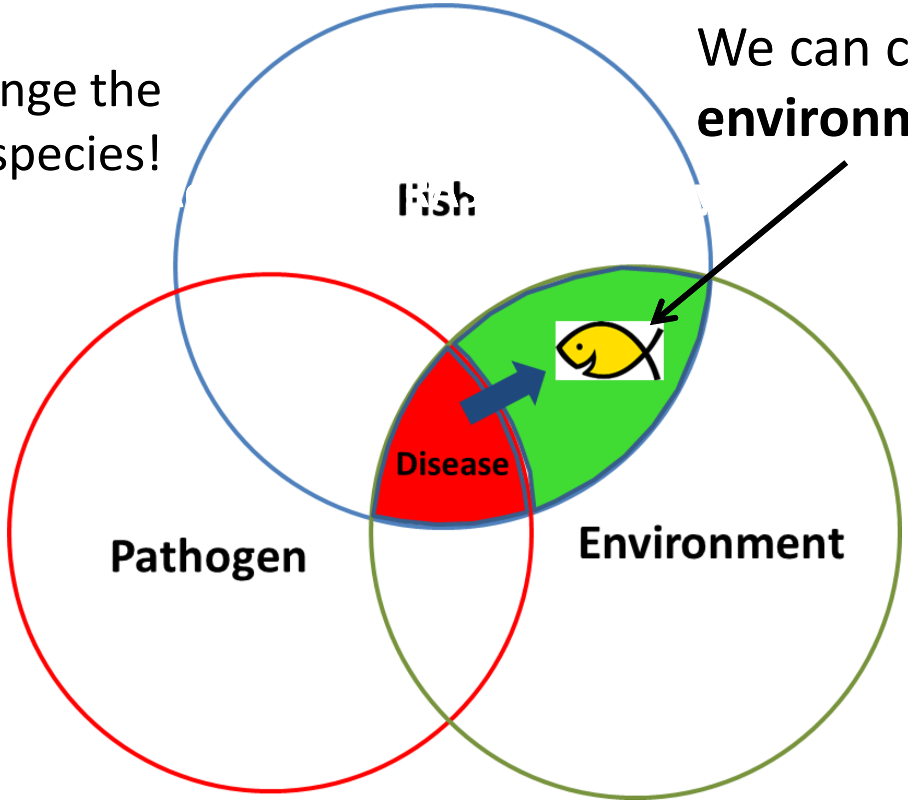
## Step 1 - Dynamic Salinity:
## Interaction of Host-Pathogen-Environment

**The Epidemiological Triad, Snieszko (1976)**

We can't change the species!

Where the disease is coming from?

Disinfecting of incoming water & live feeds failed to prevent outbreaks

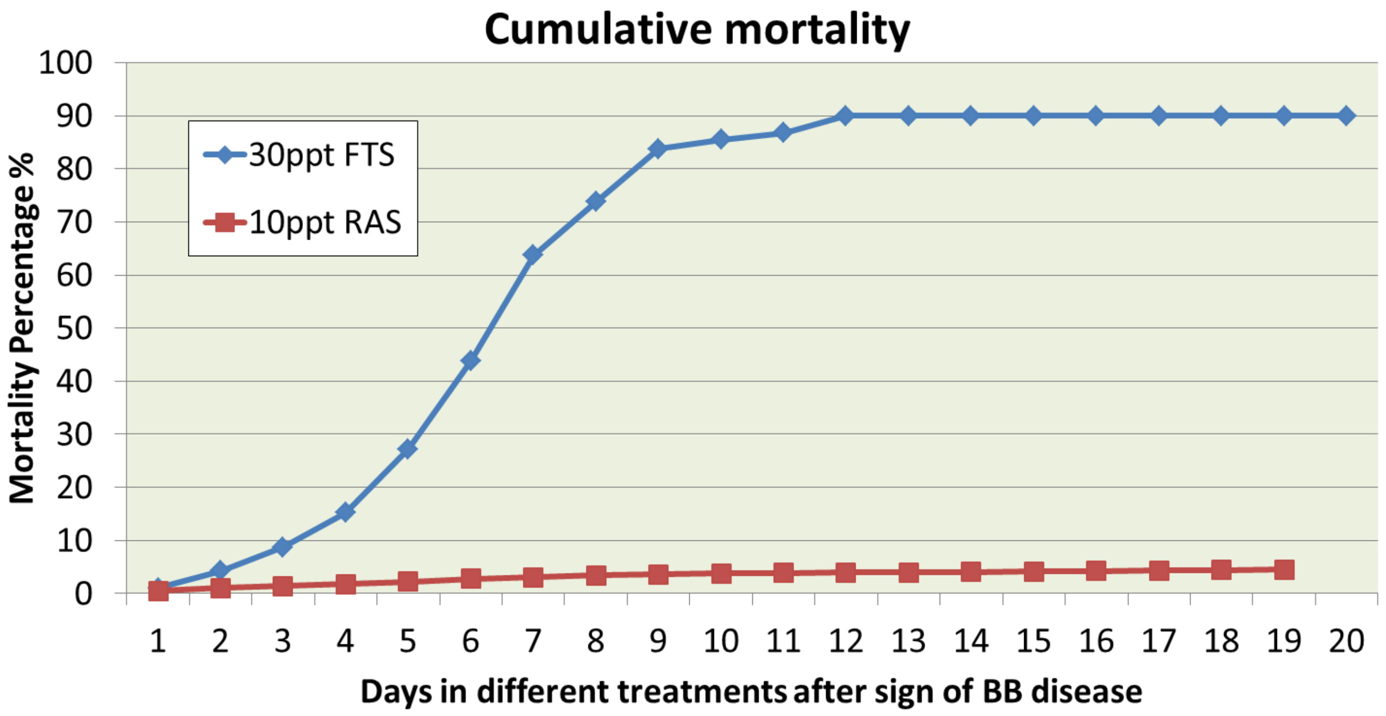



We can create an "**ideal environment**" using a RAS!

**Lowering Salinity**
Seabass is an euryhaline species and BB is **marine bacteria**

# Adoption of RAS Technology: Dynamic Salinity

## Survival rates of BB-affected fry: 30ppt flow-through vs 10ppt RAS



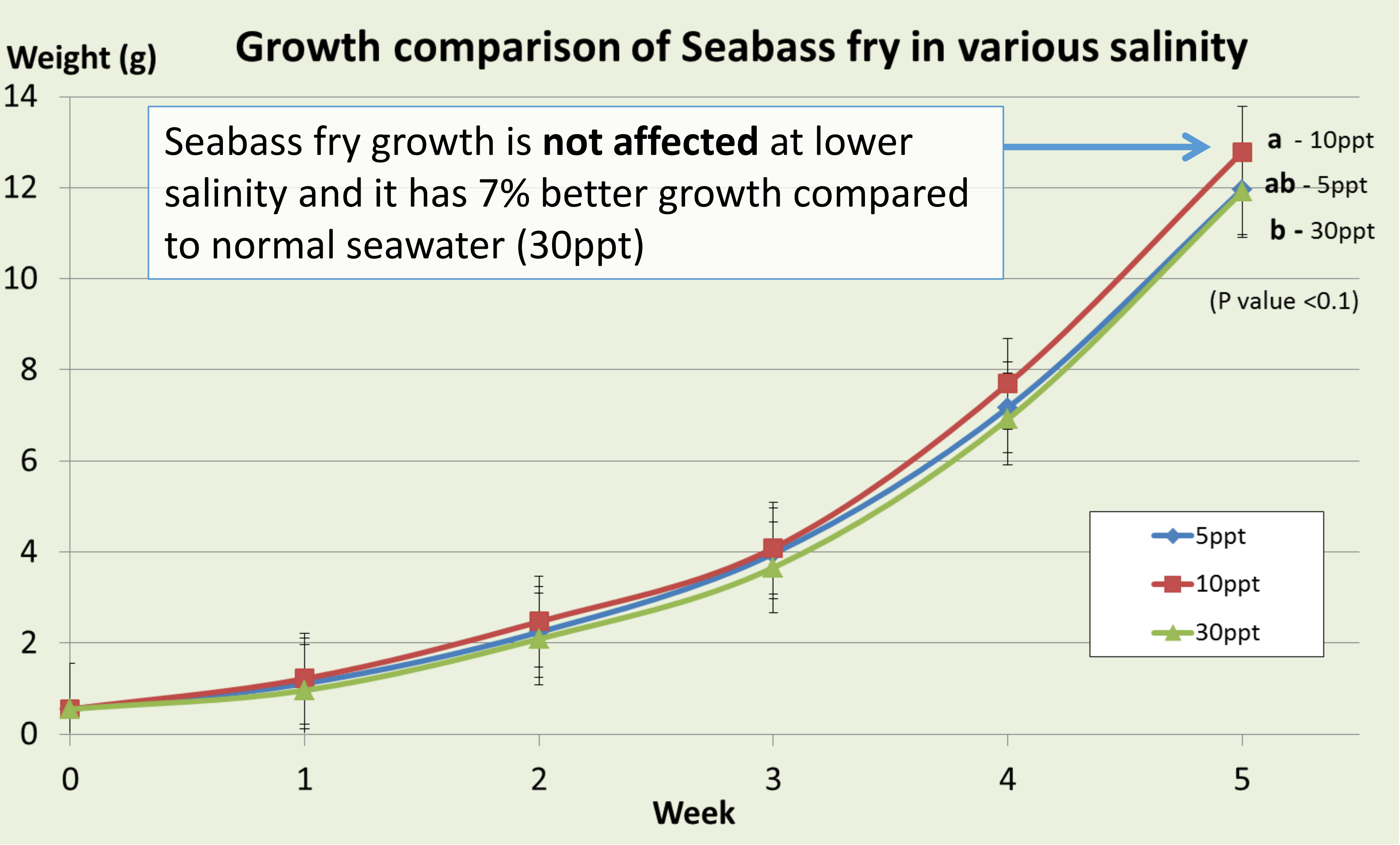

**Big-belly disease can be controlled** by reducing the salinity from 30ppt to 10ppt

**Result**:

Cumulative mortality for 30ppt-FTS group reached 90% but the group in 10ppt-RAS remained below 10%

# Adoption of RAS Technology: Dynamic Salinity

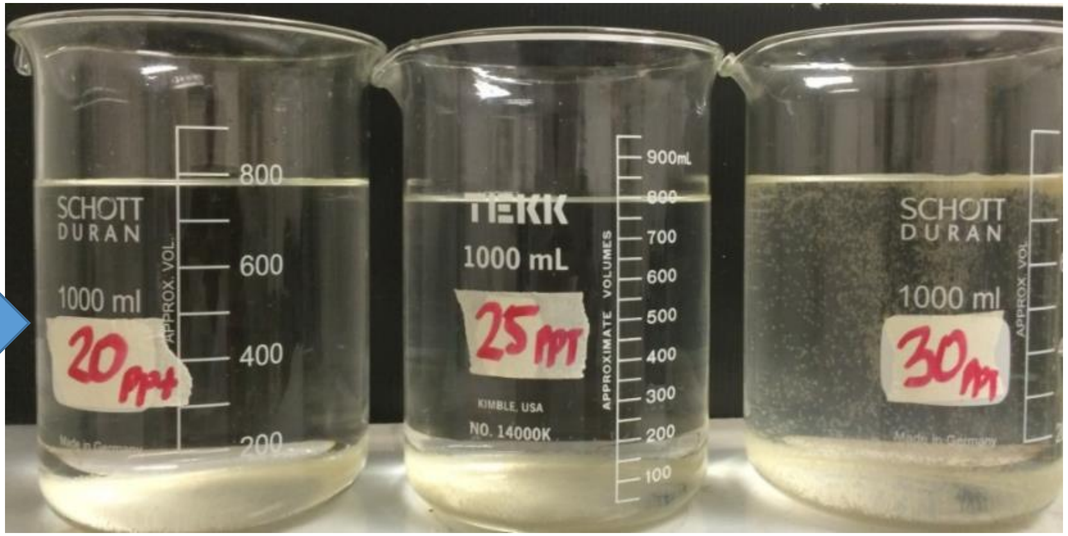
## Growth of seabass fry is not affected at low salinity



# Adoption of RAS Technology: Dynamic Salinity

## Effect of salinity on hatching rate & buoyancy of larvae

Buoyancy test of newly hatched larvae

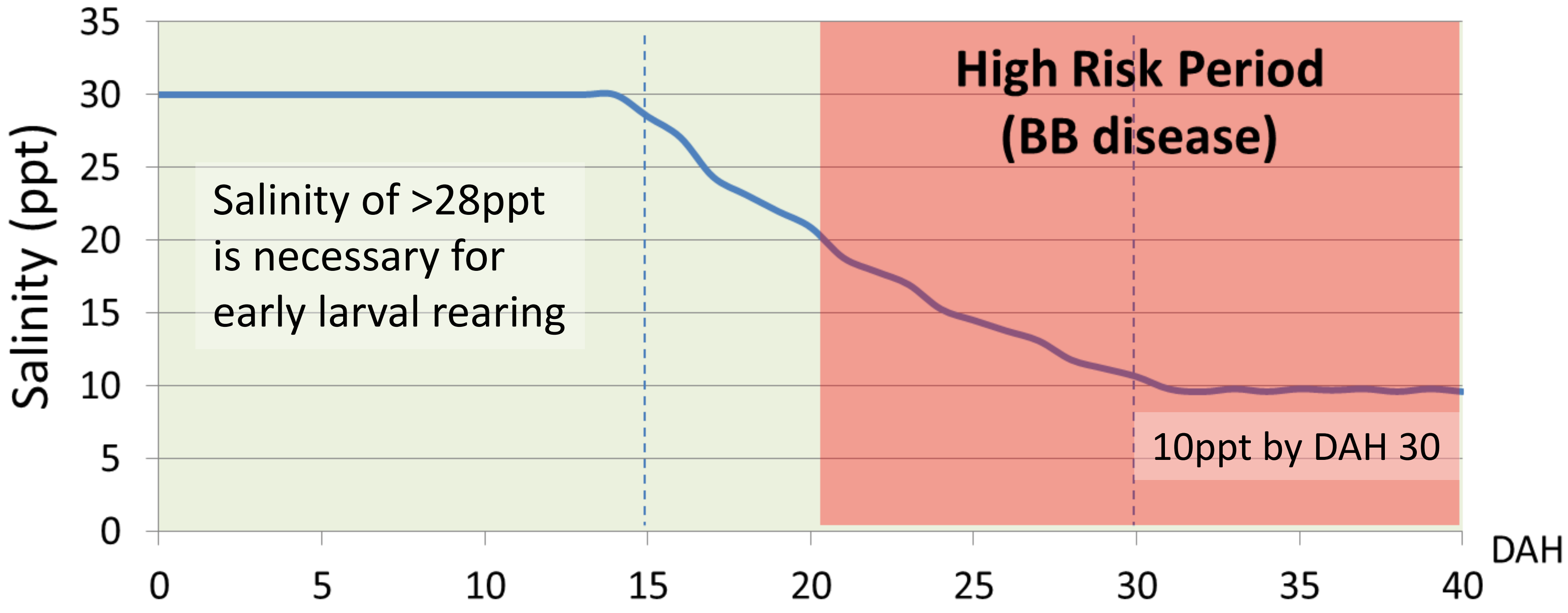



| Salinity (ppt) | 20 | 25 | 30 |
|---|---|---|---|
| Hatching Rate | 68% | 71% | **81%** |
| Buoyancy of hatched larvae (at mid-water column) | 0% | 10% | **90%** |

**28-30ppt** is required for Day 0-3

# Adoption of RAS Technology: Dynamic Salinity

## Solution: Dynamic Salinity (RAS) for control of BB




- Salinity adjustment to control Big Belly for seabass fry production
- If there is any clinical sign of BB, lower the salinity **5ppt** to suppress disease manifestation

# Adoption of RAS Technology: Mass Balance

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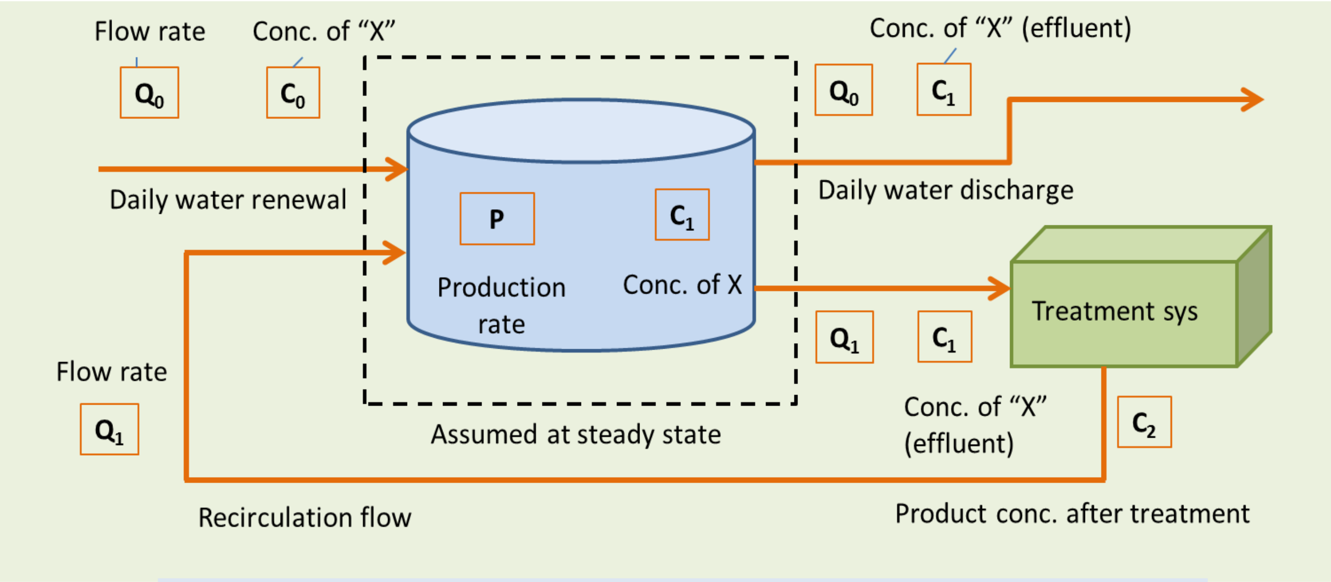
## Step 2 (Mass balance analysis):
## Determine the desired water qualities

Use of Mass Balance Analysis to size up the RAS for larviculture




| Parameters | Optimum level | Units |
|---|---|---|
| Temp | 28-31 | $^{O}C$ |
| $O_2$ | >5 | mg/l |
| $CO_2$ | <150 | mg/l |
| $NH_3/NH_4$ | <1 | mg/l |
| $NO_2$ | <2 | mg/l |
| $NO_3$ | <150 | mg/l |
| pH | 7-8 | mg/l |
| Salinity | 10-30 | ppt |

| **Transport in of "x"** | **+** | **production of "x"** | **=** | **transport out of "x"** |
|---|---|---|---|---|
| $Q_1 C_2 + Q_0 C_0$ | + | P | = | $Q_0 C_1 + C_1 Q_1$ |

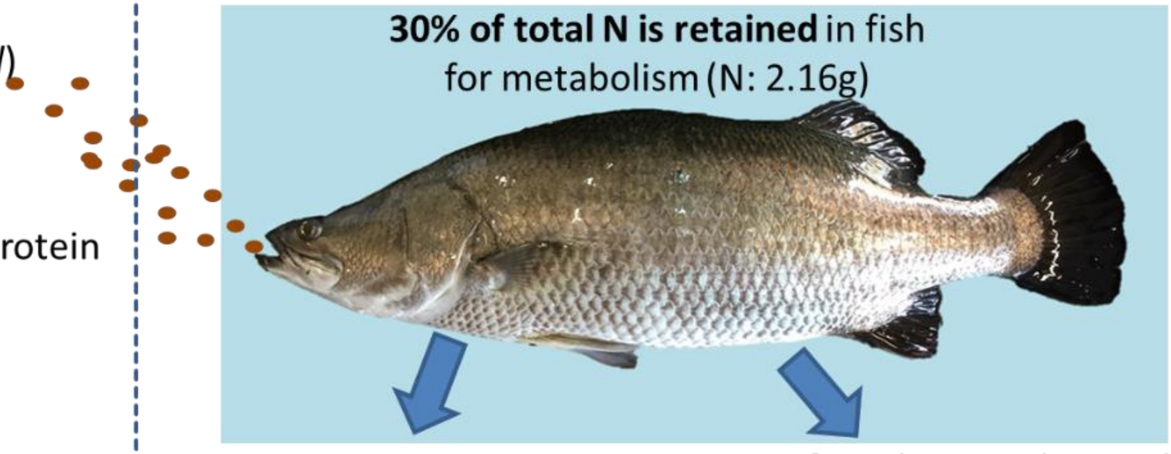
Ref: Timmons. M, et al., 2010, Recirculating Aquaculture 2nd Edition

# Adoption of RAS Technology: Mass Balance

## Waste Production – Total ammonia-nitrogen (TAN)

100g of Fish Feed
(*45% protein level*)

N is ~16% of protein
(Total N: 7.2g)




55% of total N is discharged from gills as ammonia (N:4g)

15% of total N is undigested & discharged as faeces or uneaten feeds (N:1.1g)

**61% of total N is wasted & released into the water** (N:4.3g)

At 1.2 $NH_3$ : 1N, there will be ~**5.2g of ammonia** released into the system per 1 kg of feed

6% of total N, waste matters degrade into ammonia in water (N:0.4g)

**9% of total N is removed from the system** via mechanical filter (N:0.6g)

Ref: Sommerville, C. et al., 2014, Small Scale aquaponic food production, integrated fish and plant farming ,FAO Technical paper No. 589

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# Adoption of RAS Technology: Mass Balance

## Methods in calculating the total ammonia-nitrogen (TAN):

*Sommervilles. C, et al., 2014, Small-scale aquaponic food production, Intergrated fish and plant farming,FAO Technical Paper No. 589*

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 100 | kg | Feed | x | 45 | % | protein in feed | x | 16 | % | nitrogen in protein | x | 61 | % | wasted nitrogen | | | | | x | 1.2 | g | $NH_3$ converted per nitrogen | = | 5.27 | kg | of TAN | | |
| | | | | | | | | | | | | | | | | | | | | | | | | 5.27 | % | of feed | | |

*Timmons. M, et al., 2010, Recirculating Aquaculture 2nd Edition*

| | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 100 | kg | Feed | x | 45 | % | protein in feed | x | 16 | % | nitrogen in protein | x | 80 | % | nitrogen is assimilate | x | 80 | % | excreted nitrogen | 90 | % | Nitrogen excreted as $NH_3$ | = | 4.15 | kg | of TAN | |
| | | | | | | | | | | | | | | | | | | | | | | | 4.15 | % | of feed | |


The maximum daily amount of ammonia is est. at **4-5%** of peak feed load

# Adoption of RAS Technology: Mass Balance

## Example of a Mass Balance Analysis Table

| TAN Mass Balance Calculations | | Units |
|---|---|---|
| Feed protein content | 52 | % |
| Total Ammonia Nitrogen (TAN) production rate | 0.103 | Kg/day |
| % TAN from feed (Range 3-5%) | 4.78 | % |
| Desired TAN concentration in recir water **($NH_3$/$NH_4$)** | 2.00 | mg/L |
| Passive Nitrification | 10.00 | % |
| Tan available after passive nitrification | 0.093 | Kg/day |
| Passive denitrification | 0.00 | % |
| Maximum nitrate-Nitrogen concentration desired **($NO_3$)** | 200 | mg/L |
| **Daily new water replacement to maintain nitrate conc. ($NO_3$)** | **465.00** | **L/day** |
| Tan available to biofilter after effluent removal | 0.0921 | Kg/day |
| Flow rate to remove TAN to desired concentration | 93,000.96 | L/day |
| **Flow rate to maintain at desired ammonia ($NH_3$/$NH_4$) conc.** | **3.88** | **m3/hr** |
| Water turnover per hour | 48.21 | % |

Ref: Wayne Hutchinson, et. al, 2004, Recirculating Aquaculture Systems: Minimum Standards for Design, Construction and Management.

# Adoption of RAS Technology: Mass Balance

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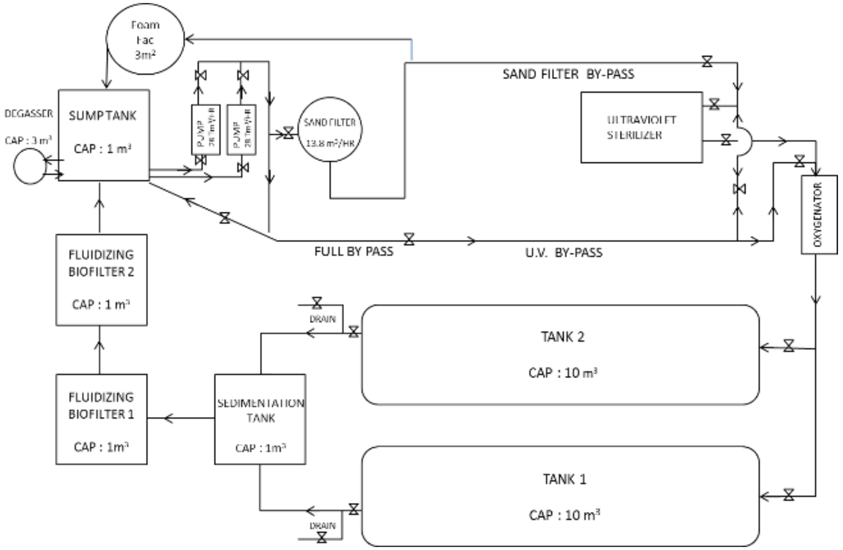
## Design of RAS-larviculture at MAC




**Removal of Solid Waste**

Larger waste particles are removed by filtering through series of sand beds

**Removal of Dissolved Organic Waste**

Dissolved organic materials are trapped by charged fine bubbles

**Biological Filtration**

Nitrifying bacteria colonized on bio-media reduce the ammonia to less toxic forms

**$CO_2$ removal/ Degassing**

Harmful dissolved carbon dioxide are removed through generous aeration of water

**Water Disinfection**

Filtered Water is sterilized using UV to eliminate microbes

# Adoption of RAS Technology: Mass Balance

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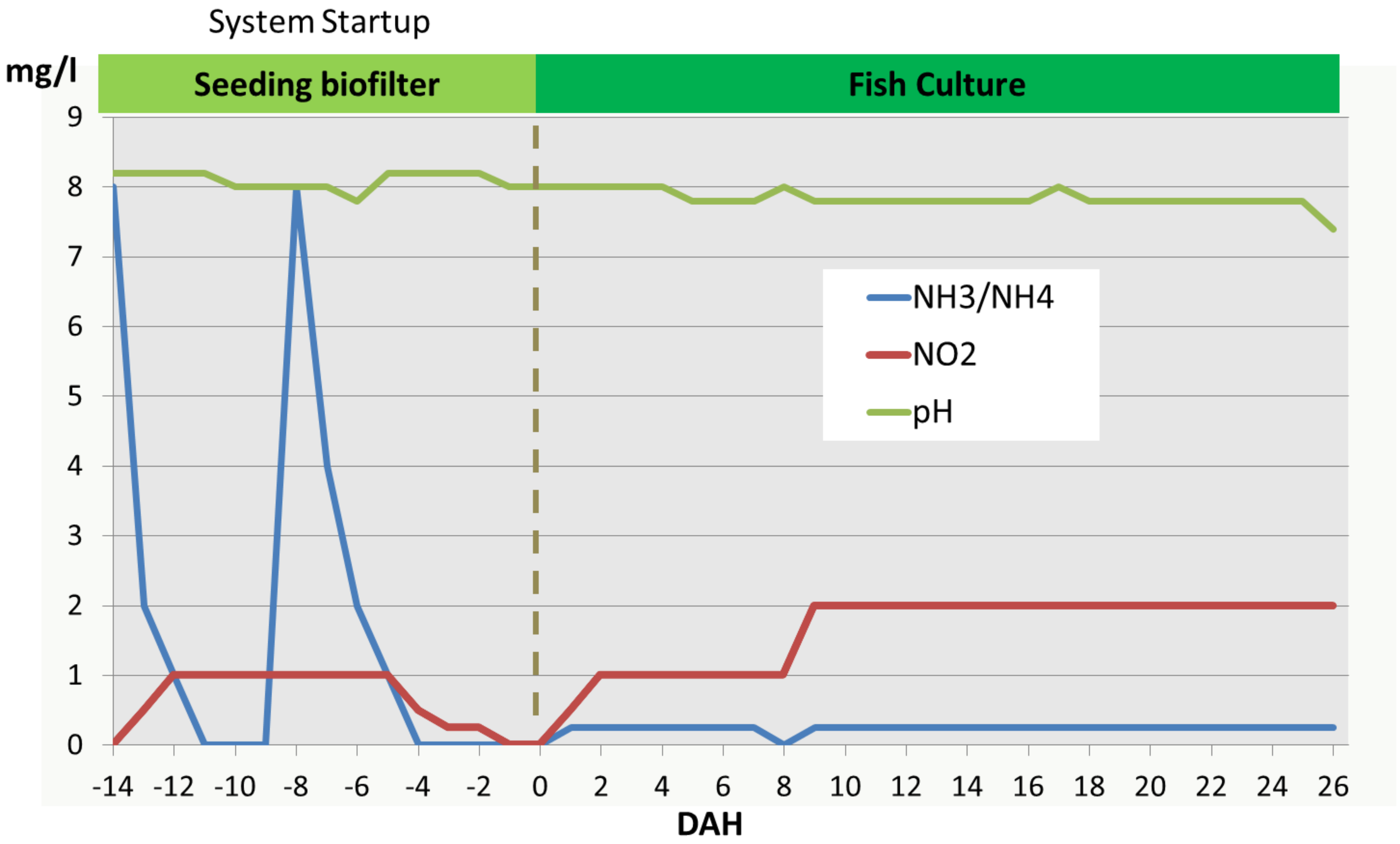
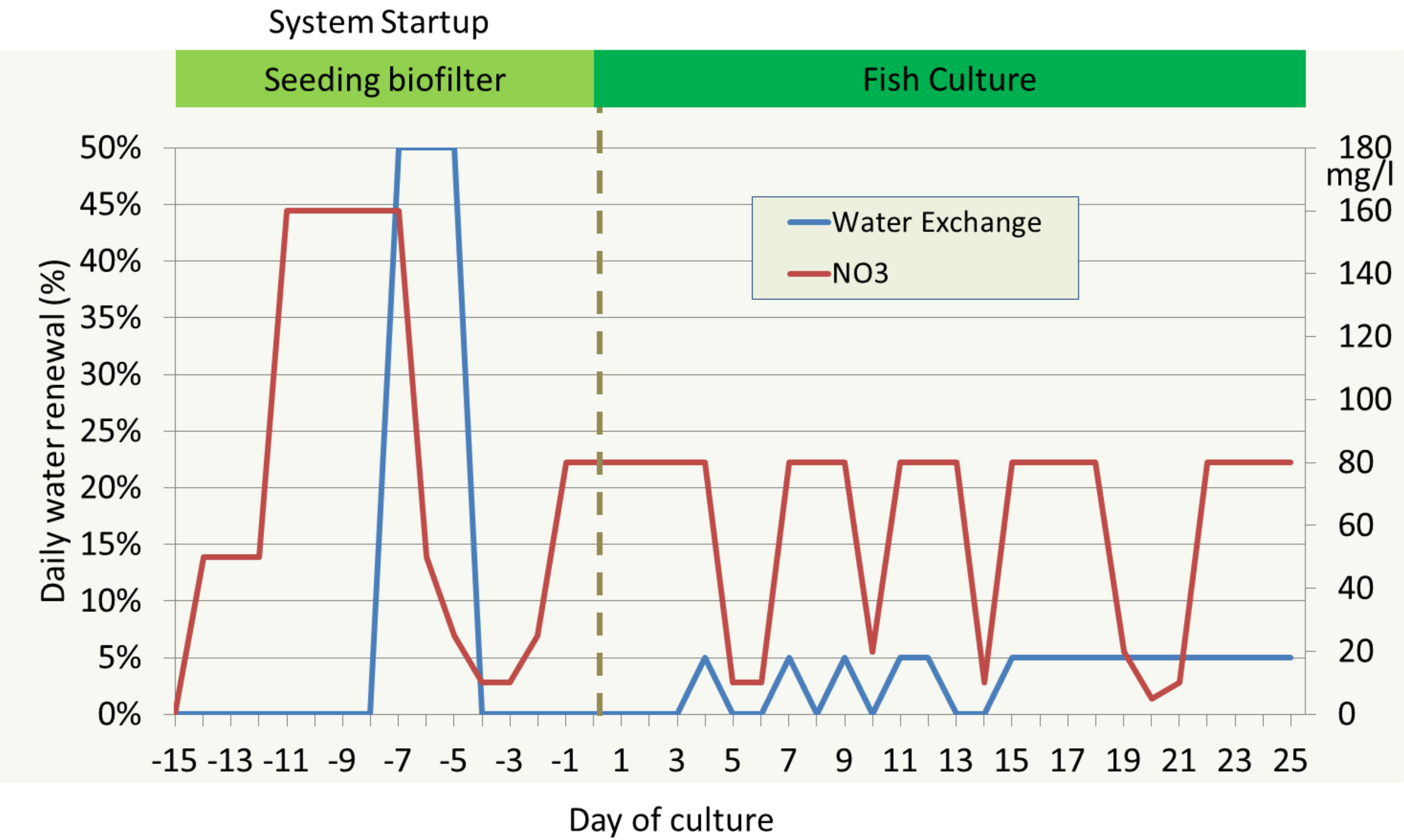
## Water Quality: Ammonia & Nitrite levels



# Adoption of RAS Technology: Mass Balance

## Water Quality: Nitrate & Water change



# Adoption of RAS Technology: Mass Balance

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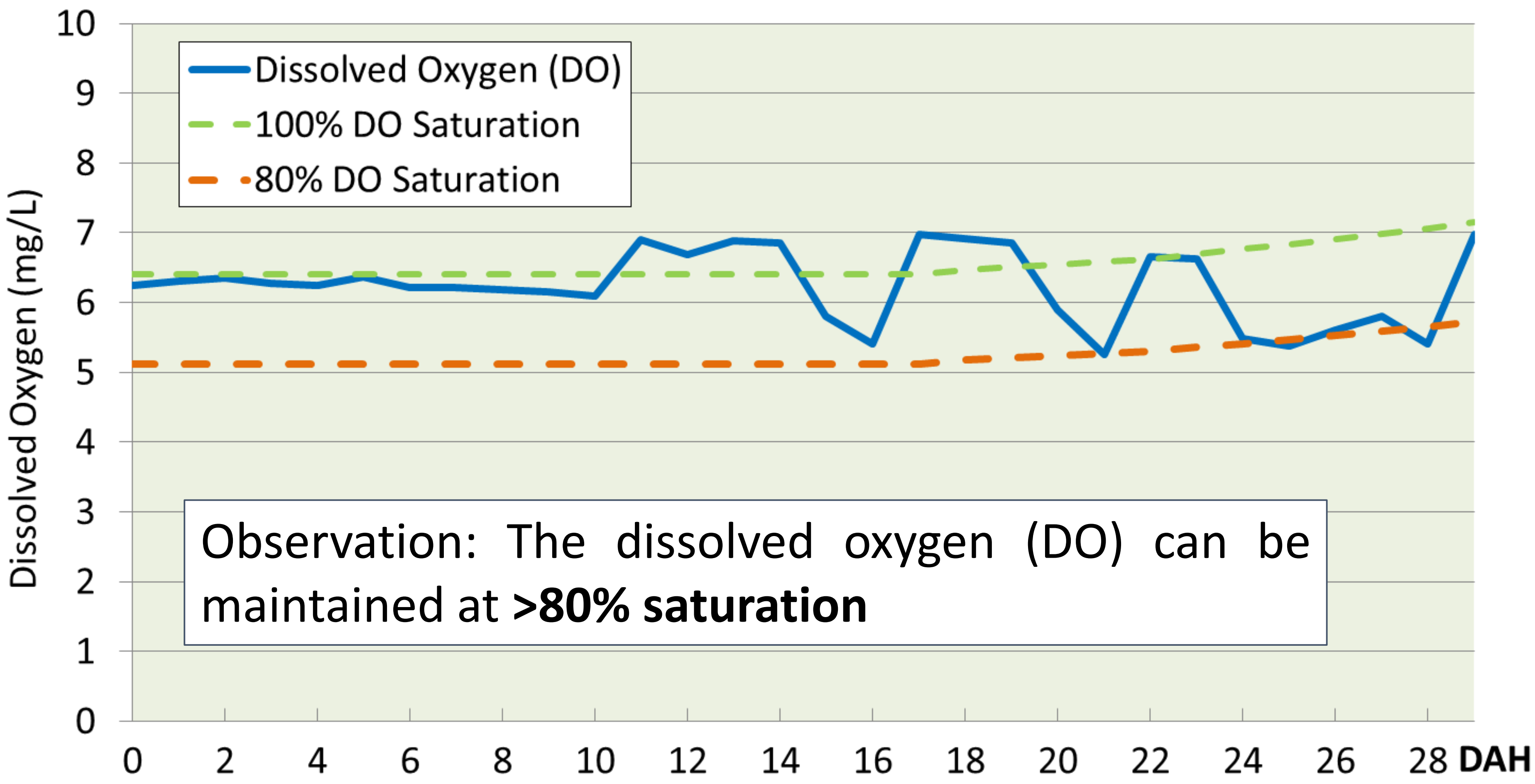
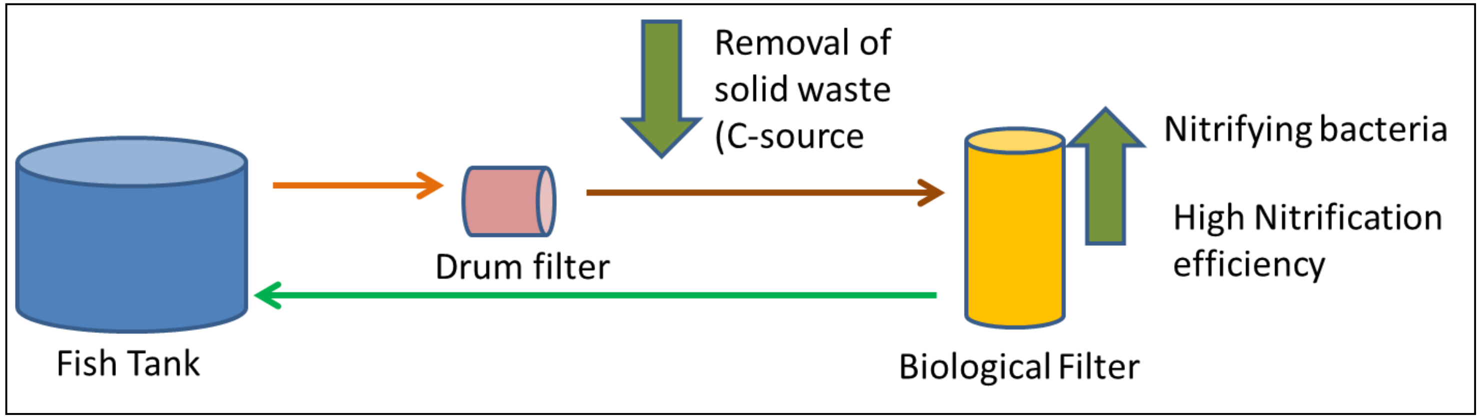
## Water Quality – Dissolved Oxygen




Observation: The dissolved oxygen (DO) can be maintained at **>80% saturation**

# Adoption of RAS Technology: Microbial Control

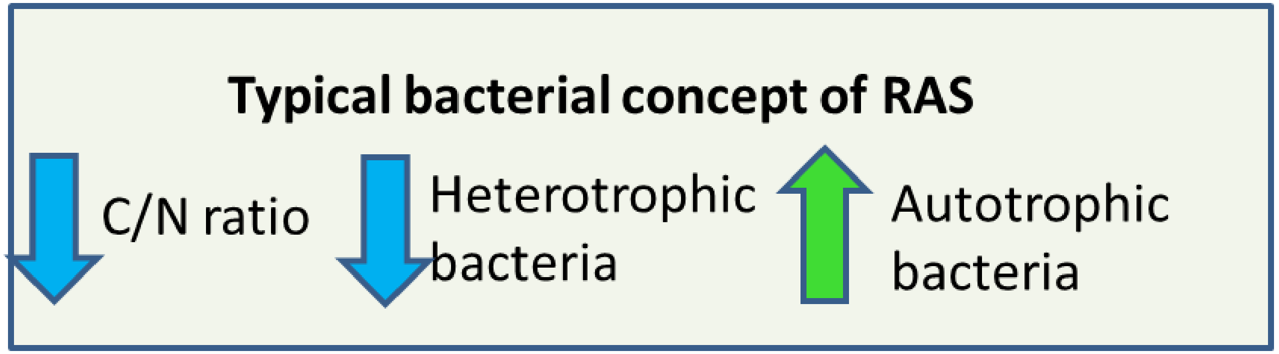
## Step 3: Microbial Control Strategy

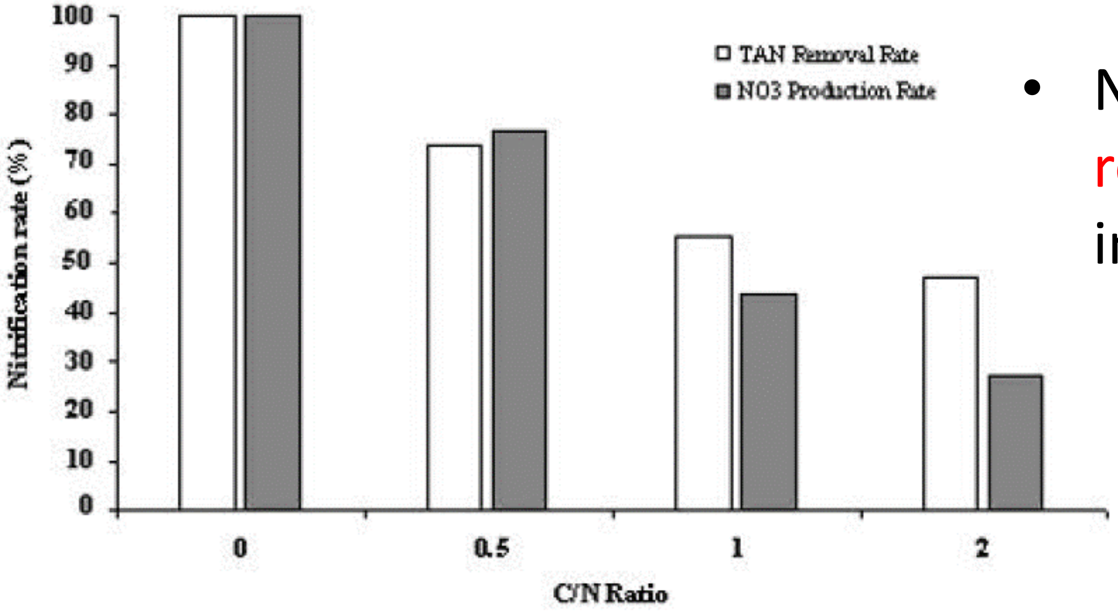



- **Nitrifying bacteria** convert the toxic inorganic nitrogen to less toxic form (nitrate)
- To ensure **high nitrification efficiency**, need to reduce/remove the carbon load via the mechanical filter, before water enters the biological filter
- Removal of carbon source will **prevent the heterotrophic bacteria** from dominating the biological filter

# Adoption of RAS Technology: Microbial Control

## Effect of C/N ratio in RAS




- Nitrification efficiency reduces as the C/N ratio increase

Ref: Luigi Michaud, 2007, Microbial communities of recirculating aquaculture facilities

# Adoption of RAS Technology: Microbial Control

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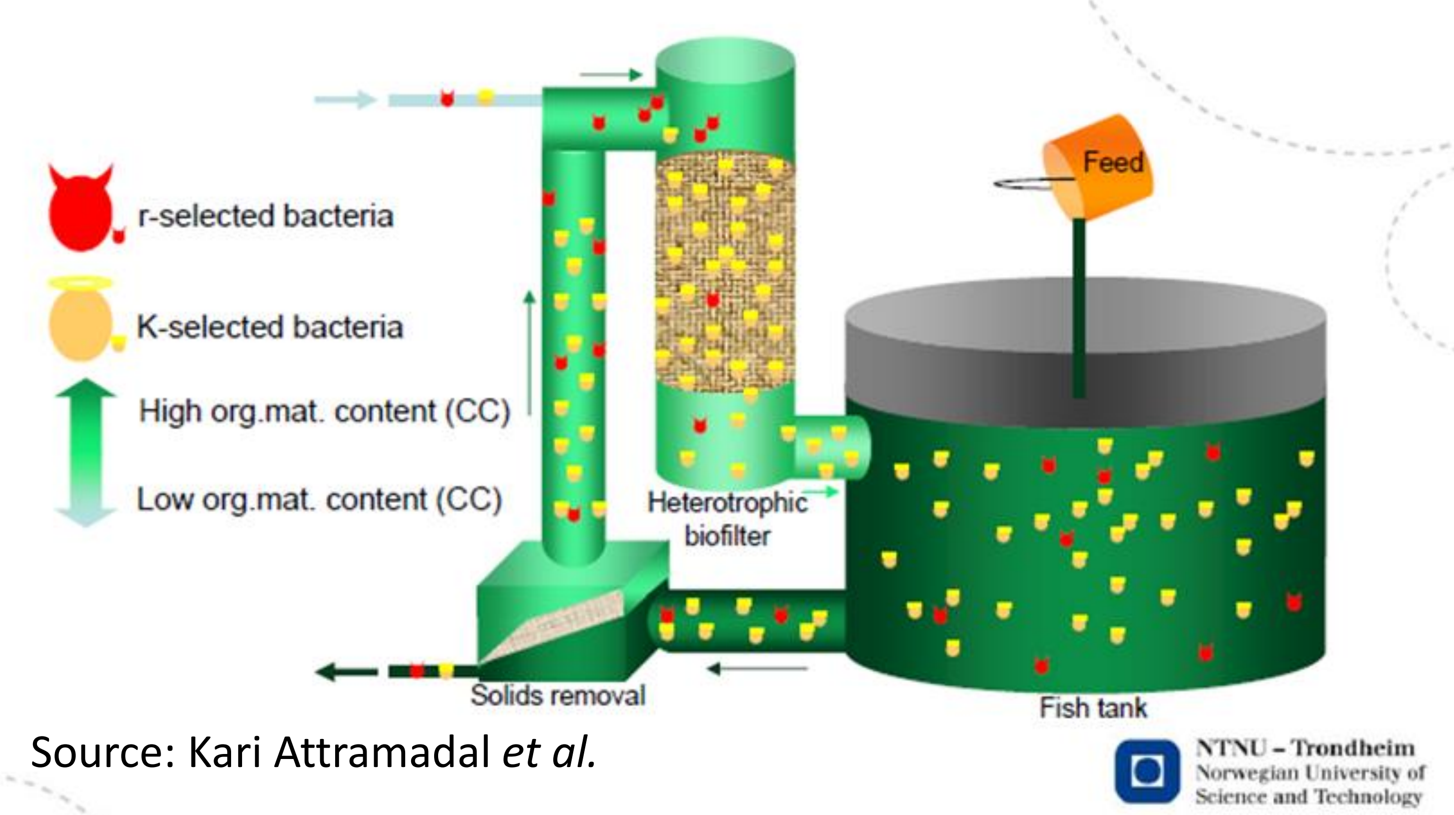
## Competitive Exclusion by K-selective bacteria




Source: Kari Attramadal *et al.*

**RAS system:**

Matured bacterial content in water less likely to be perturbed by bacterial from inputs such as rotifer, feed

# Adoption of RAS Technology: Microbial Control

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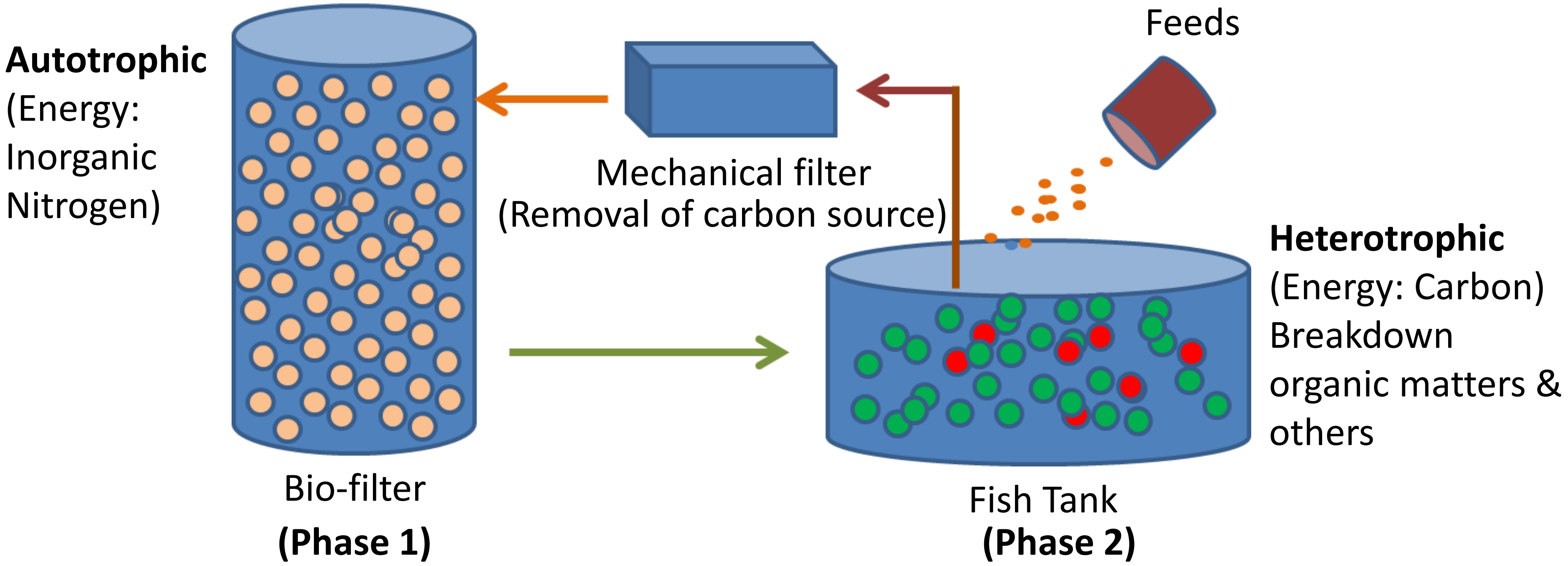
## Establishing autotrophic & heterotrophic bacteria

- **Phase 1**: Allow autotrophic bacteria (nitrifying bacteria) to be established at the startup of RAS (**w/o any carbon source**)
- **Phase 2**: Focus on heterotrophic bacteria to occupy the "water column/wall" in the culture tank



# Adoption of RAS Technology

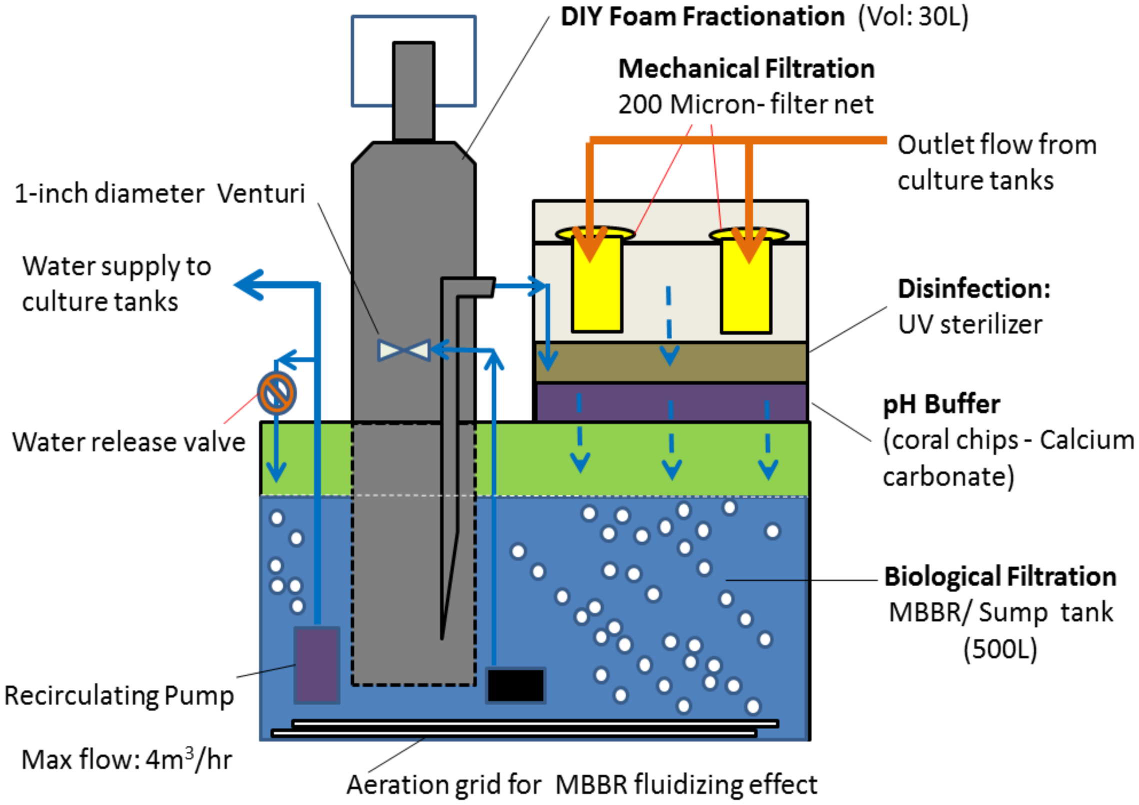
## Technology transfer to industry

- Farms faced **disease challenges** in producing fish fry using outdoor tank flow-thru system
- With adoption of RAS, **high biosecurity** can be maintained and **better control of diseases**
- Achieved **consistent production** of 1-inch seabass fry, consecutive 6 batches with survival rates between 30%-40%



# Adoption of RAS Technology

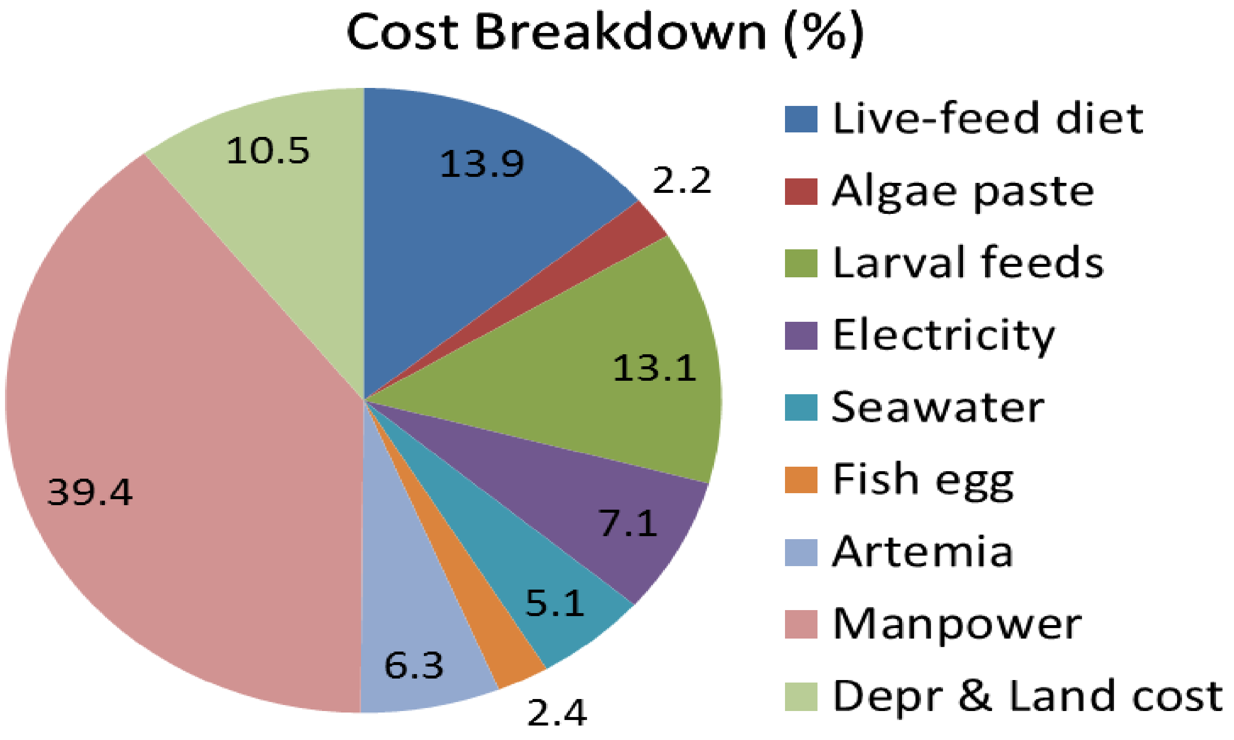
## Development of Compact RAS for Hatchery




The compact water treatment system is only 12% of the total footprint (9m$^2$) used, supporting a production of 120K seabass fry

# Adoption of RAS Technology

## Estimated cost of production of Seabass Fry in commercial-scale RAS




**~0.8-0.9 cents per fry**

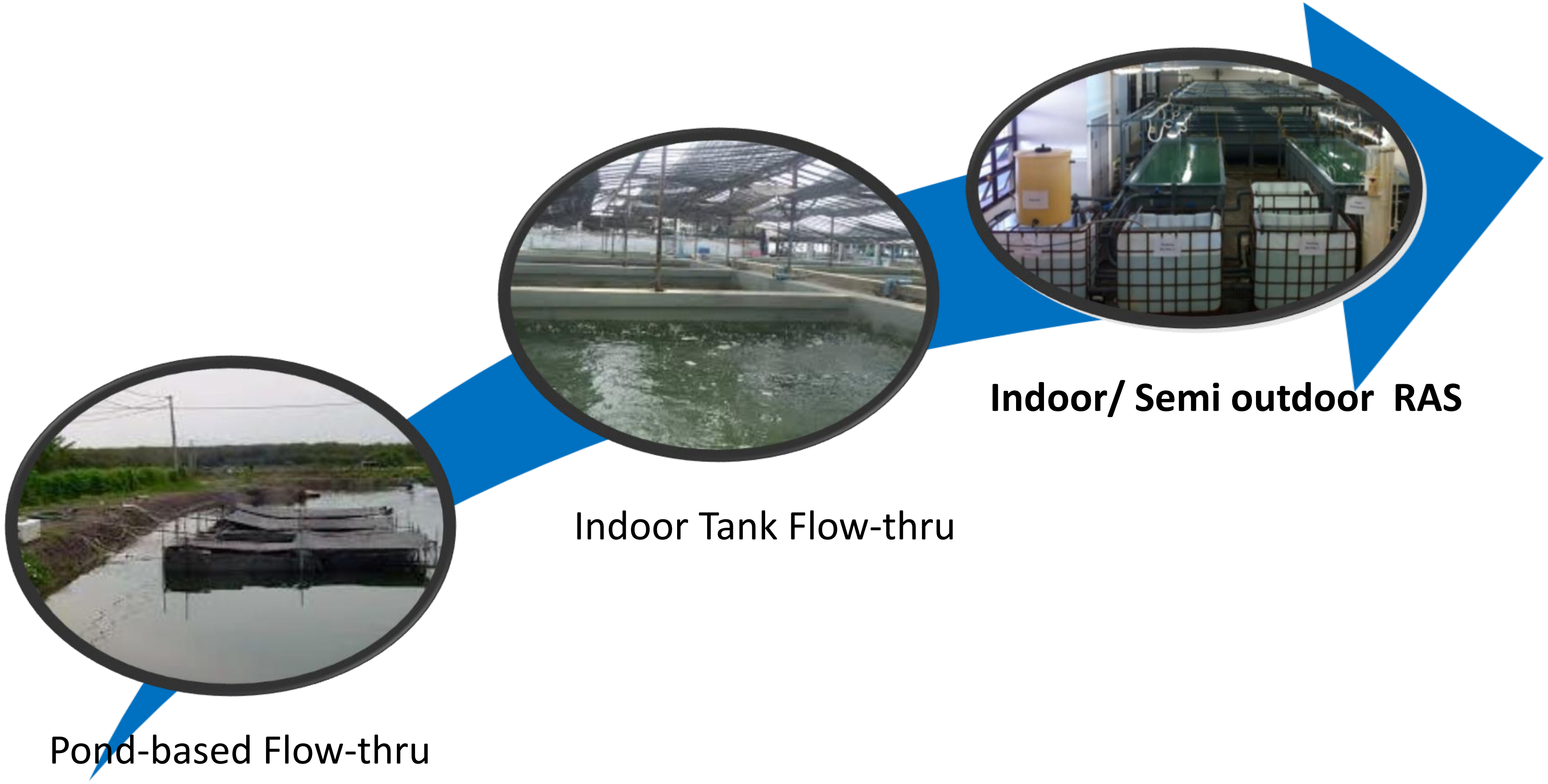
# Summary

- RAS can **overcome the limitations** of FTS (water quality control & biosecurity/diseases).
- RAS-based hatchery production can be based **anywhere** and even in multi-level farm-factories, far from the natural water bodies
- RAS technology for fry production is **well established and ready for industry adoption**

# Summary

## Future development of Hatchery Technology

RAS-based fry production is **economically viable** and also **future-proof** (climate change/ limited land & resources)



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# Thank you