



WINTER SCHOOL
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Torino (Italy)

Emerging threats for trout farms



Paolo Pastorino



The PRIMA programme is an Art.185 initiative supported and funded under Horizon 2020, the European Union's Framework Programme for Research and Innovation

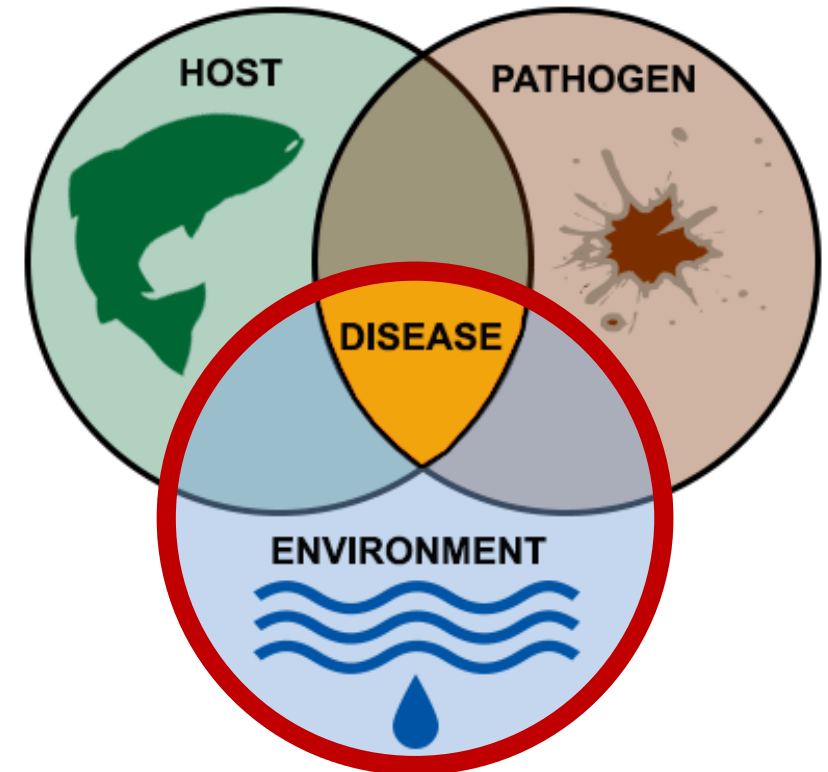


AQUACULTURE AND ENVIRONMENT

AQUACULTURE ↔ ENVIRONMENT



I NEED HIGH
WATER
QUALITY!!



Conceptually, it is useful to think of fish disease or fish health as a set of interactions among the host, pathogen and environment.

Humans impact the physical environment in many ways....



GLOBAL CHANGE

What does the term “global change” mean?

We understand global change to comprise a wide range of biophysical, ecosystem and socio-economic changes that alter the functioning of Earth as a system on a planetary scale (changes in climate, land and ocean productivity, atmospheric and water chemistry, ecosystems). **The result is a change in Earth’s ability to support life.**

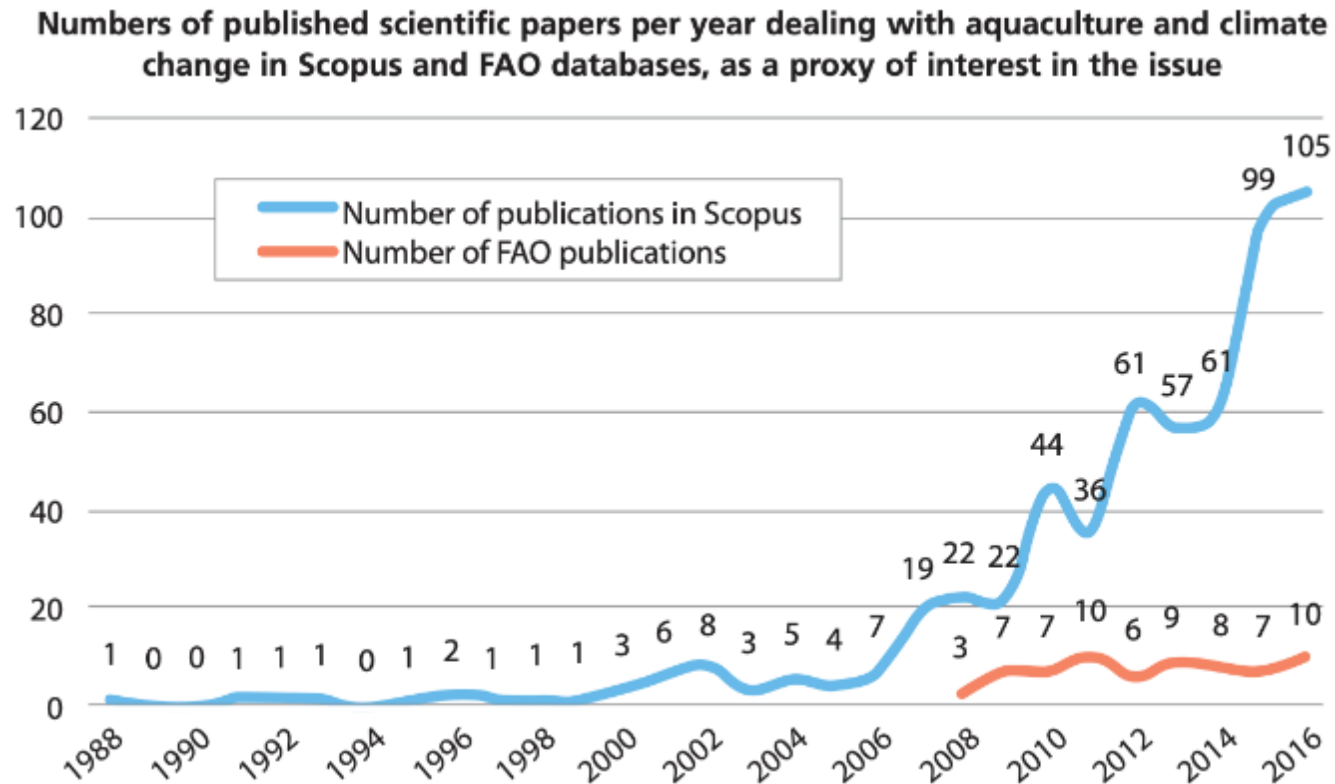
THREATS TO TROUT FARMS

- Climate change
- Water scarcity
- (Re)emerging diseases
- Emerging contaminants



CLIMATE CHANGE AND AQUACULTURE

The first mention of research on climate change and aquaculture in the scientific literature was at the end of the 1980s (Sherwood, 1988) but it took more than 15 years after that for researchers to invest significant effort on the topic.



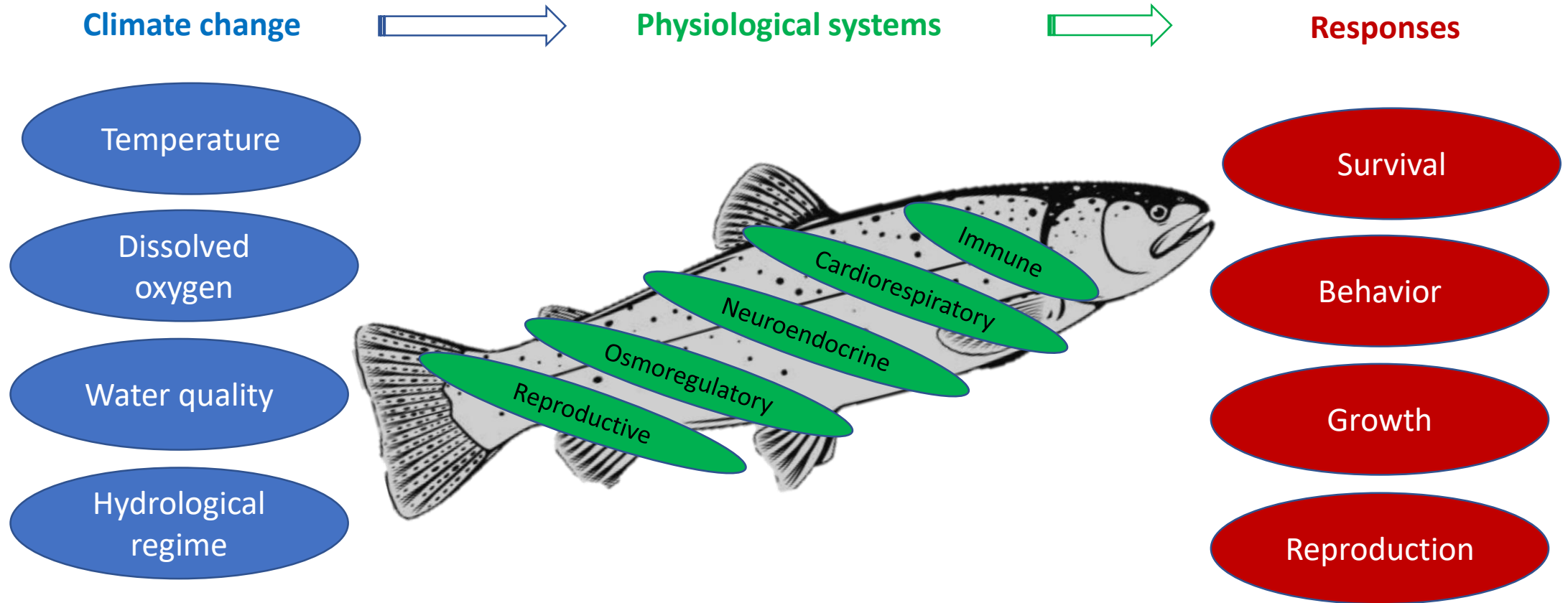
CLIMATE CHANGE AND AQUACULTURE

- Direct and indirect climate change drivers can be responsible for changes in aquaculture, whether in the short- or long-term.
- Short-term impacts include loss of production or infrastructure due to extreme events (i.e., flood, tornadoes), diseases, toxic algae and parasites.
- Long-term examples include scarcity of wild seed, limited access to freshwater for farming, limited access to feeds from marine and terrestrial sources, decreased productivity due to suboptimal farming conditions, eutrophication and other perturbations.

CLIMATE CHANGE AND AQUACULTURE

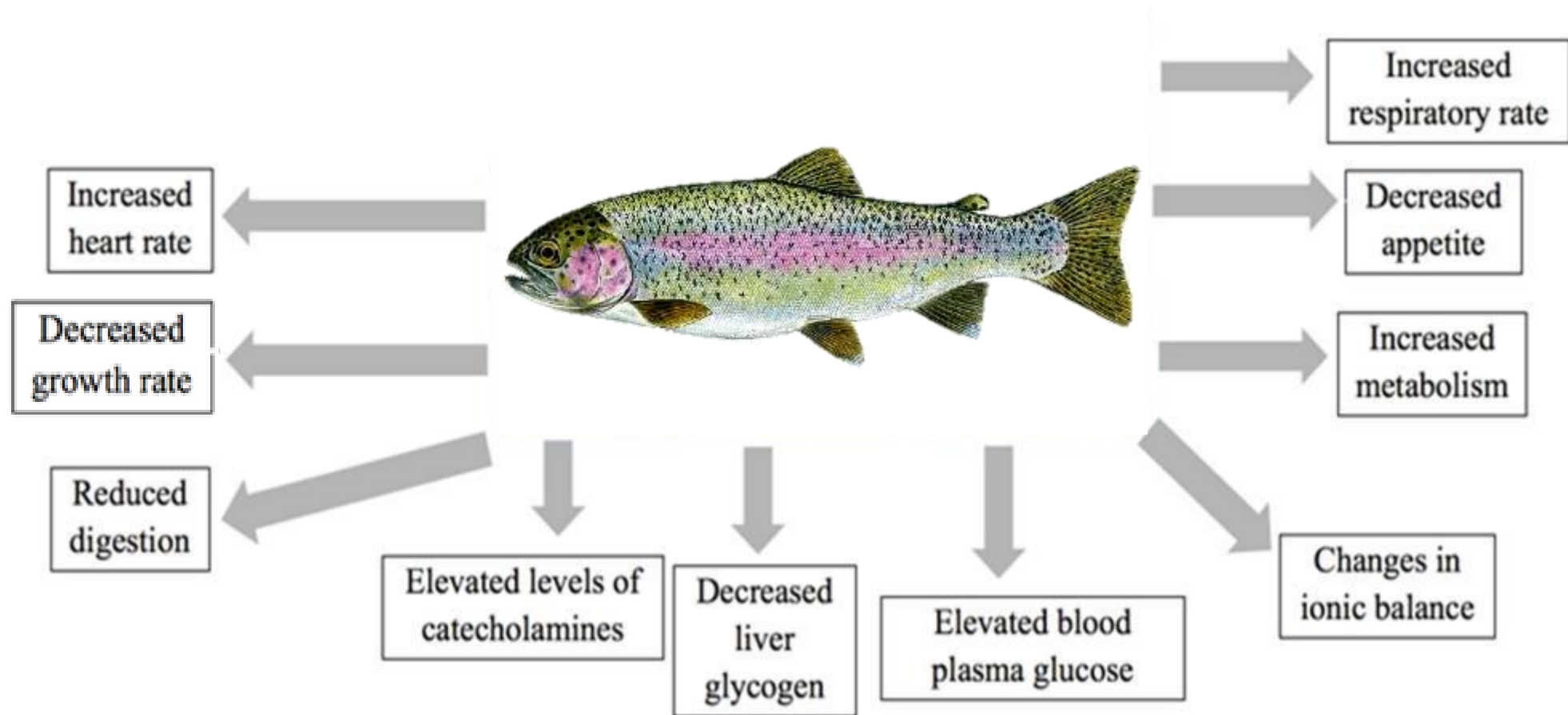
Drivers	Aquatic organisms	People	Farming system
Warming	<ul style="list-style-type: none"> Increased metabolism and growth rate 	<ul style="list-style-type: none"> Increased production, improved feed conversion and shorter production cycles should translate into a more profitable sector and higher income 	<ul style="list-style-type: none"> Increased farm production Improved feed conversion efficiency for species with higher thermal tolerance Shift to shorter production cycle aquaculture; intensified production
	<ul style="list-style-type: none"> Increased plankton respiration and proliferation Changes in mollusc spatfall Changes in reproduction and sex ratios Increased/decreased transmission of some diseases 	<ul style="list-style-type: none"> Relocation of some farming facilities (e.g. seaweed, finfish, shellfish) to cooler/deeper areas in the sea may create new safety risks Moving facilities may affect livelihoods and increase production cost 	<ul style="list-style-type: none"> HABs may force farm movement/closure or installation of depuration facilities Effects of increased jellyfish blooms on marine farms Lower feed conversion efficiency for species subjected to increased stress
	<ul style="list-style-type: none"> Species with a narrow thermal range may no longer be farmed Increased sensitivity to other drivers (e.g. acidification, pathogens) Increased Harmful Algal Blooms (HABs) 	<ul style="list-style-type: none"> Adopt guidelines on decent work in aquaculture (e.g. FAO, 2016b) 	<ul style="list-style-type: none"> Selective breeding for thermal tolerance Adjustment in farming calendar/practices Change of farmed species Climate-smart facilities (e.g. deeper ponds, etc.)
Adaptation measures	<ul style="list-style-type: none"> Farm species and/or strains with higher thermal tolerance Move farming facilities to cooler/deeper offshore or inland areas 	<ul style="list-style-type: none"> Adopt guidelines on decent work in aquaculture (e.g. FAO, 2016b) 	<ul style="list-style-type: none"> Selective breeding for thermal tolerance Adjustment in farming calendar/practices Change of farmed species Climate-smart facilities (e.g. deeper ponds, etc.)

Conceptual model describing the responses of fish physiological systems to climate change



The left column lists abiotic characteristics of freshwater ecosystems that are influenced by climate change, which, in turn, influence five physiological systems within an individual fish. The right column describes how scientists or managers could measure different responses resulting from climate change effects on fish physiology.

Schematic representation of the major climate change stressors in rainbow trout farming



Climate change affects and alters environmental factors influencing the welfare of rainbow trout (*O. mykiss*) and, therefore, problems in the growth rate and welfare of the fish may occur

Water scarcity

Water makes life possible since life without water is not sustainable. Water provides many beneficial functions, both for the earth and for humans—that help produce the abundance of life around us every day.

Water covers about 70% of the planet, giving it the unique ability to foster and sustain life. Yet, <2.5% is freshwater, available for consumption, of course after processing.

The three major sources of freshwater are rainwater, surface water and groundwater.



Water scarcity is the deficiency of adequate water resources that can meet the water demands for a particular region.

By one definition, human populations face water scarcity when annual renewable water supplies in a region fall below $1,000 \text{ m}^3 \text{ person}^{-1}$, which currently occur throughout most countries in Northern Africa and the Arabian Peninsula.

Contribution of 'The water we eat!!' to water scarcity

- We «eat» 3496 litres of water every day!!
- 'Eating water' – it might sound strange, but it's the reality. This is the water associated with the **production of food we consume daily**. In fact the water we essentially need for drinking is only about 0.01% of the water we require to produce our food.
- Most of the world's freshwater goes to agriculture, mainly for the production of crops, livestock and aquatic organisms, such as fish and plants, as well as for the processing and preparation of these foods and products.
- At present agriculture accounts for nearly 70% of all freshwater withdrawn from rivers, lakes, and aquifers globally (FAO, 2011) which is equivalent to 3600 km³ year⁻¹ (Including losses).

Estimated water consumption in inland aquaculture

Aquaculture in inland freshwater contribute maximum to the world aquaculture production (contributed 63% of the world aquaculture production in 2016) and at the outlook, inland **freshwater aquaculture seems as a highly water-intensive endeavour**, requiring much more water than conventional agriculture, withdrawing on average 16.9 m³ water for per kg production (FAO, 2017).

	Water use variable	Water consumption per kg production	World total water use in inland aquaculture (in km ³ /yr)
A. System associated water consumption			
1.	Evaporation losses	5,200 L (5.2 m ³)	131
2.	Infiltration losses	6,900 L (6.9 m ³)	175
3.	To regulate water exchange	3,100 L (3.1 m ³)	79
B. Feed associated water consumption		1,700 L (1.7 m ³)	44.1
C.	Gross total water consumption (A + B)	16,900 L (16.9 m ³)	429.1
D.	1. Recycled water (Infiltration losses + water exchange losses) (A2 + A3)	10,000 L (10 m ³)	254
E.	2. Net total water consumption (C – D)	6,900 L (6.9 m ³)	175.1

(FAO, 2017)

World Bank has estimated that, aquaculture’s contribution to world’s fish consumption will rise from current 40% to roughly 62% by 2030; and thus consequently water demand for the sector will rise significantly (Hussan et al., 2019).

The potential of rainbow trout farming in aquaponics

- Nowadays, rapidly elevated levels of carbon dioxide (CO₂) have threatened rainbow trout aquaculture and global food security.
- To increase the global aquaculture production, despite climate change, an expansion of sustainable aquaculture systems (e.g., aquaponic systems etc.) is needed in the context of a circular economy.
- Aquaponics, in combination with selective breeding and thermal acclimation, are promising management strategies that may contribute to the development of a new form of rainbow trout farming.
- Modern freshwater aquaculture relies mainly on closed aquaculture systems (RAS), which reuse the same volume of water. In these systems, the rate of water reuse ranges between 80 and 99%, therefore reducing water requirements and the environmental impact of aquaculture.
- The unification of closed aquaculture systems (RAS) and hydroponics—known as aquaponics—improves sustainability and ensures food sufficiency, providing various significant economic and social benefits.
- These innovative sustainable aquaculture systems, already implemented, are characterized as integrated multi-trophic aquaculture (IMTA) or polycultures.

Aquaponics as a promising strategy to mitigate impacts of climate change on rainbow trout culture

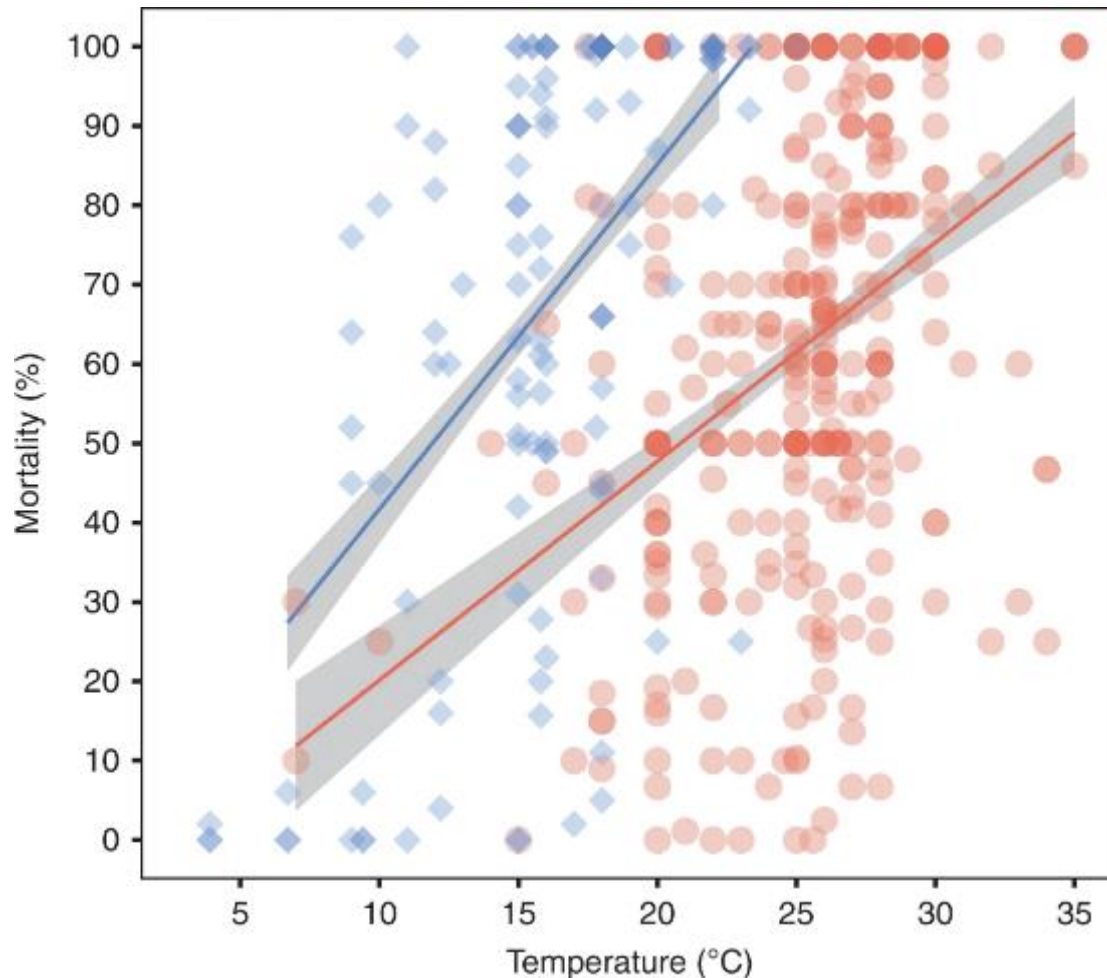
However, the rainbow trout has not been commercially reared so far in such systems.

It should be noted that rainbow trout may represent an optimum candidate for an aquaponic system



Experimental research of rainbow trout aquaculture in an aquaponic system in Greece (Vasdravanidis et al., 2022).

The increase in the temperature of the water can cause thermal stress on the fish, burdening their immune systems and leading to greater susceptibility to diseases



Bacterial pathogens: *Aeromonas* spp., *F. columnare*, *Lactococcus* spp., *Streptococcus* spp., *Vibrio* spp., and *Yersinia* spp. Red indicates tropical and subtropical host species (n = 329), blue indicates temperate host species (n = 129). Dots represent the raw data and the lines the linear mixed model predictions with SE.

Reverter et al., 2020

Enteric red mouth disease

Causative agent

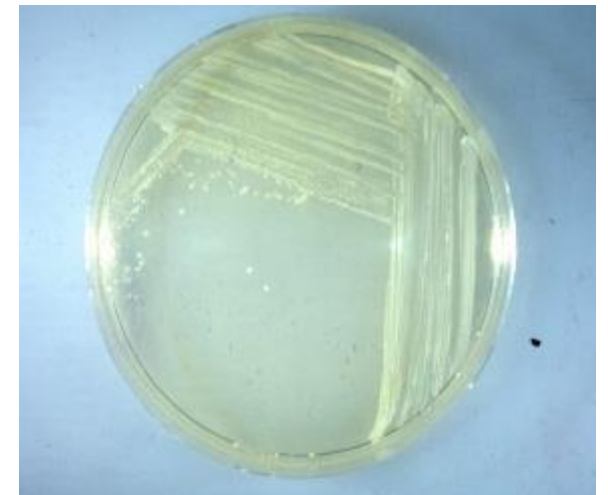
The **causative agent** of enteric red mouth disease is the bacterium *Yersinia ruckeri*.

There are several serotypes of the bacterium, and classification systems can be based upon whole-cell typing as well as individual cell-wall antigen groupings. The serotype responsible for enteric red mouth disease is the Hagerman strain, serotype O1a, which is considered to be the most virulent.

Host range

Salmonids (Atlantic salmon, Brown trout, Brook trout, Rainbow trout) + non salmonids (i.e., Common carp, Goldfish)

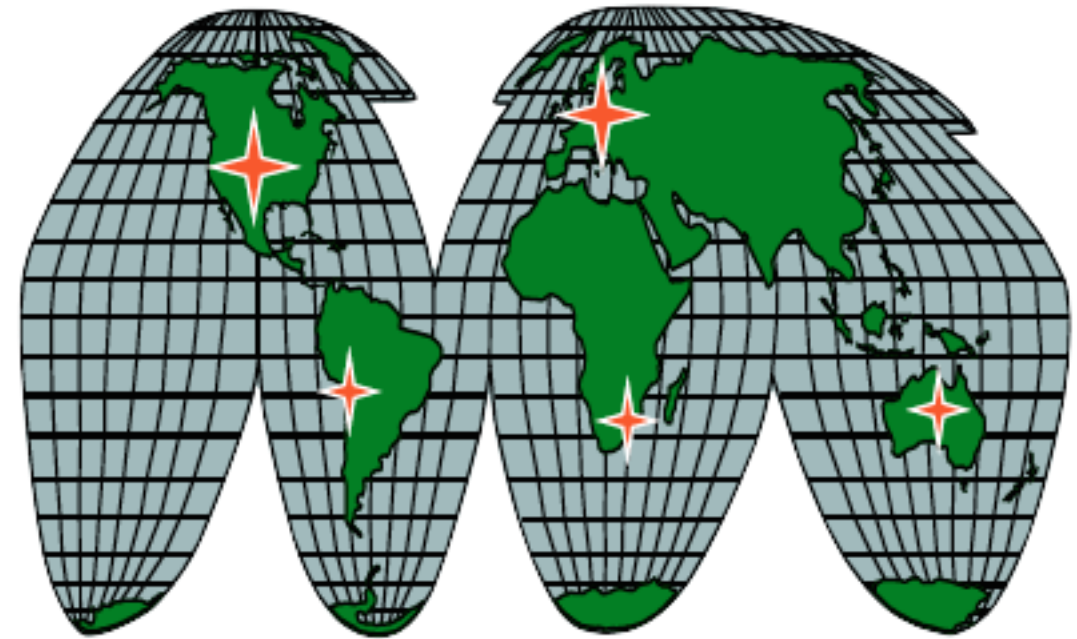
Many other aquatic species are potential carriers but show no signs (e.g. some crustaceans, including freshwater crayfish).



Enteric red mouth disease

Epidemiology

- Transmission can be horizontal, via direct contact with infected fish or carriers. Carriers are particularly important sources of infection under stressful situations (i.e., **increasing temperature**).
- The organism can survive in the environment, with some strains able to form biofilms.
- Vertical transmission (fish to egg) is suspected but is yet to be proven.
- This disease causes septicaemia (bacteria are spread through the body via the blood).
- Fish of all ages are affected, and outbreaks usually begin with low mortalities that slowly escalate.



Geographical range

First report in USA (1958)
Europe (1981)
America
Australia
South Africa

Enteric red mouth disease

Gross pathological signs

- haemorrhages at base of fins
- reddening (subcutaneous haemorrhages) of the gill cover, corners of mouth, gums, palate and tongue
- exophthalmos (popeye) and orbital haemorrhages
- loss of appetite
- swollen abdomen
- ascites (fluid in the abdominal cavity)
- pinpoint haemorrhages on the liver, swim bladder and lateral musculature surfaces
- enlarged spleen
- inflamed lower intestine containing thick yellow fluid.



Enteric red mouth disease

Diagnosis

Isolation of the bacterial pathogen from samples of spleen or kidney in generic freshwater agar based media like tryptone soy agar (TSA), and the species confirmation can be made by phenotypic profiling or molecular assays as PCR. Further histological examination can provide evidence of the level of infection in the fish organs and tissues



Enteric red mouth disease

Preventative measures

Prevention measures are essential to control ERM.

Vaccination has helped to control the significant mortalities due to ERM, especially those that act against several biotypes of *Y. ruckerii*.

Monovalent vaccines have been developed to control the disease in areas where specific bacterial strains are more prevalent than others, based on epidemiological studies of bacterial populations in fish farms.

Treatment relies mainly on in-feed oral use of antibiotics, including routinely used oxolonic acid and florfenicol. Since *in vitro* testing has shown rapid resistance development against several antibiotics, considering the limited availability of antibiotics in fish medicine it is highly recommended to carry out **antibiotic sensitivity testing** to check the ability of the specific bacterium strain to survive in the presence of the antibiotic in question and alleviate the **growing drug-resistance problem**.



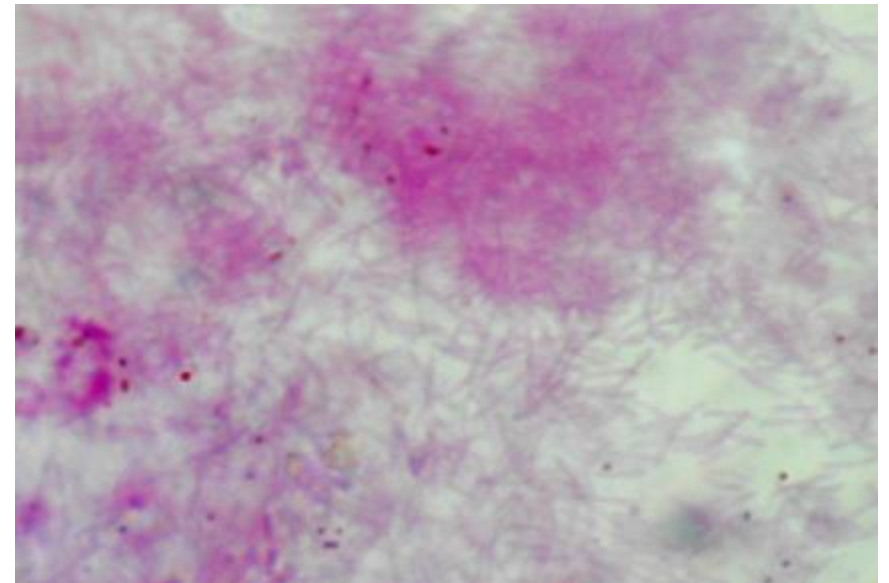
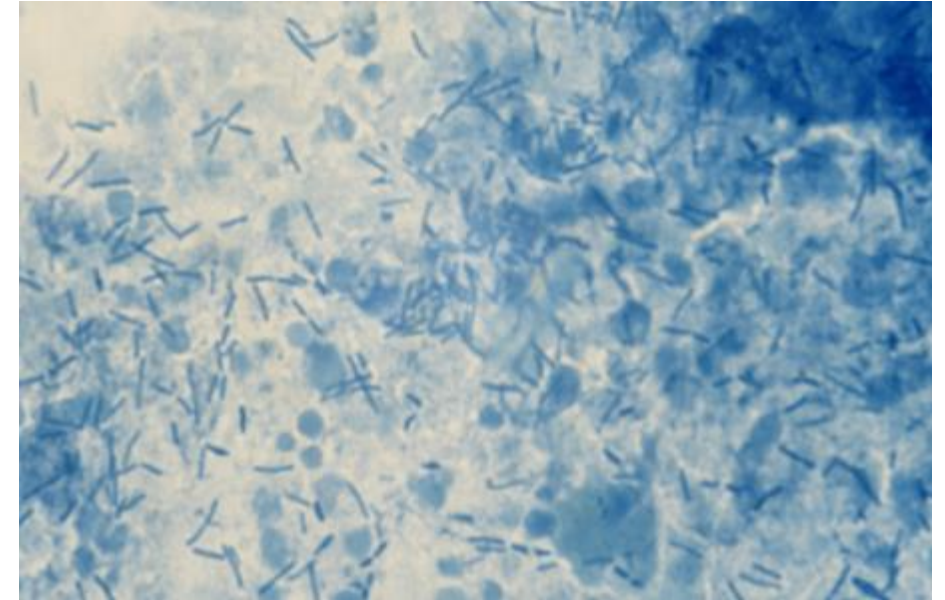
Columnaris Disease

Causative agent

Columnaris disease is caused by the Gram-negative bacterium *Flavobacterium columnare* (Bernardet et al., 1996).

Host range

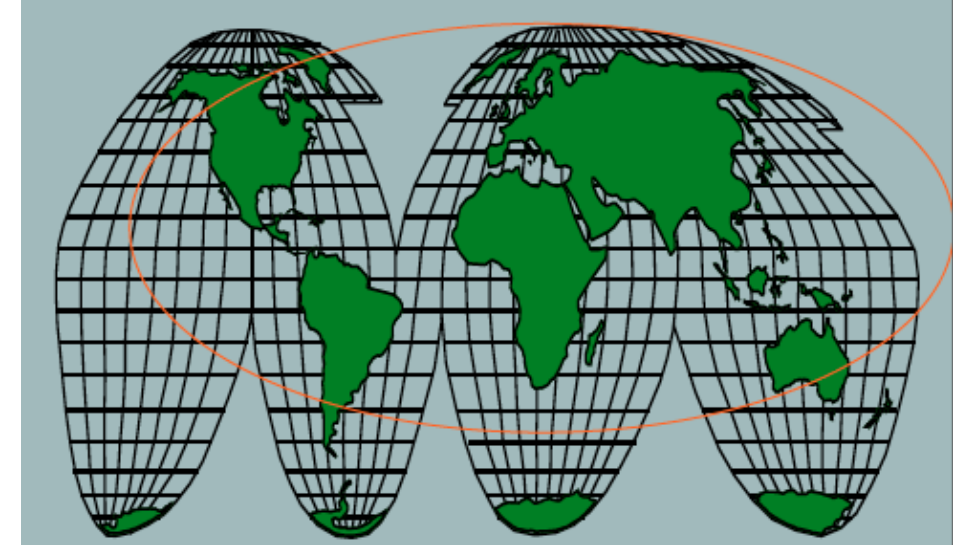
Most freshwater fishes (cultured and wild) are considered susceptible to *F. columnare*.



Columnaris Disease

Epidemiology

- Columnaris disease is transmitted horizontally from fish to fish and can affect fish of all ages but is more prevalent in young fish.
- **The severity and occurrence of columnaris disease is generally greater at warmer water temperatures (>20 °C);** however, the disease can occur in salmonids reared at 12-15 °C.
- Columnaris disease can occur in fish without any predisposing conditions but outbreaks are commonly associated with stressful rearing conditions.
- Additionally, handling and injuries to the skin/mucosa may predispose fish to columnaris disease.
- Mortality patterns can be acute, sub-acute, or chronic, depending on the the fish species involved.
- Mortality rates can be extremely high, with 60 to 90% mortality common.



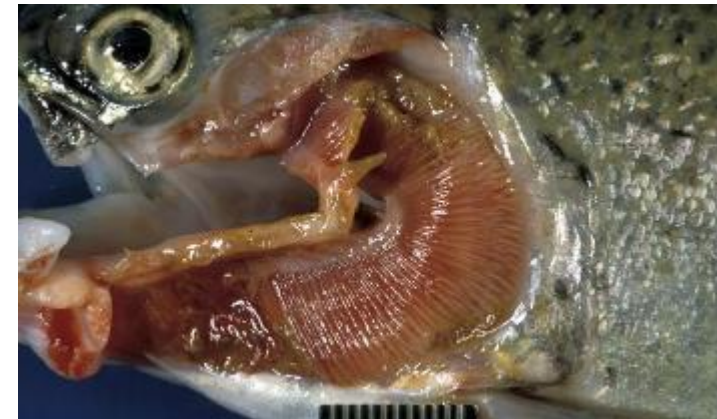
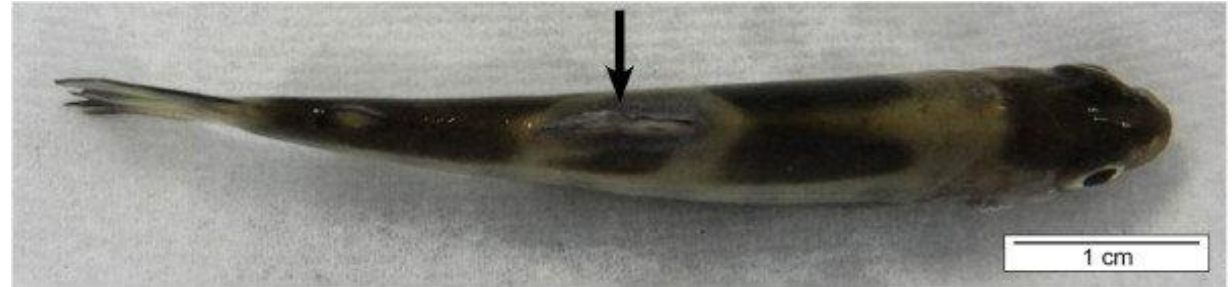
Geographical range

It is generally assumed that it has a worldwide distribution.

Columnaris Disease

Gross pathological signs

- frayed fins
- depigmented lesions on the skin
- necrotic gill lesions
- Skin lesions often begin around the dorsal fin and then increase in size and result in a gray to white lesion that has the appearance of a saddle (i.e., saddleback lesion)
- These lesions can also form on other portions of the caudal peduncle
- In some cases the margin of the lesion may be yellow in appearance due to the proliferation of the yellow-pigmented bacterium.
- Internal lesions are usually not present. Although these are the classical signs, in some cases diseased fish may die without any gross signs.



Columnaris Disease

Diagnosis

- Primary isolation of *F. columnare* should be from the gills and lesions.
- The head kidney and spleen are good organs to target for isolation of *F. columnare* and it may be easier to obtain pure cultures from these.
- Specialized low nutrient microbiological media are needed to support the growth of *F. columnare*, such as tryptone yeast extract salts agar (TYES), cytophaga agar
- Cultures should be incubated at 20-30 °C for 1 to 3 days.
- *Flavobacterium columnare* produces flat, rhizoid, yellow colonies with irregular margins that tightly adhere to the agar.
- Colony morphologies and adherence to agar can vary depending on the isolate and moisture content of agar plates used.



Species confirmation can be made by molecular assays as PCR.

Columnaris Disease

Preventative measures

General preventive measures should be applied in order to minimize stress and avoid the outbreak of parasitic and infectious diseases in farmed fish:

- good husbandry practices and optimal environmental conditions
- careful fish handling
- regular feeding with a right amount of well conserved feed
- appropriate stocking density
- periodical sanitization of equipment (cage nets, landing nets, etc.) by cleaning, disinfecting, washing and drying in sunlight
- vaccination (if authorized)

Fish Streptococcosis

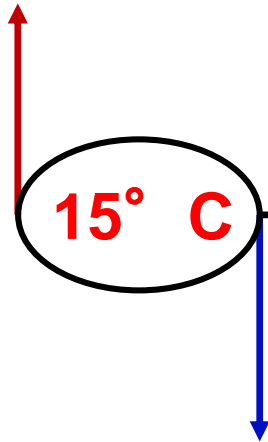
Warmwater Streptococcosis

Lactococcus garvieae/L. petauri

Streptococcus iniae

Streptococcus agalactiae

Streptococcus parauberis

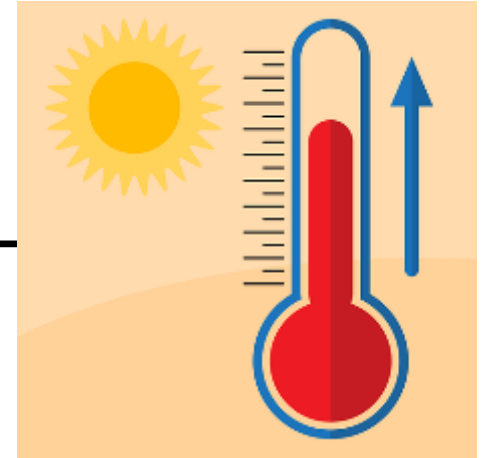


Coldwater Streptococcosis

Vagococcus salmoninarum

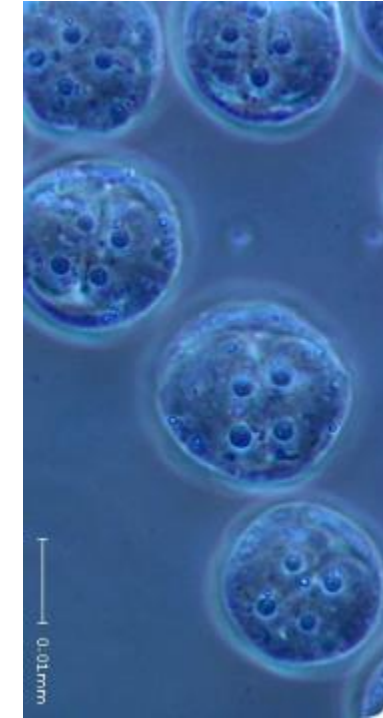
Lactococcus piscium

Carnobacterium maltaromaticum

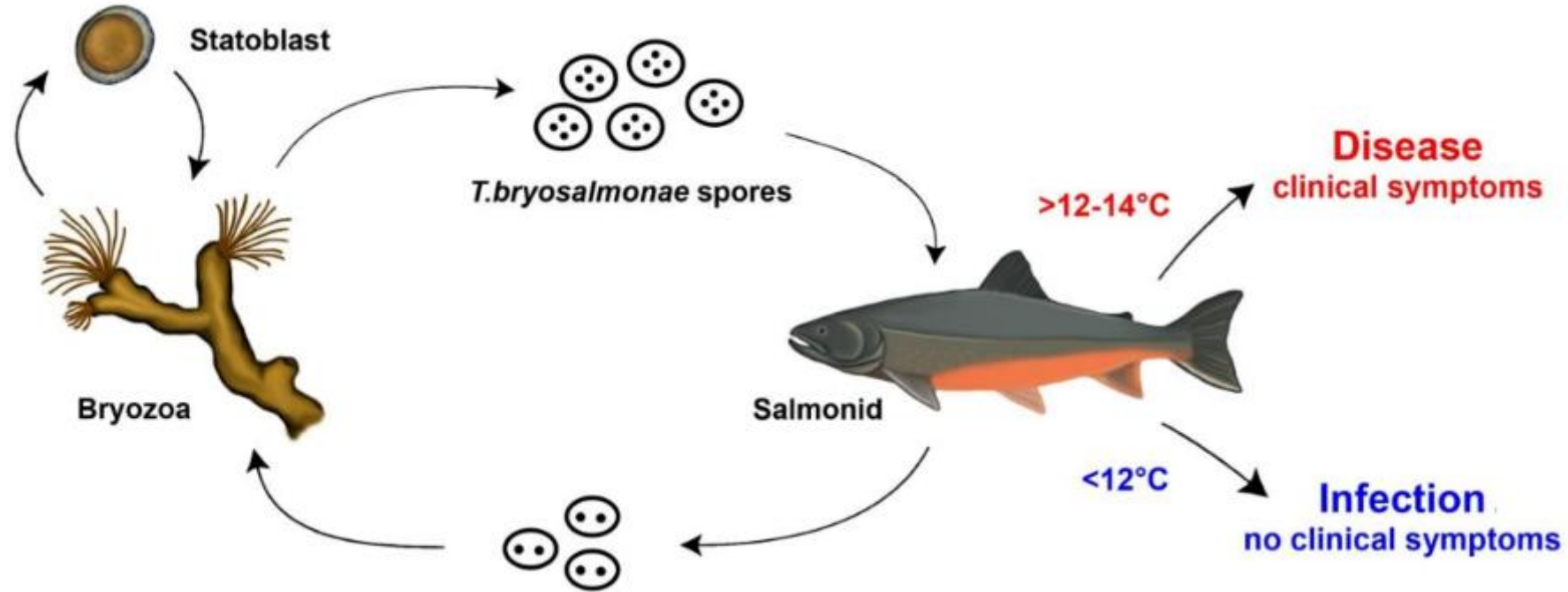


Proliferative Kidney Disease

- Proliferative kidney disease (PKD) is a disease affecting salmonid populations in European and North-American rivers.
- It is caused by the endoparasitic myxozoan *Tetracapsuloides bryosalmonae*, which exploits freshwater bryozoans (*Fredericella sultana*) as primary host.
- Clinical PKD is associated to a massive inflammatory response caused by the proliferation of parasite stages in the kidney and spleen of infected salmonid fish.
- Mortality can reach 90-100%. Moreover, **it is known that the development and pathology of PKD are influenced by temperature.** PKD has been present in brown trout population for a long time but has recently increased rapidly in incidence and severity.
- Environmental changes are likely to cause PKD outbreaks in more northerly regions as **warmer temperatures promote disease development.**



Proliferative kidney disease



As for other myxozoans, the life cycle of *T. bryosalmonae* is complex: the parasite needs two hosts to complete its life cycle with salmonids as their intermediate hosts and bryozoans as their definite hosts

Proliferative kidney disease

Host range

PKD has been reported in both wild and captive salmonids and several other species including whitefish and northern pike in the Pacific Northwest; brown trout, Atlantic salmon and grayling in Europe, including Finland and Sweden; Arctic char in Iceland. In Alaska, the parasite has been reported in lake-reared juvenile sockeye salmon.



Gross pathological signs

External signs are non-specific and may include:

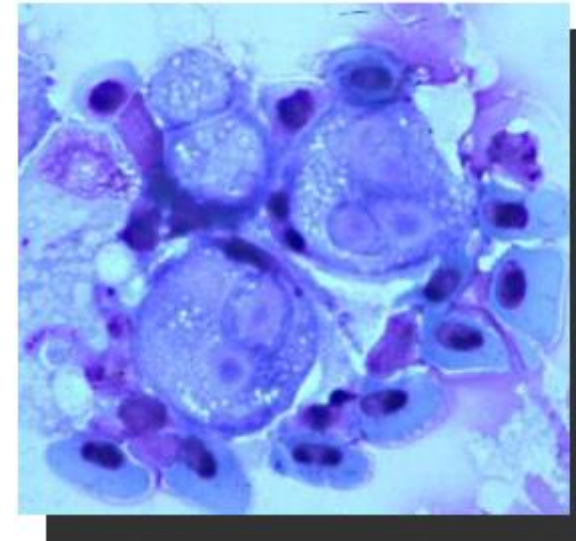
- Swollen abdomen
- Pale gills
- Enlarged spleen and kidney
- Exophthalmos



Proliferative kidney disease

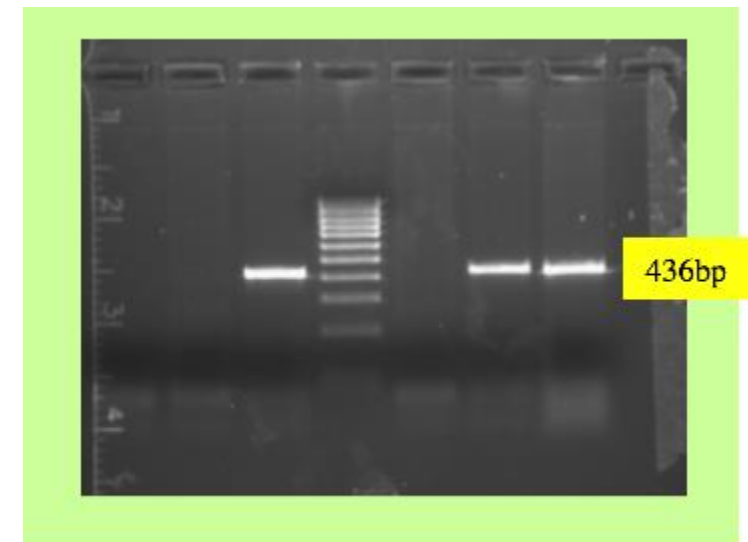
Diagnosis

- Microscopic diagnosis is made by Giemsa-stained imprints
- Parasite DNA can be detected in all organs by PCR and the parasite cells can be observed in kidney, spleen and liver of infected fish by immunohistochemistry



Preventive measures

- Use of well water (absence of bryozoa)
- it is necessary to ensure that the susceptible fish come into contact with the infective stages only in late summer (lower infective load of spores due to lower water temperature): the fish can contract mild infections without the emergence of serious disease and can develop immunity protection for the following year



Emerging contaminants

Water contamination is a serious problem, with 22% of surface water bodies and 28% of groundwater in the European Union being significantly affected by diffuse pollution.

- Contaminants of emerging concern (CECs) are typically divided into chemicals, as they are properly called, and biological CECs, such as pathogens.
- CECs comprise a vast array of contaminants that have only recently appeared in water, or that are of recent concern because they have been detected at concentrations significantly higher than expected, and/or their risk to human and environmental health may not be fully understood
- CECs span natural and artificial chemical substances and their by-products, comprising pharmaceuticals, personal care products (PPCPs), flame retardants (FRs), pesticides, nanoparticles, microplastics and their transformation products, but also antibiotic resistant bacteria (ARB), antibiotic resistant genes (ARG)



Fibres



Pellets



Films



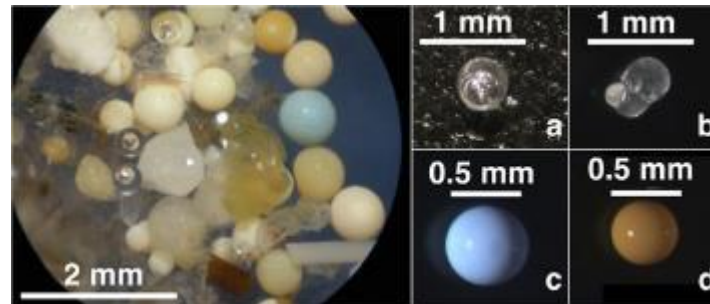
Fragments



Foam



Microbeads



PRIMARY MICROPLASTICS

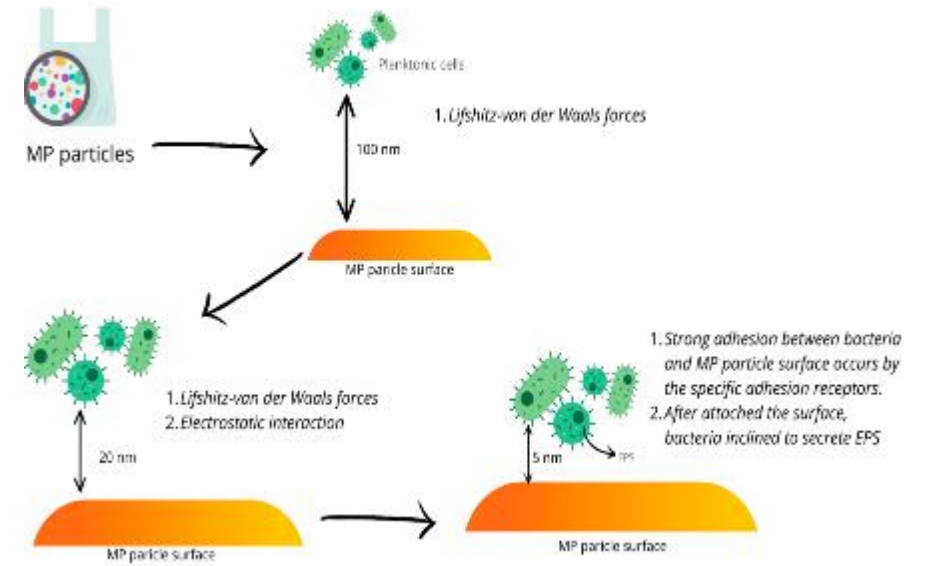
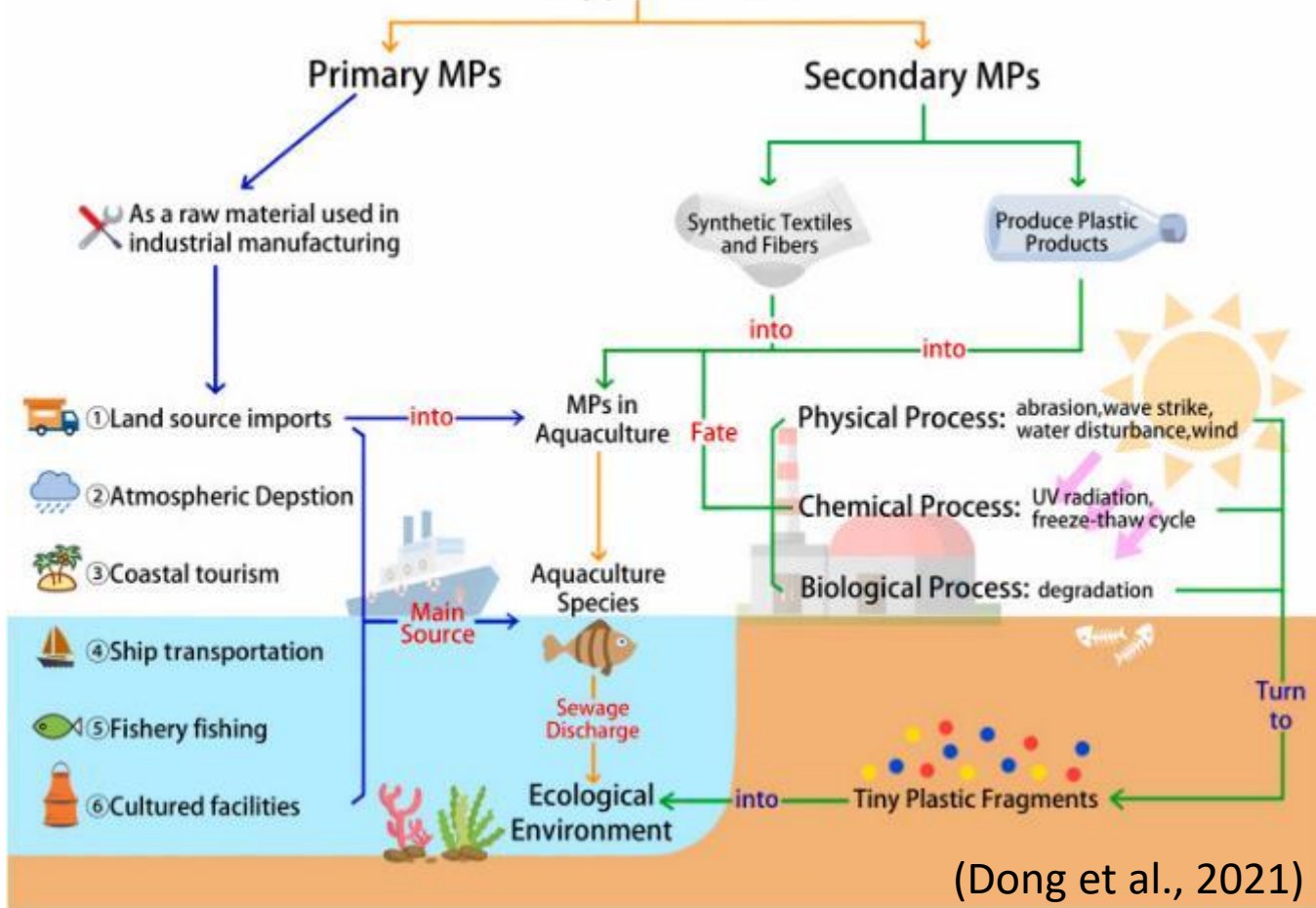
Those which enter the aquatic environment in their « micro » size

SECONDARY MICROPLASTICS

Resulting from the breakdown of larger plastics in the marine environment

The sources, distributions, and behavior characteristics of MPs in the aquaculture

The Types of MPs



Cholewinska et al., 2022

Pastorino et al. *Water Emerg Contam Nanoplastics* 2022;1:3
DOI: 10.20517/wecon.2022.01

Water Emerging Contaminants & Nanoplastics

Review

Open Access

Check for updates

High-mountain lakes as indicators of microplastic pollution: current and future perspectives

Paolo Pastorino¹, Marino Prearo¹, Elisabetta Pizzul², Antonia Concetta Elia³, Monia Renzi², Antoni Ginebreda⁴, Damià Barceló^{4,5}

The sources, distributions, and behavior characteristics of MPs in the aquaculture

Table 1

The concentrations and characteristics of microplastics in the aquaculture systems.

Site	Source	Abundance	Size	Shape	Composition	Color	Reference
Xiangshan Bay, China	seawater	8.9 ± 4.7 items/m ³	Ave: 1.54 ± 1.53 mm.	fiber, film, fragment, foam	PE, PP, PS, PA, PET, cellulose	N/A	Chen et al. (2018)
	sediment	17.39 ± 21.53 items/kg	Ave: 1.33 ± 1.69 mm	fiber, film, fragment, foam	PE, PP, PET, Rubber, cellulose		
Xiangshan Bay, China	sediment	33-113 items/kg, Ave: 74 items/kg	345–4998 µm, Ave: 1830 µm	fiber, film, fragment	Cellulose, PA, AN, PP, PET	N/A	Wu et al. (2020)
Fish farms in Mediterranean, Spain	sediment	0 to 213 items	0.128–5 mm	fiber, fragment, pellet	PE, PP, PA, cellulose	black, transparent, blue, yellow, red	Krüger et al. (2020)
Fish ponds in Changzhou, China	freshwater	13 to 27 items/L	<0.1–5 mm	fiber, film, fragment, pellet	PE, PP, PS, PA, PET	transparent, white, green, yellow, gray	Wang et al. (2020)
Maowei Sea, China	seawater	1.2–10.1 items/L, Ave: 4.5 ± 0.1 items/L	<0.25–5 mm	fiber, flake, foam, fragment	PES, PP, PE, PA, PS, POM, PU, PBT	white, yellow, blue, green, red, black	Zhu et al. (2019)
Fish ponds in Carpathian basin, Europe	freshwater	3.52–32.05 items/m ³ , Ave: 13.79 ± 9.26 items/m ³	N/A	N/A	PE, PP, PS, PTFE, PAC, PES	N/A	Bordós et al. (2019)
	sediment	0.46 to 1.62 items/kg, Ave: 0.81 ± 0.37 items/kg					
Fish ponds in Guangzhou, China	freshwater	42.1 items/L	<0.1–3 mm	fiber, film, granule, fragment, pellet	PP, PE	blue, purple, transparent, white, black, green, yellow, red	Ma et al. (2020)
Rice-fish co-culture system in Shanghai, China	freshwater	0.4 ± 0.1 items/L	<1–5 mm	fiber, film, granule, fragment	PE, PVC, PP	black, transparent, blue, white	Lv et al. (2019)
	sediment	10.3 ± 2.2 items/kg					
Mussels farming in Jurujuba Cove	seawater	16.4/m ³	<1–5 mm, dominant: < 1 mm	fragment, fiber, sheet, pellet	PE, PP	blue, green, red, yellow, orange, black	Castro et al. (2016)
Eel culture stations, Shanghai	water	1.0 ± 0.4 items/L	<0.1–5 mm	film, fiber, fragment, granule	PE, PP, EA	yellow, green, white, black, blue, translucent	Lv et al. (2020)
Milkfish ponds in Muara Kamal	water	27.1 ± 7.0 items/kg	N/A	fiber, film, fragment, granule	N/A	N/A	Priscilla and Patria. (2019)
	sediments	111680 ± 13204 items/kg					
Milkfish ponds in Marunda	water	90.7 ± 17.4 items/L					Chen et al. (2020a)
	sediments	82480 ± 11226 items/kg					
Shrimp-culturing farm in Longjiao Bay, China	seawater	250-5150 items/m ³ , mean: 1594 items/m ³	0.3–5 mm (92.03%) <0.3 mm (7.97%)	fiber, fragment, foam, film, granule	PE, PET, PS, PP, PC, PA, PAA	granule, fibers, white, yellow, black	
Artificial reefs in Ma'an Archipelago, China	seawater	0.2 ± 0.1–0.6 ± 0.2 items/L	1–5 mm	fibers, fragments,	PA, PE, PP, PS, cellulose, cellophane	blue, transparent, black, red, green, yellow, white	Zhang et al. (2020b)
	sediment	30.0 ± 0.0–80.0 ± 14.1 items/kg	0.05–1 mm (dominant)	films			
Eight sea cucumber farms along the Bohai Sea and the Yellow Sea in China	sediment	20 - 1040 items/kg	<1 mm (82%) 1–5 mm (18%)	fibers, fragments, films	cellophane, polyester, PET, PE, PP, PA, PVA, PAN	blue, transparent, black, red, purple, brown	Mohsen et al. (2019)



Aquaculture

Volume 512, 15 October 2019, 734322



Comparison of microplastic contamination in fish and bivalves from two major cities in Fujian province, China and the implications for human health

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Science of The Total Environment

Volume 658, 25 March 2019, Pages 62-68



Microplastic pollution in the Maowei Sea, a typical mariculture bay of China

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Accumulation of microplastics in typical commercial aquatic species: A case study at a productive aquaculture site in China

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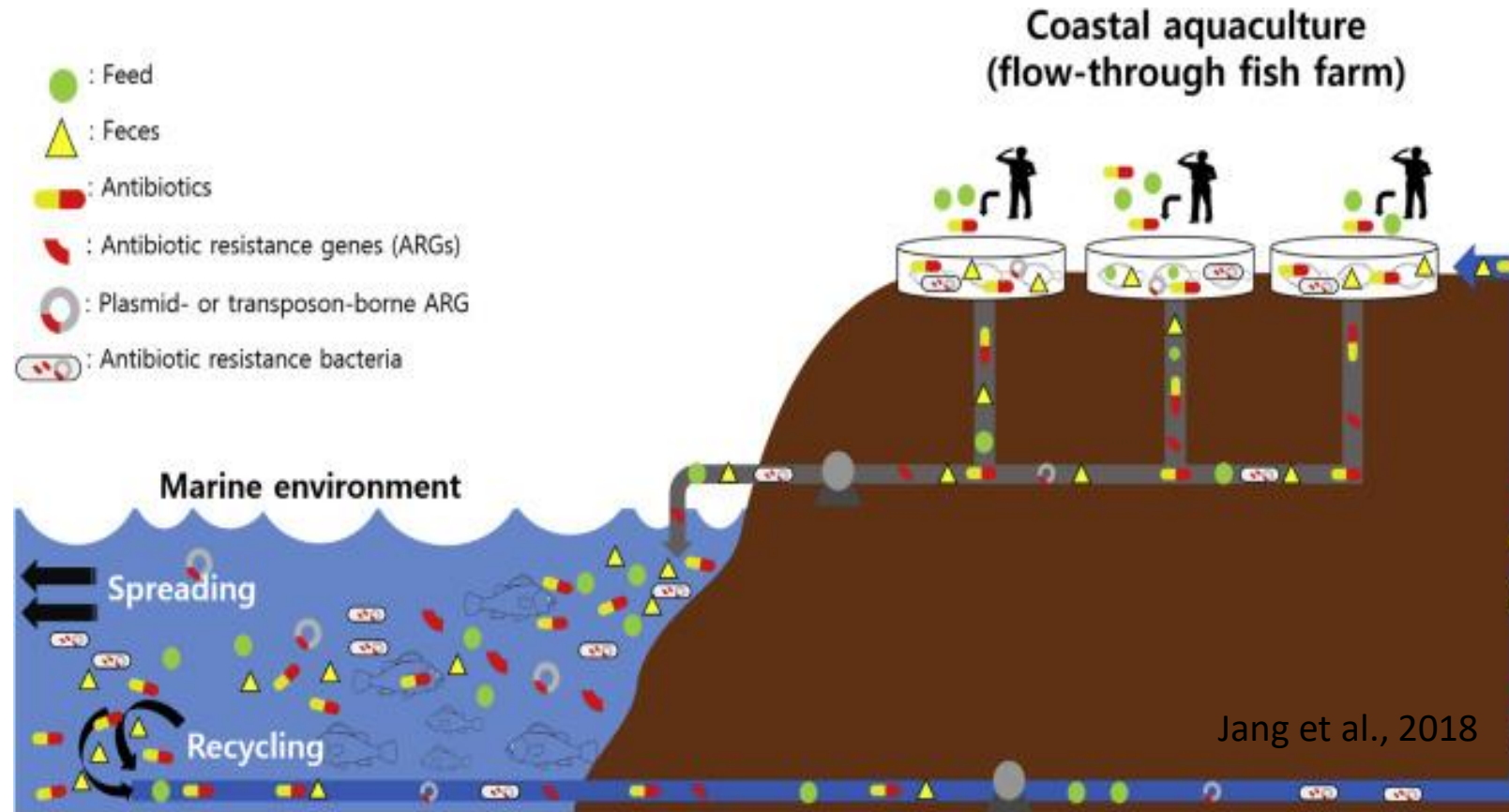


Microplastic ingestion in reared aquaculture fish: Biological responses to low-density polyethylene controlled diets in *Sparus aurata* ☆

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The wide use of antibiotics in aquaculture for therapeutic purpose can potentially lead to the prevalence of antibiotic resistance genes (ARGs).



Drugs contained in fish feed can persist in the aquatic environment for a long time and rapidly spread throughout water systems, exerting selective pressure in ecosystems.

AMR in closed Aquaculture Systems

Closed flow-through systems produce wastewater containing suspended solids and nitrogen, phosphorous, and high microbial loads, which will either enter the municipal wastewater system after a number of treatment steps, flow to wetlands, or be treated to produce a sludge that **can be added to land as a fertilizer.**

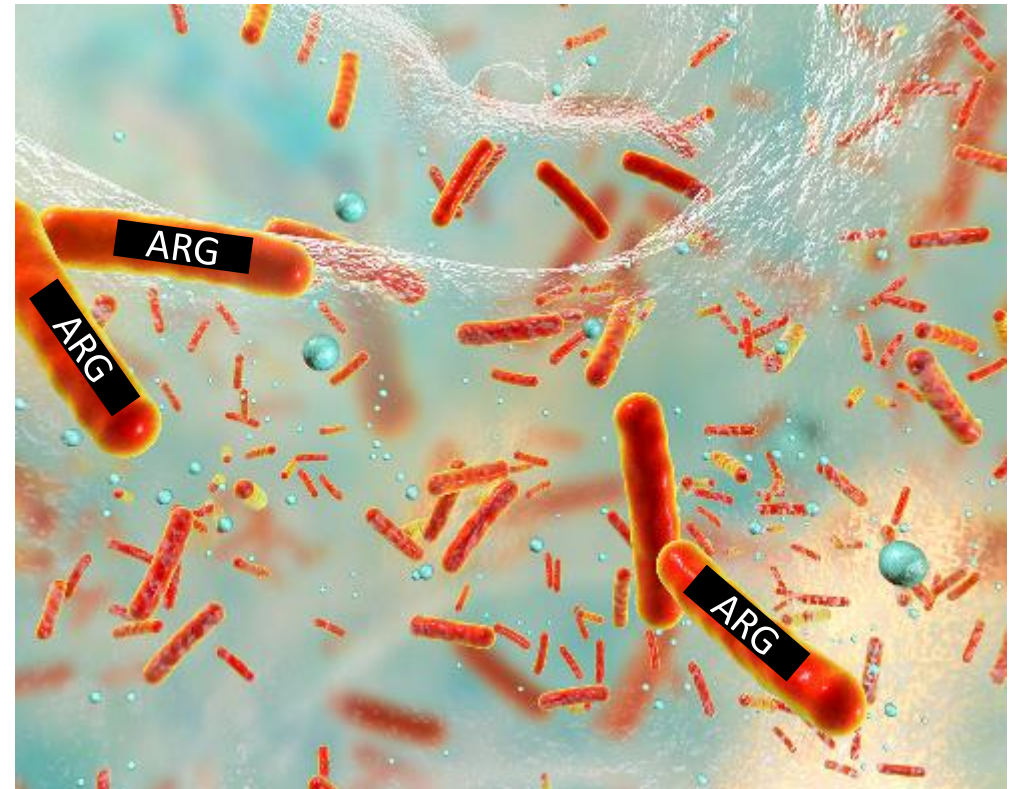
This use of **aquaculture sludge** has numerous **implications for the concentration and spread of AMR** genes onto food crops and into the soil system.



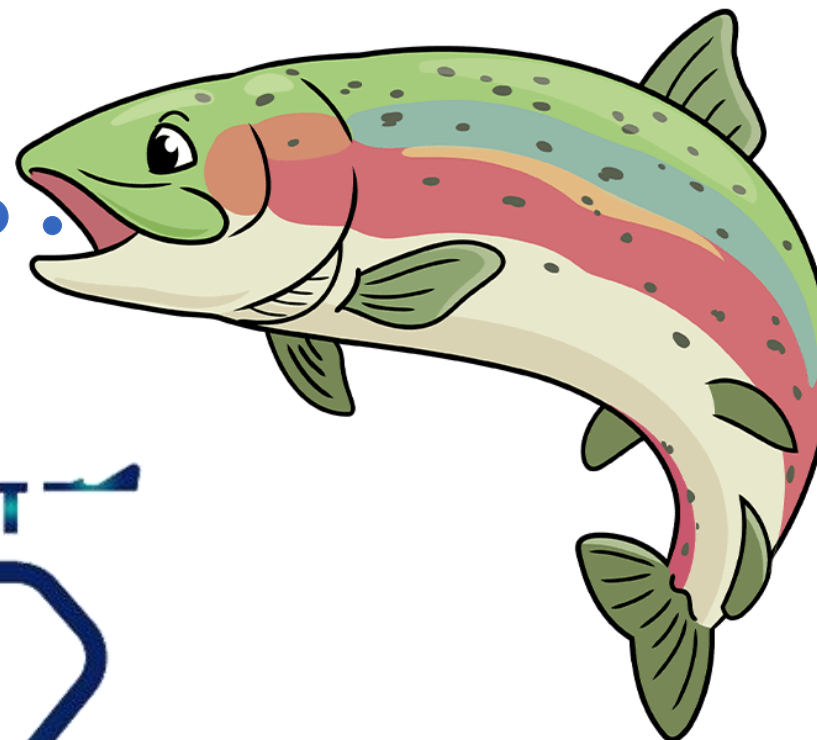
Near zero-discharge recirculating aquaculture systems (RASs) are designed to produce species at high density and minimize environmental impact by effectively managing, collecting, and treating wastes that accumulate during fish growth for both freshwater and marine systems.

Li et al. (2017) found **that biofilms from RAS mixed bed biofilters are a reservoir for antibiotic resistance genes, including tetO, qnrA, and tetE.**

Biofilms, however, are generally resistant to penetration by antibiotics, which, makes the treatment of pathogens difficult (Blancheton et al., 2013).



THANKS FOR YOUR ATTENTION!!!



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