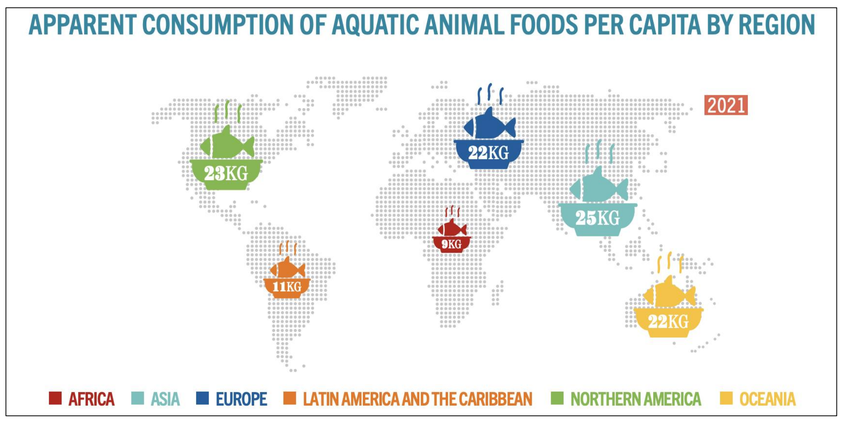
Oral vaccine delivery for fish using C3b tagged chitosan as a drug delivery vehicle

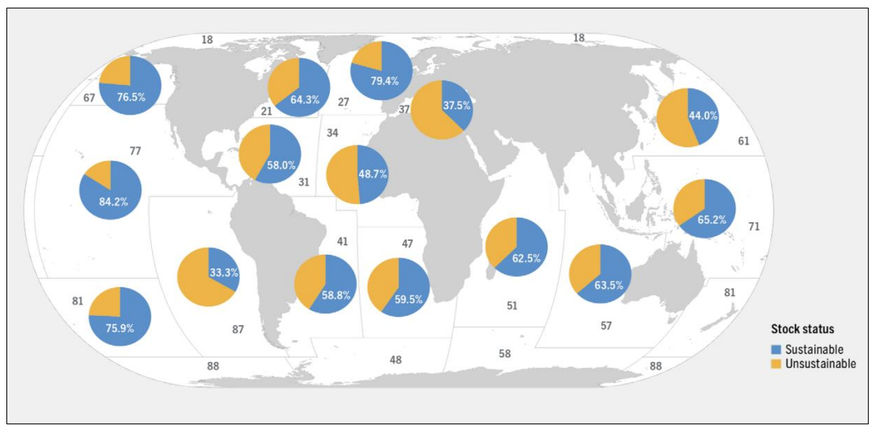
Akhil Kizhakkumpat, Harsha Prakash, Tomonori Somamoto, Takahiro Nagasawa, Miki Nakao

Faculty of Agriculture, Kyushu University

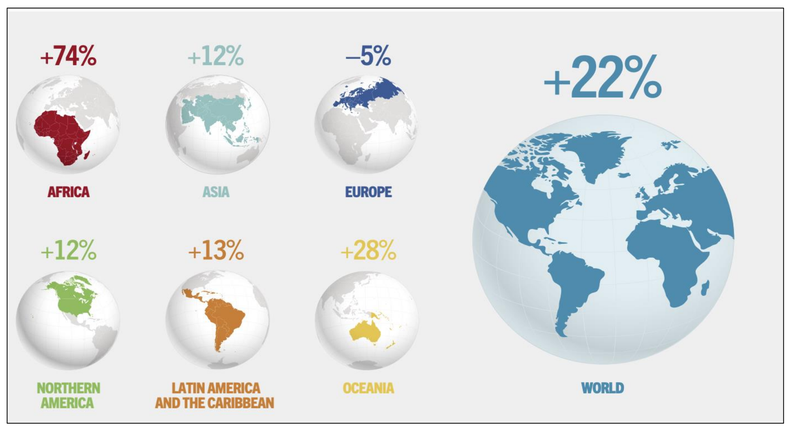
Significance of Aquaculture



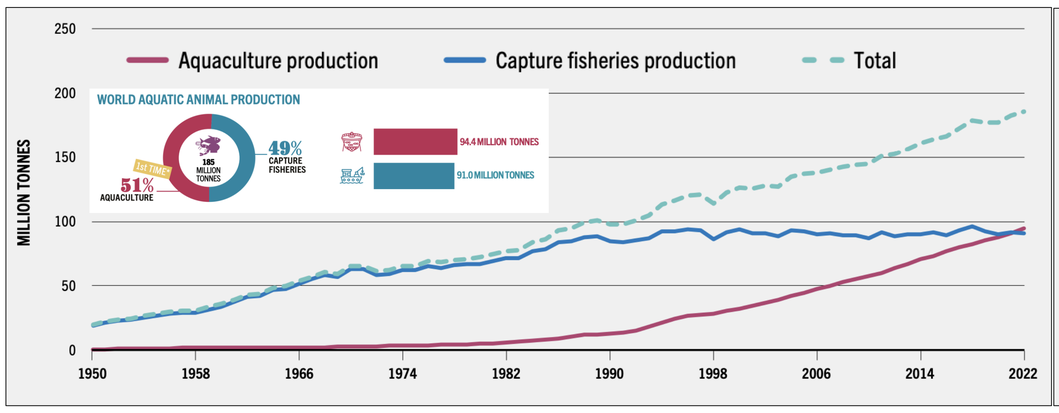
Fish constitutes one sixth of the world`s protein with Asia being one of the biggest consumer[1]



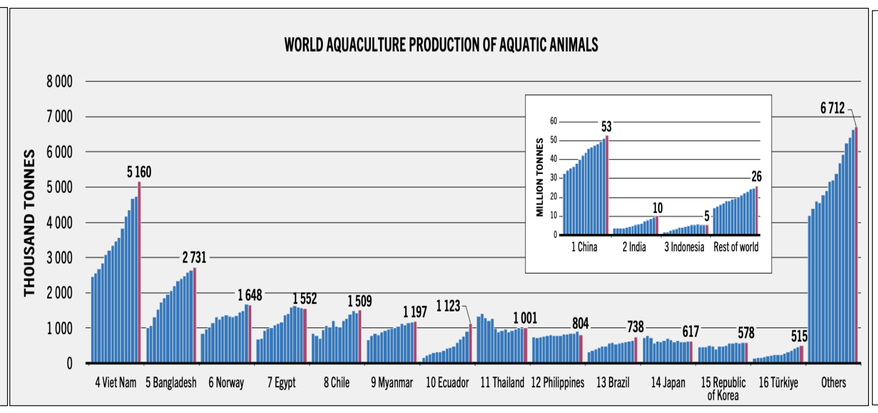
Sustainable and unsustainable fisheries stock in major fishing areas[1]



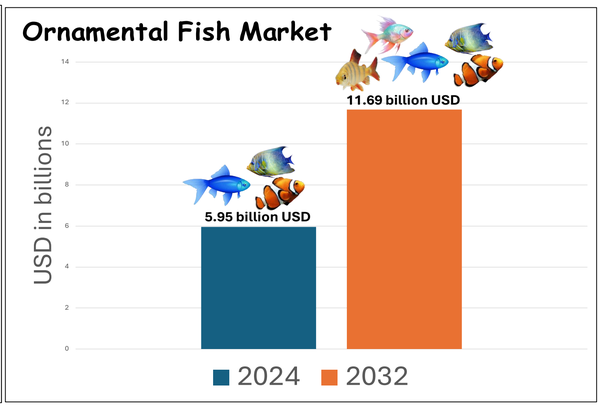
Required growth in aquatic food supply to sustain 2022 per capita consumption levels through to 2050 estimated by FAO[1]



Aquaculture surpassed traditional fishing as a major fish source for the first time in 2022[1]



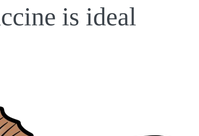
Aquaculture production in the world. Red bar shows the data in 2022[1]



Ornamental Fish Market is expected to grow at a CAGR of 7.8% with ASIA-PACIFIC being the largest market [2]

Fish Diseases : One of the major problem in commercial fish farming

Disease can be caused by bacteria, virus, fungus or parasites and results in an estimated economic impact **of $10 billion USD** annually for the aquaculture industry on a global scale[3]. **Developing effective vaccines are significant for making fish farming sustainable**

Types of Vaccine (Based on administration route)		
Injection Vaccination	Immersion Vaccination	Oral Vaccination
		
• Labor intensive • Highly Stressful for fish • Only systemic immune response is elicited	• Labor intensive • Stress for fish • Higher amount of Antigen	• Easy to administer • Less stressful for the fish. • Both systemic as well as mucosal immune response is elicited

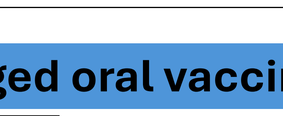
Although most of the commercially available vaccines are administered via injection[3], an effective oral vaccine is ideal

Shortcomings of oral vaccination: 1) The oral vaccine gets degraded at the gut conditions before reaching the posterior intestine which is the site where the immune cell population is the highest in gastro intestinal tract. 2) The vaccines are not effectively absorbed by the intestinal cells including intestinal macrophages[4]

✓ Degradation of vaccine due to gut environment

Encapsulate the vaccine in a protective vaccine vehicle

Chitosan[5]

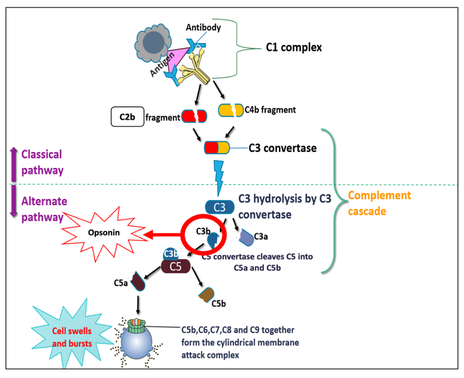


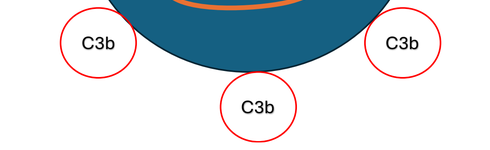
- **Controlled release of vaccine**
- **Low immunogenicity**
- **Easy to synthesize chitosan particles and encapsulate the vaccine**
- **High Para cellular permeability**
- **Non-toxic, Biodegradable**

✓ Decreased uptake of vaccine by the intestinal cells

Complement Pathway[6]

- **Complement system includes proteins which are imminent for the immune system.**
- **It has three pathways which will be activated based on the property of the antigen**
- **C3b is a complement protein formed during the activation of complement pathway, which binds to the antigen and enhances the uptake of uptake of antigen by the immune cells including macrophages**

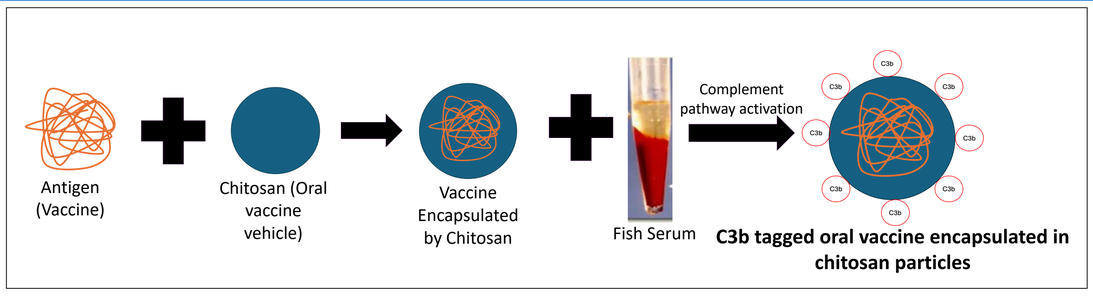




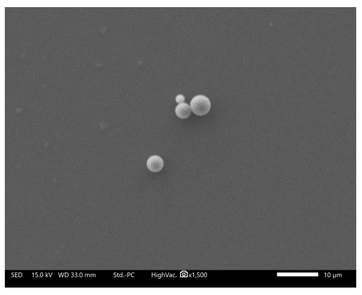
C3b tagged oral vaccine encapsulated in chitosan particles

- ✓ Chitosan protects the vaccine from the harsh gut environment
- ✓ C3b tagging enhances the absorption of vaccine by the intestinal immune cells

Development of the C3b tagged oral vaccine encapsulated in chitosan particles

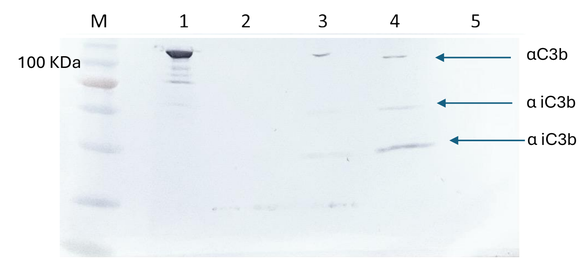


Vaccine encapsulated with chitosan is synthesized via ionic gelation. Vaccine encapsulated with chitosan is further incubated with fish serum which activates complement pathway and results in the deposition of C3b

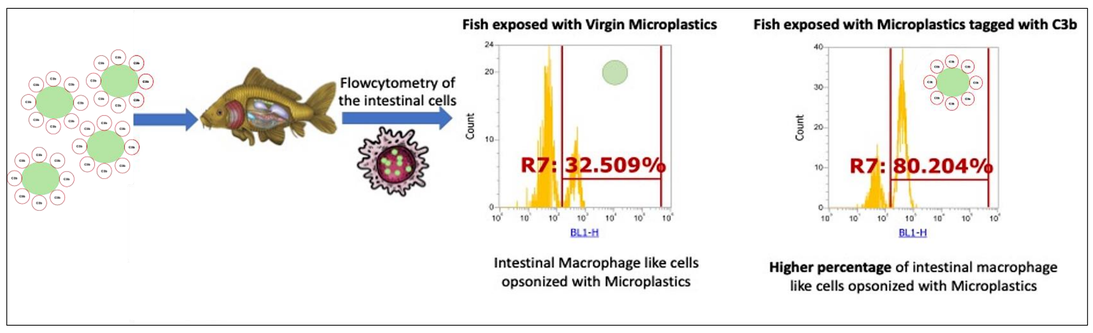


Scanning electron microscopy image of Ovalbumin (model antigen) encapsulated chitosan particle

Carp serum



Western blot showing deposition of C3b
Lane: **M**-Marker, **1**-C3, **2**-Chitosan particles, **3**-Chitosan particles incubated with serum, **4**-Microplastics with serum, **5**-Microplastics



Experiment showcasing heightened opsonization of microplastics by intestinal macrophage like cells in fish exposed microplastics tagged with C3b

References

1. FAO. (2024). State of World Fisheries and Aquaculture. Rome: FAO.
2. Straits Research. (2024). Ornamental fish market.
3. Rathor, G. S., & Swain, B. (2024). Fish vaccination advancements. Appl. Sci., 14, 5672.
4. Mutoloki, S., Munang'andu, H. M., & Evensen, Ø. (2015). Oral fish vaccination. Front. Immunol., 6, 519.
5. Sangnim, T. et al. (2023). Chitosan in oral drug delivery formulations: A review. Pharmaceutics, 15, 2361.
6. Bavia, L. et al. (2022). Advances in the complement system of a teleost fish, Oreochromis niloticus. Fish & Shellfish Immunology, 123, 61-74.

Acknowledgement

The study is funded by JSPS Kakenhi Early Career Research Grant **2024年度 若手研究 (**JAG4K17960)

Summary

1. In 2022, aquaculture surpassed traditional fishing as the primary source of fish, with the Asia-Pacific region being the largest market for ornamental fish.

2. Fish diseases pose a significant challenge in commercial aquaculture, and developing vaccines is crucial since they are more effective than other treatments. Oral vaccines are ideal, though most are currently administered via injection.

3. Oral vaccines face problems such as degradation in the gut environment and decreased uptake by intestinal cells.

4. A proposed solution is to use chitosan and complement protein c3b as an oral vaccine vehicle to address these issues.

5. Chitosan protects the vaccine from gut degradation, while the deposition of C3b improves vaccine uptake by intestinal immune cells.

6. In the current study, a chitosan-encapsulated vaccine was developed, which activated the carp alternative complement pathway upon incubation with carp serum, leading to c3b deposition.

7. The increased uptake by intestinal cells was confirmed through flow cytometry using c3b-coated microplastics, and further research will continue.