

Spotlight 3: TUMSAT spotlight

DNA vaccines for poxvirus infections in koi and ayu

Regional Workshop for WOAHA Focal Points for Aquatic Animals

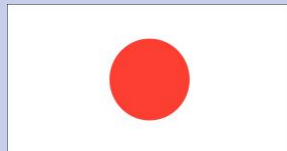
Motohiko Sano, Ph.D

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Prof. TUMSAT



World Organisation
for Animal Health



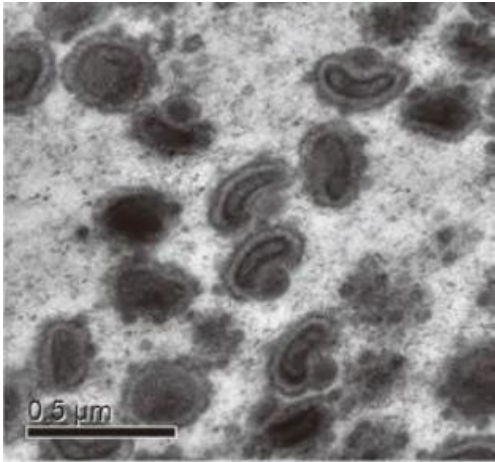
Australian Government
Department of Agriculture,
Fisheries and Forestry



国立大学法人
東京海洋大学
Tokyo University of Marine Science and Technology



History of Fish Poxviruses



Typical poxvirus particle morphology: PaPV

- **1970s - Japan:** Viral edema of carp / koi sleepy disease caused by carp edema virus (CEV).
- **1980s - Japan:** Atypical cellular gill disease in ayu caused by *Plecoglossus altivelis* poxvirus (PaPV).
- **Late 1990s - Norway:** Salmon gill poxvirus (SGPV) disease in Atlantic salmon.
- **Recent Reports (2017-2025):** Detections were reported in the Cape seahorse (2017), Atlantic Cod (2025), black bullhead (2025), sailfin tang (2025), and red seabream (2025).
- These emerging pathogens predominantly affect the gills and skin.

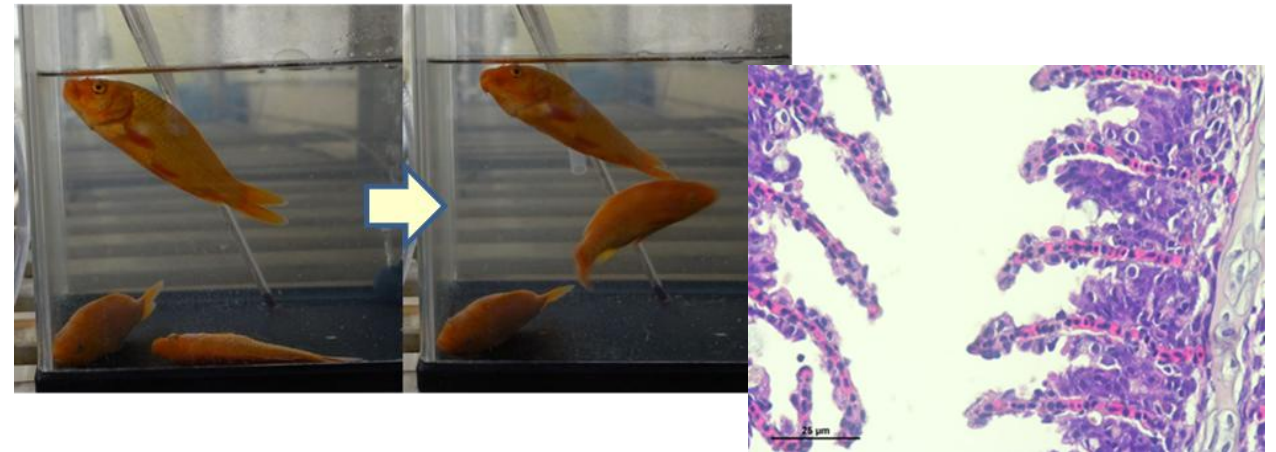


Pathology of Fish Poxviruses

- Unlike many systemic virus infections, fish poxviruses primarily target epithelial cells in the gills and skin.
- Primary symptoms: Extensive pathology of the gills—such as epithelial hyperplasia and clubbing—leads to gill dysfunction, resulting in respiratory and osmotic impairments manifested by lethargy and hyperventilation. The subsequent hypoosmotic state induces severe edema in freshwater fish.



PaPV infection in ayu: swelling, congestion, and formation of atypical cells with cytoplasmic inclusions in the gills. From JFRCA : “ACGD Manual, Ver 2”



CEV infection in koi: lethargic and sleeping behavior, and gill epithelium hyperplasia.



International Regulatory Context

- **The Network of Aquaculture Centres in Asia-Pacific (NACA) in 2017:** Infection with CEV listed as a “notifiable disease”.
- **The WOAHA Aquatic Animal Health Standards Commission (AAHSC) in 2020:** infection with CEV designated as an “emerging disease”. (Mandatory reporting)
- **WOAHA AAHSC in 2023:** Status revised; new scientific evidence required for future listing.

WOAHA Technical Disease Card for “Infection with CEV”
<https://www.woah.org/en/disease/carp-edema-virus/>



Current Countermeasures

- **Saltwater Bathing (0.5%) & Heating (above 28°C):** Alleviates edematous symptoms by normalizing osmotic pressure and reduces virus propagation.
- **The Pitfall:** These treatments do not directly inactivate the virus.
- **Outcome:** Surviving fish can acquire immunity against the disease; however, they become asymptomatic carriers and serve as sources of infection. If naive fish are introduced into a pond where a virus-carrier fish has been reared, they will develop the disease.



Koi rearing ponds



Salt stock for emergency treatment



Economic Impact on Koi Aquaculture

- Koi export is a major Japanese aquaculture industry facing stringent biosecurity hurdles.
- **The “CEV-Free” Mandate:** To export koi to China, governmentally registered koi farms must be certified free of CEV, as well as koi herpesvirus (KHV) and spring viremia of carp virus (SVCV).
- **Research Motivation:** Developing an effective vaccine is a crucial pathway to mitigating virus-carrier risks and ensuring trade sustainability.



Shoes disinfection bath



UV in rearing water



Gill biopsy for PCR test

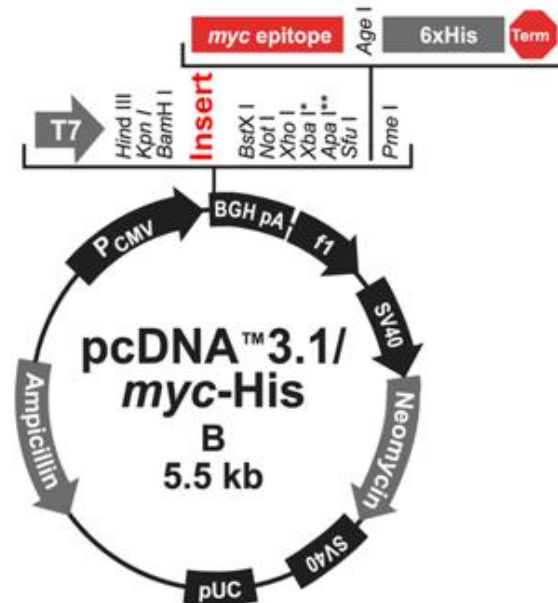


Biosecurity in a local market

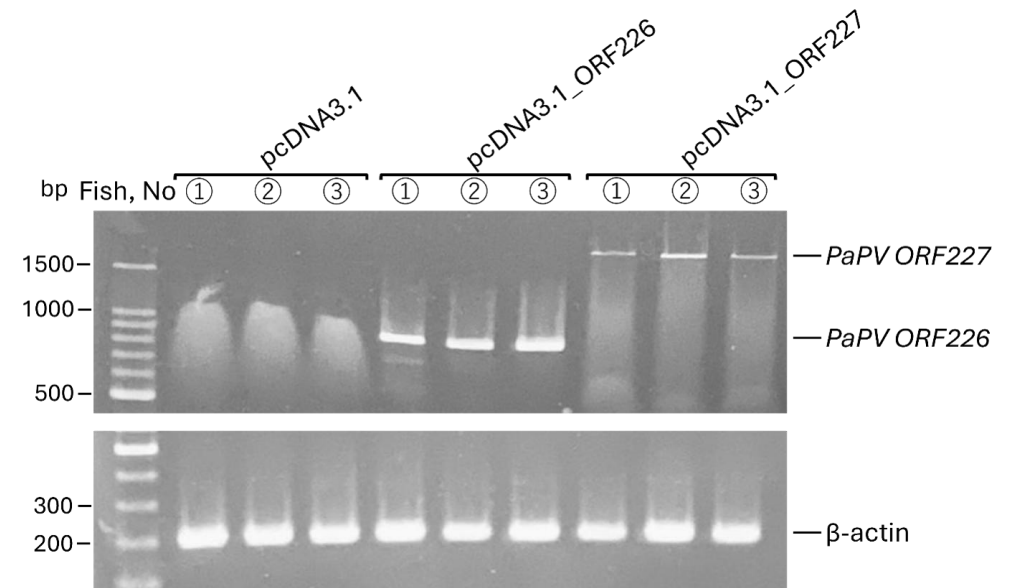


Vaccine Development Strategy

- Focused on vaccinia virus (VV) protective antigen homologs:
VV L1R homolog (envelope protein): ORF226 (PaPV), ORF173 (CEV)
VV D13L homolog (core protein): ORF227 (PaPV), ORF174 (CEV)
- Vector System: Constructed DNA vaccines using pcDNA3.1 myc/His B vector plasmid.



From <https://documents.thermofisher.com>

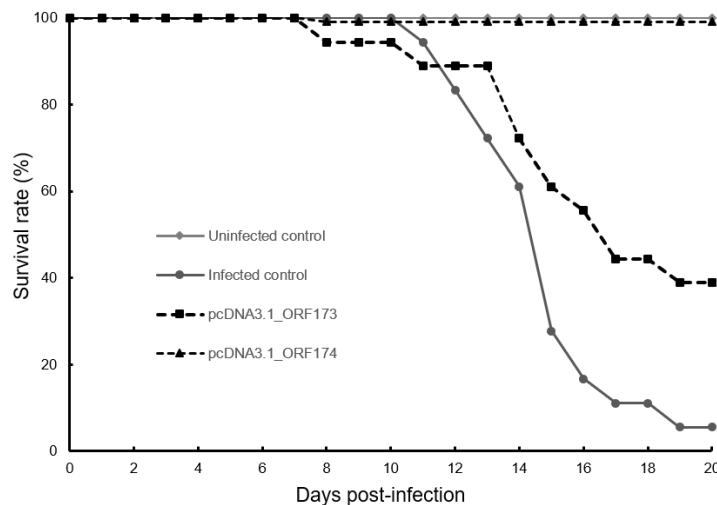


Expression of the DNA vaccines in fish



Vaccine Protective Efficacy

- Protective efficacy of the DNA vaccines against virus challenge (relative percent survival, RPS):
VV L1R homolog: ORF226 (PaPV): 40%, ORF173 (CEV): 35%
VV D13L homolog: ORF227 (PaPV): 85%, ORF174 (CEV): 100%
- VV D13L homolog DNA vaccines induced robust protection in both ayu and koi. Notably, these target genes are highly conserved within the subfamily *Chordopoxvirinae*.



Fish survival after virus challenge

Baba et al., Development of an effective DNA vaccine against ayu atypical cellular gill disease using draft genome information of the causative agent, *Plecoglossus altivelis* poxvirus, *Virology* 612 (2025): 110671

Ogawa et al., Development of an effective DNA vaccine against viral edema of carp/koi sleepy disease caused by carp edema virus, *Journal of Fish Diseases* 49 (2026): e70119



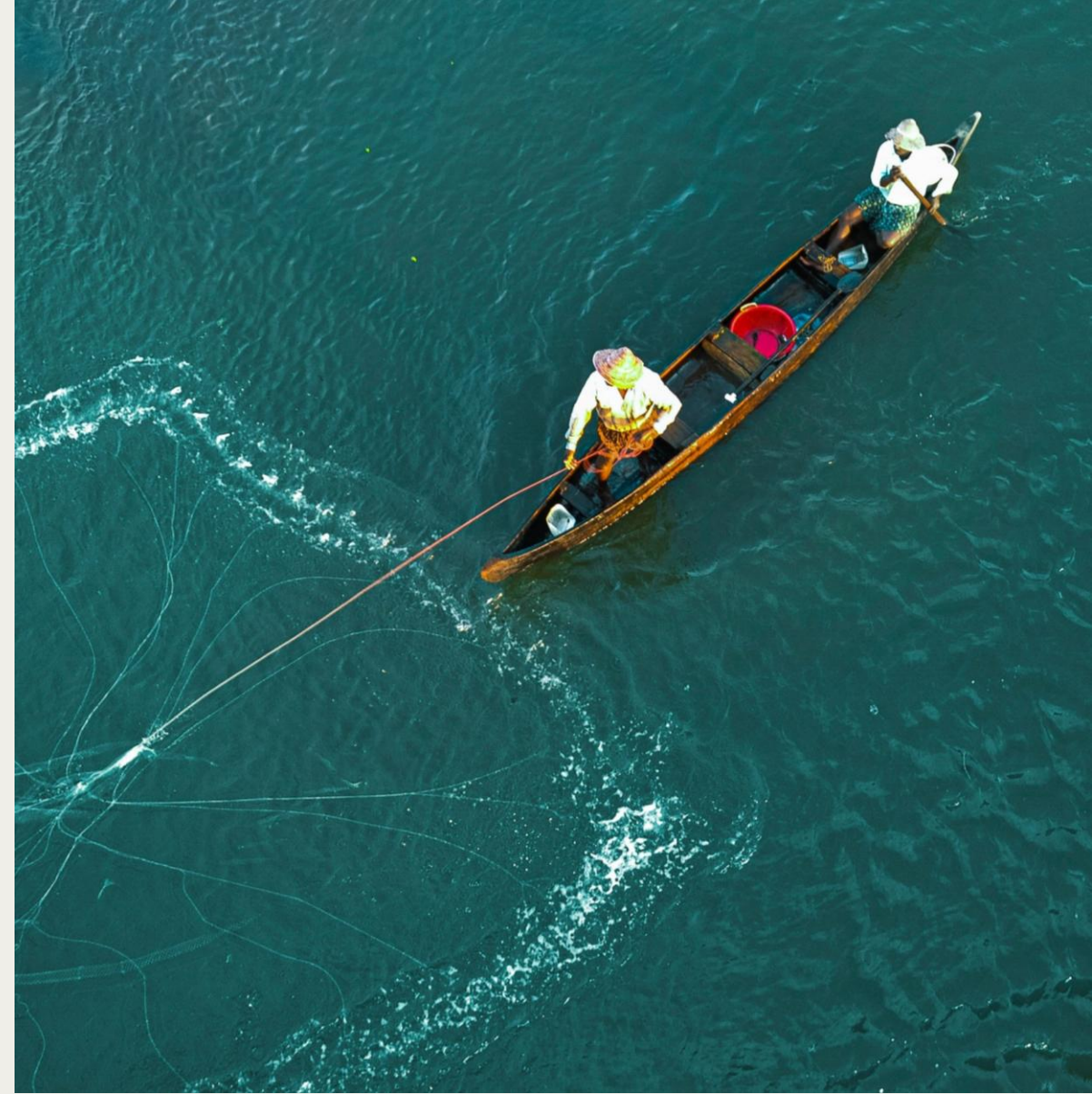
Conclusion & Future Perspectives

- **Practical Application:** Accelerating the development of the DNA vaccine against CEV infection (viral edema of carp / koi sleepy disease) for the koi aquaculture industry to facilitate and secure international export.
- **Advancing Mucosal Immunity:** This study provides key insights into protecting fish mucosal tissues against emerging poxviruses.

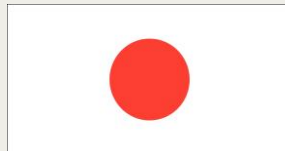


“Koi Show” in Japan

Thank you for your attention



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