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Infection with Decapod iridescent virus 1, an emerging disease in Decapod

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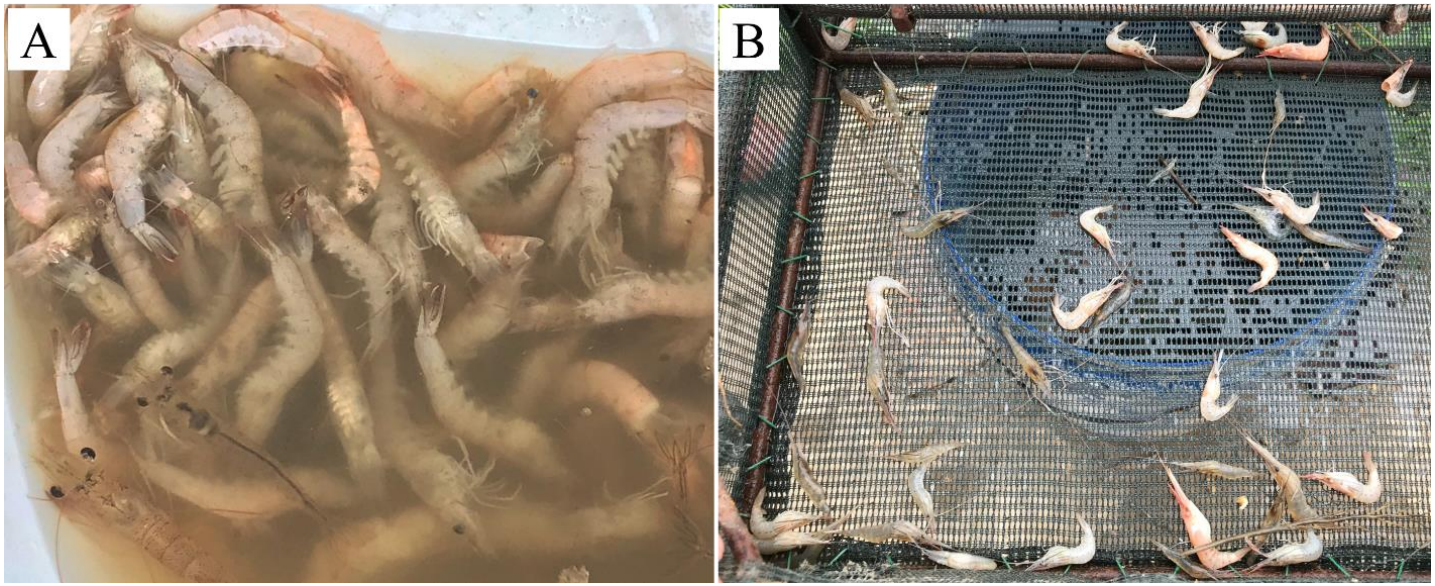
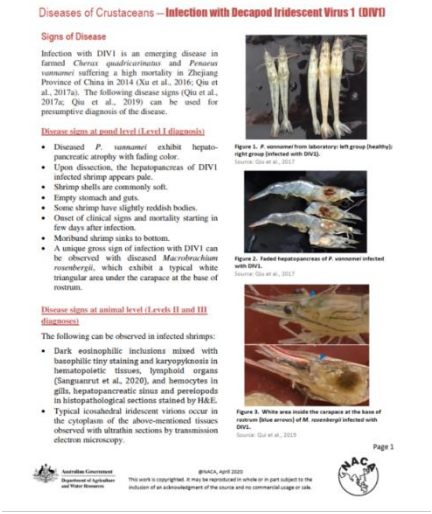
Introduction

- The shrimp industry has been beset by many devastating diseases in the last three decades, which has caused severe production and economic losses and even caused the collapse of the industry in some countries.
- As an emerging viral disease of decapod, infection with DIV1 has caused substantial mortalities in farmed shrimps, prawns, and crayfishes in certain areas.



Introduction

- It has been included in the Quarterly Aquatic Animal Disease Report (QAAD) by the Network of Aquaculture Centres in Asia-Pacific (NACA) in 2016 and listed by the World Organization for Animal Health (WOAH) in 2021.
- The new iridescent virus, Decapod iridescent virus 1 (DIV1), was proven to be the etiological agent of this new disease. The disease is also known as the 'white head' or 'white spot' disease because of its typical clinical symptoms in the giant freshwater prawn, *Macrobrachium rosenbergii* (Qiu et al., 2019a).


Diseases of Crustaceans – **Infection with Decapod Iridescent Virus 1 (DIV1)**

Signs of Disease

Infection with DIV1 is an emerging disease in farmed *Cherax quadricarinatus* and *Penaeus vannamei* suffering a high mortality in Zhejiang Province of China in 2014 (Xu et al., 2016; Qiu et al., 2017a). The following disease signs (Qiu et al., 2017a; Qiu et al., 2019) can be used for presumptive diagnosis of the disease.

Disease signs at pond level (Level I diagnosis)

- Diseased *P. vannamei* exhibit hepatopancreatic atrophy with fading color.
- Upon dissection, the hepatopancreas of DIV1 infected shrimp appears pale.
- Shrimp shells are commonly soft.
- Empty stomach and guts.
- Some shrimp have slightly reddish bodies.
- Onset of clinical signs and mortality starting in few days after infection.
- Moribund shrimp sinks to bottom.
- A unique gross sign of infection with DIV1 can be observed with diseased *Macrobrachium rosenbergii*, which exhibit a typical white triangular area under the carapace at the base of rostrum.

Disease signs at animal level (Levels II and III diagnoses)

The following can be observed in infected shrimps:

- Dark eosinophilic inclusions mixed with basophilic tiny staining and karyopyknosis in hematopoietic tissues, lymphoid organs (Sanguanrut et al., 2020), and hemocytes in gills, hepatopancreatic sinus and pereiopods in histopathological sections stained by H&E.
- Typical icosahedral iridescent virions occur in the cytoplasm of the above-mentioned tissues observed with ultrathin sections by transmission electron microscopy.

Figure 1. *P. vannamei* from laboratory: left group (healthy); right group (infected with DIV1).

Figure 2. Faded hepatopancreas of *P. vannamei* infected with DIV1.

Figure 3. White area inside the carapace at the base of rostrum (blue arrows) of *M. rosenbergii* infected with DIV1.

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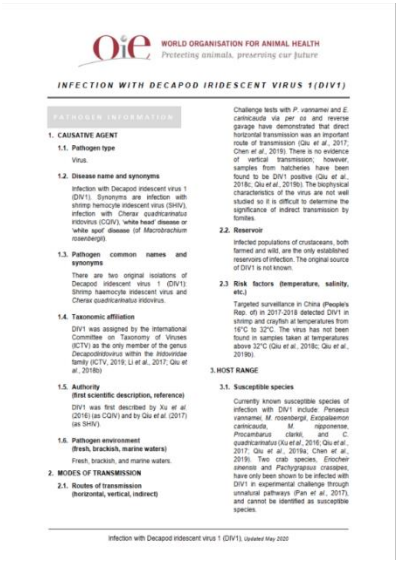
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INFECTION WITH DECAPOD IRIDESCENT VIRUS 1(DIV1)

PATHOGEN INFORMATION

1. CAUSATIVE AGENT

1.1. Pathogen type
Virus.

1.2. Disease name and synonyms
Infection with Decapod iridescent virus 1 (DIV1). Synonyms are infection with shrimp hemocyte iridescent virus (SHIV), infection with *Cherax quadricarinatus* iridovirus (CQIV), 'white head' disease or 'white spot' disease (of *Macrobrachium rosenbergii*).

1.3. Pathogen common names and synonyms
There are two original isolations of Decapod iridescent virus 1 (DIV1): Shrimp haemocyte iridescent virus and *Cherax quadricarinatus* iridovirus.

1.4. Taxonomic affiliation
DIV1 was assigned by the International Committee on Taxonomy of Viruses (ICTV) as the only member of the genus *Decapodiridovirus* within the *Iridoviridae* family (ICTV, 2019; Li et al., 2017; Qiu et al., 2018b)

1.5. Authority (first scientific description, reference)
DIV1 was first described by Xu *et al.* (2016) (as CQIV) and by Qiu et al. (2017) (as SHIV).

1.6. Pathogen environment (fresh, brackish, marine waters)
Fresh, brackish, and marine waters.

2. MODES OF TRANSMISSION

2.1. Routes of transmission (horizontal, vertical, indirect)
Challenge tests with *P. vannamei* and *E. calmicaudo* via *per os* and reverse gavage have demonstrated that direct horizontal transmission was an important route of transmission (Qiu *et al.*, 2017; Chen *et al.*, 2019). There is no evidence of vertical transmission; however, samples from hatcheries have been found to be DIV1 positive (Qiu et al., 2018c; Qiu *et al.*, 2018b). The biophysical characteristics of the virus are not well studied so it is difficult to determine the significance of indirect transmission by fomites.

2.2. Reservoir
Infected populations of crustaceans, both farmed and wild, are the only established reservoirs of infection. The original source of DIV1 is not known.

2.3 Risk factors (temperature, salinity, etc.)
Targeted surveillance in China (People's Rep. of) in 2017-2018 detected DIV1 in shrimp and crayfish at temperatures from 16°C to 32°C. The virus has not been found in samples taken at temperatures above 32°C (Qiu et al., 2018c; Qiu et al., 2018b).

3. HOST RANGE

3.1. Susceptible species
Currently known susceptible species of infection with DIV1 include: *Penaeus vannamei*, *M. rosenbergii*, *Exopalaemon carinicauda*, *M. nipponense*, *Procambarus clarkii*, and *C. quadricarinatus* (Xu *et al.*, 2016; Qiu *et al.*, 2017; Qiu *et al.*, 2019a; Chen *et al.*, 2019). Two crab species, *Eriocheir sinensis* and *Pachygrapsus crassipes*, have only been shown to be infected with DIV1 in experimental challenge through unnatural pathways (Pan *et al.*, 2017), and cannot be identified as susceptible species.

Infection with Decapod iridescent virus 1 (DIV1), *Updated May 2020*

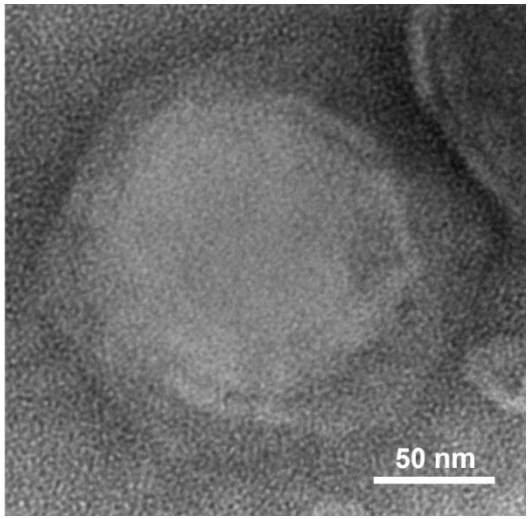
Aetiology

- The virus was independently found by two groups:

  CQIV (Cherax quadricarinatus iridovirus) was found from red claw crayfish *Cherax quadricarinatus* in Xiammen, China in 2014 by Xu et al. (2016);

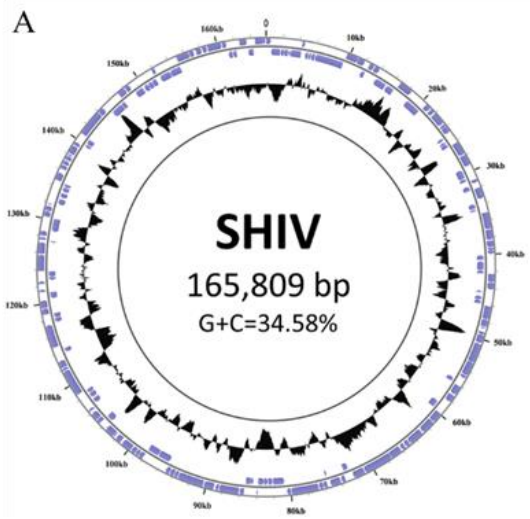
  SHIV (Shrimp hemocytes iridescent virus) was found from farmed white leg shrimp *Penaeus vannamei* collected in Zhejiang in 2014 by Qiu et al. (2017).

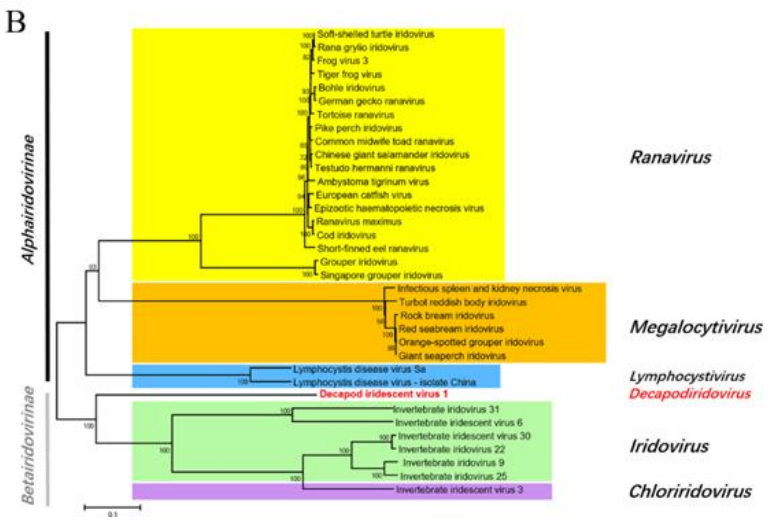
- Purified DIV1 virus was observed to form an enveloped icosahedral particle under TEM, with diameters of approximately 158.6 nm (v-v) and143.6 nm (f-f).



Aetiology

- DIV1 has a double-stranded DNA genome of about 166 kbp (Li et al. 2017; Qiu et al., 2018a).
- The genomic sequences of the two original isolates can be obtained from NCBI (Genbank No. NC_040612.1 and NC_055165.1).
- The complete genome sequences of SHIV 20141215 and CQIV CN01 share 99.97% identity.





Aetiology

- In March 2019, ICTV approved the proposal to add a new species, DIV1, in a new genus, *Decapodiridovirus*, of family *Iridoviridae,* with SHIV 20141215 and CQIV CN01 as the original isolates.

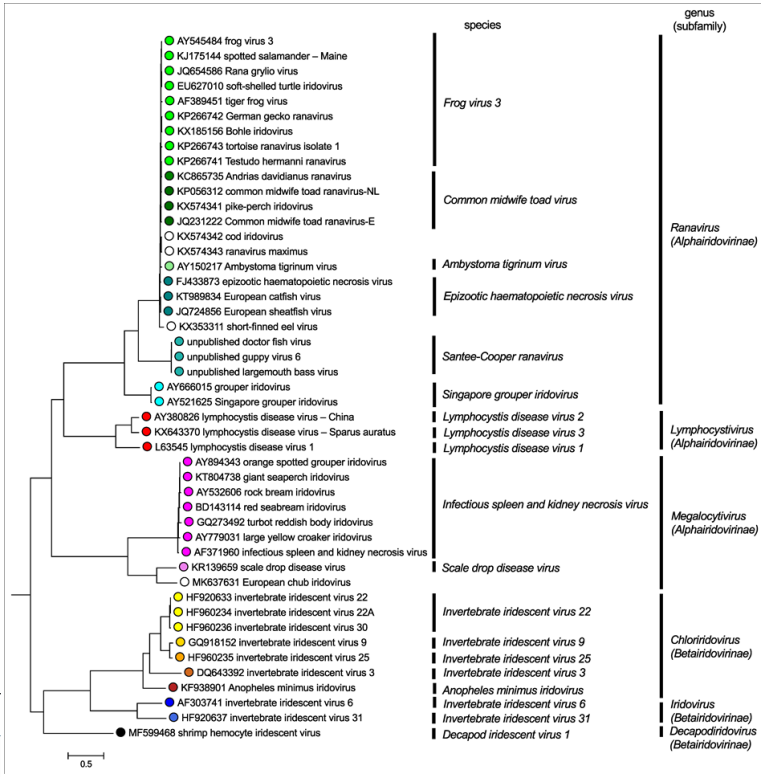
Member taxa

- *Alphairidovirinae*
  - *Lymphocystivirus*
  - *Megalocytivirus*
  - *Ranavirus*
- *Betairidovirinae*
  - *Chloriridovirus*
  - *Decapodiridovirus*
  - *Iridovirus*

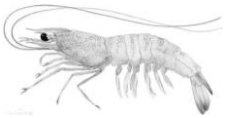
Member species

★ Exemplar isolate of the species

	Species	Virus name	Isolate	Accession number
★	*Decapod iridescent virus 1*	shrimp hemocyte iridescent virus	20141215	MF599468
	Decapod iridescent virus 1	Cherax quadricarnatus iridovirus	CN01	MF197913



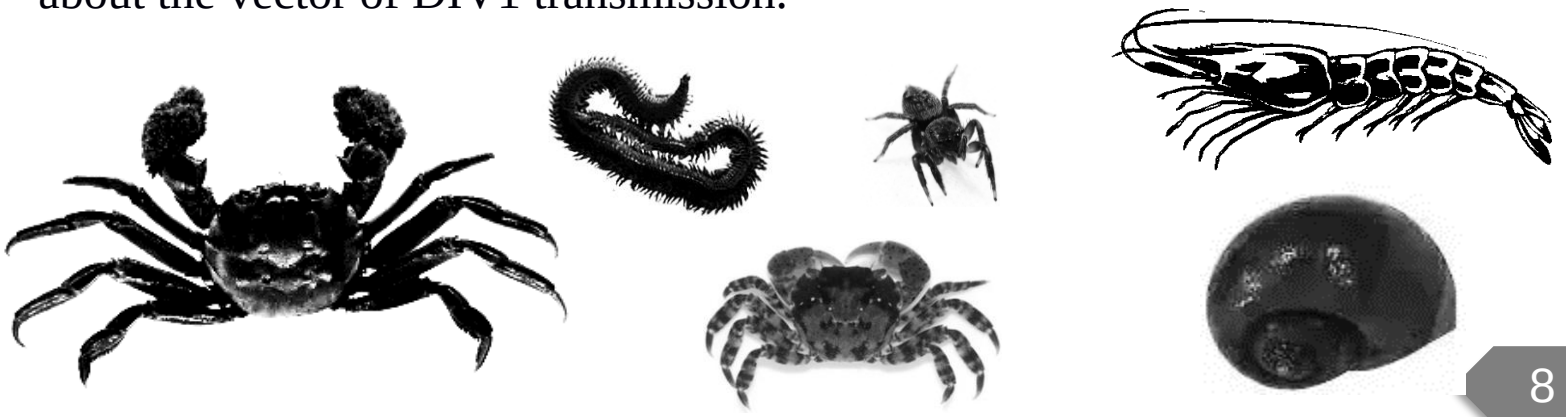
Susceptible species



Common (scientific) name	Infection type	Mortality	Reference
Pacific white shrimp (*Penaeus vannamei*)	Natural and experimental	Yes	Xu et al., 2016; Qiu et al., 2017; Qiu et al., 2020a
Giant freshwater prawn (*Macrobrachium rosenbergii*)	Natural	Yes	Qiu et al., 2019
Ridgetail white prawn (*Exopalaemon carinicauda*)	Experimental	Yes	Chen et al., 2019
Oriental river prawn (*M. nipponense*)	Natural	Yes	Qiu et al., 2019
Red swamp crayfish (*Procambarus clarkii*)	Natural and experimental	Yes	Xu et al., 2016; Qiu et al., 2019
Red claw crayfish (*Cherax quadricarinatus*)	Natural	Yes	Xu et al., 2016
Black tiger shrimp (*P. monodon*)	Natural	No	Srisala et al., 2020a
Swimming crab (*Portunus trituberculatus*)	Experimental	Yes	Qiu et al., 2022

Susceptible species

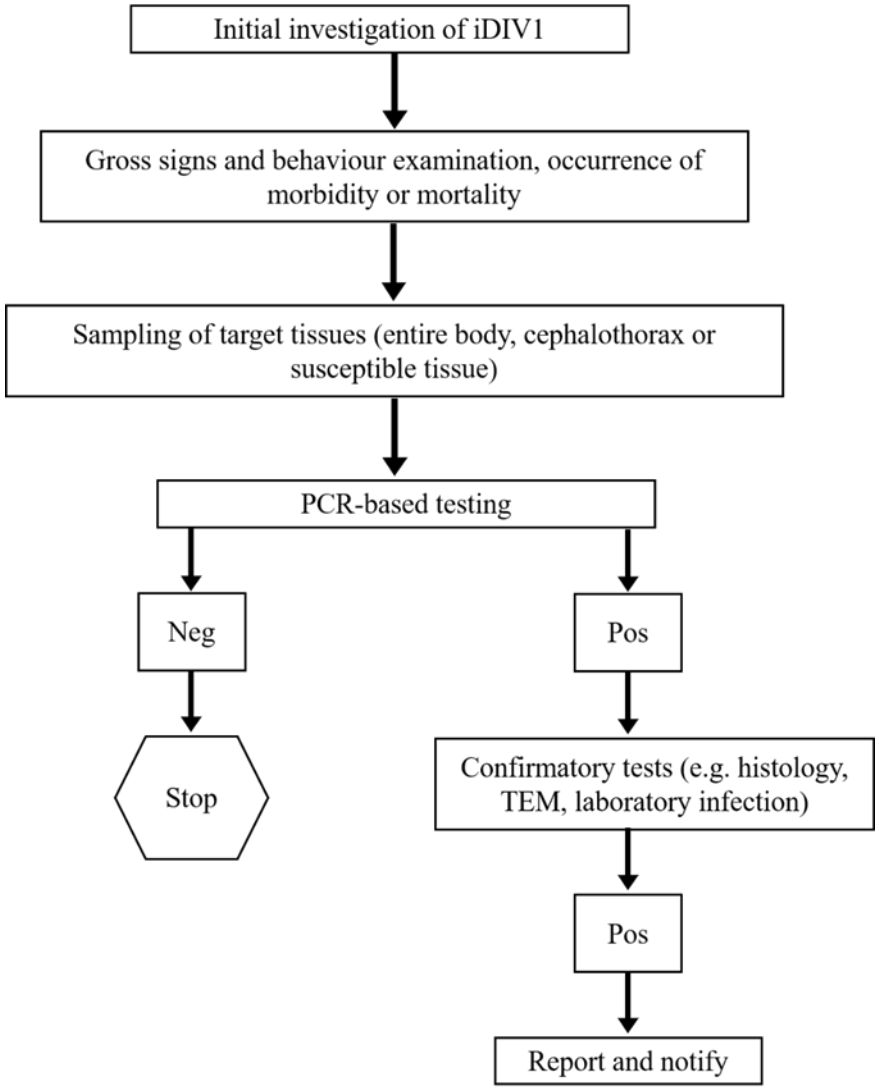
- Two species of crab, Chinese mitten crab *Eriocheir sinensis* and *Pachygrapsus crassipes*, were infected with DIV1 in experimental challenge by intramuscular injection and cannot yet be identified as susceptible species. (Pan et al., 2017)
- DIV1 was also detected in *P. chinensis*, *P. japonicus*, *M. superbum*, clam worm *Nereis succinea*, *Helice tientsinensis*, *Hemigrapsus penicillatus*, *Pomacea canaliculata*, *Plexippus paykulli* and some cladocera using only PCR method (Qiu et al., 2017, 2018b, 2019a,b, 2020b, 2021a).
- DIV1 may have a wide range of hosts and seriously affect a variety of important cultured decapods in the world. Although, with the deepening of investigation, more and more wild species (such as wild crab, *Po. canaliculata*, *Pl. paykulli*, etc.) have been detected as DIV1 positive, but there is still no direct evidence about the vector of DIV1 transmission.



Global distribution

- As early as 1993, there were two reports of suspected iridovirids infecting decapods, one in the marine crab *Macropipus depurator* in **France** (Montanie et al., 1993) and the other in the penaeid shrimp *Protrachypene precipua* in **Ecuador** (Lightner and Redman, 1993).
- The exact reports of DIV1 were reported in 2016 and 2017, respectively (Xu et al., 2016; Qiu et al., 2017). The National Aquatic Animal Disease Surveillance Program in China, from 2017 to 2021, detected DIV1 positive samples at the molecular level in **14 of 16 provinces of China** (Qiu et al., 2018b, 2019b, 2020b, 2021a).
  In 2020, OIE issued a disease notification report, indicating that DIV1 was detected in the samples of *P. monodon*, *C. quadricarinatus*, and *P. vannamei* from **Taiwan of China** (OIE, 2020).
- In 2020, NACA issued an urgent warning that DIV1 was detected in wild *P. monodon* from **the Indian Ocean**, noting that it is unlikely to have been transmitted from China (Srisala et al., 2020b).
- Due to the lack of worldwide surveillance investigations and the existence of high positive rates in wild populations in the Indian Ocean, it is speculated that the prevalence of DIV1 may be much more widespread than currently reported.

Diagnosis of disease



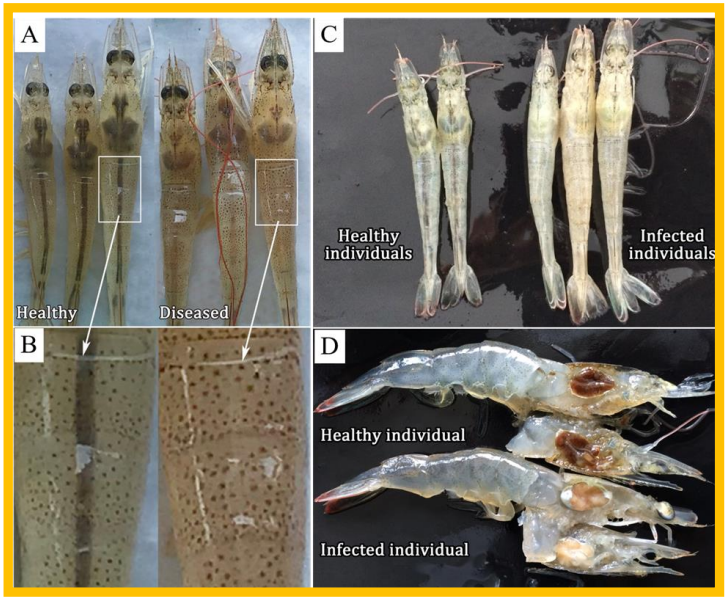
iDIV1 diagnostic flowchart. Pos: positive, Neg: negative.

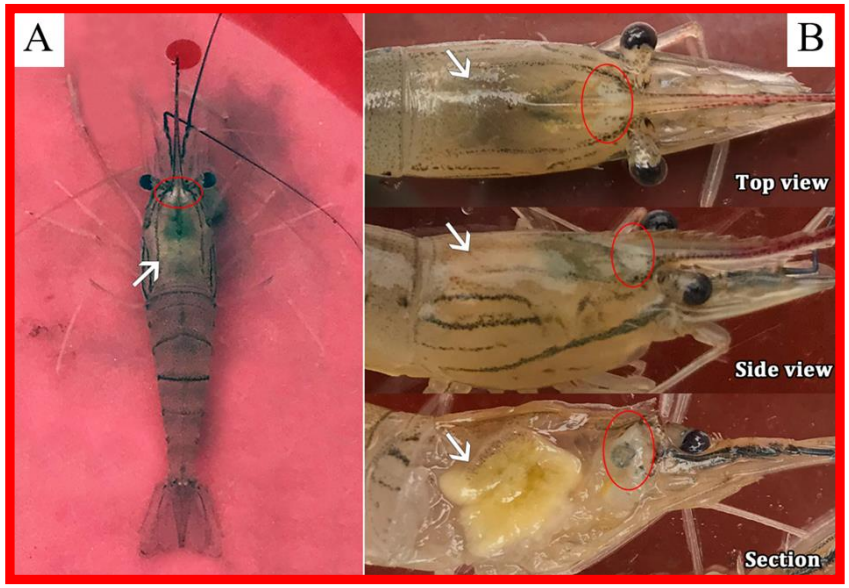
Diagnosis of disease

- The diagnostic methods of aquatic animals can be categorized into three levels as described by Bondad-Reantaso et al. (2001):
  Level I: examination of gross signs and observations of animals' behaviours;
  Level II: isolation and examination of pathogens in parasitology, bacteriology and mycology and histopathological evaluations of infected hosts;
  Level III: virus isolation, TEM examination, and molecular techniques (PCR-based assays).

Diagnosis of disease

Gross signs and behaviour (Level I)





Diagnosis of disease

iDIV1

Diagnosis of disease

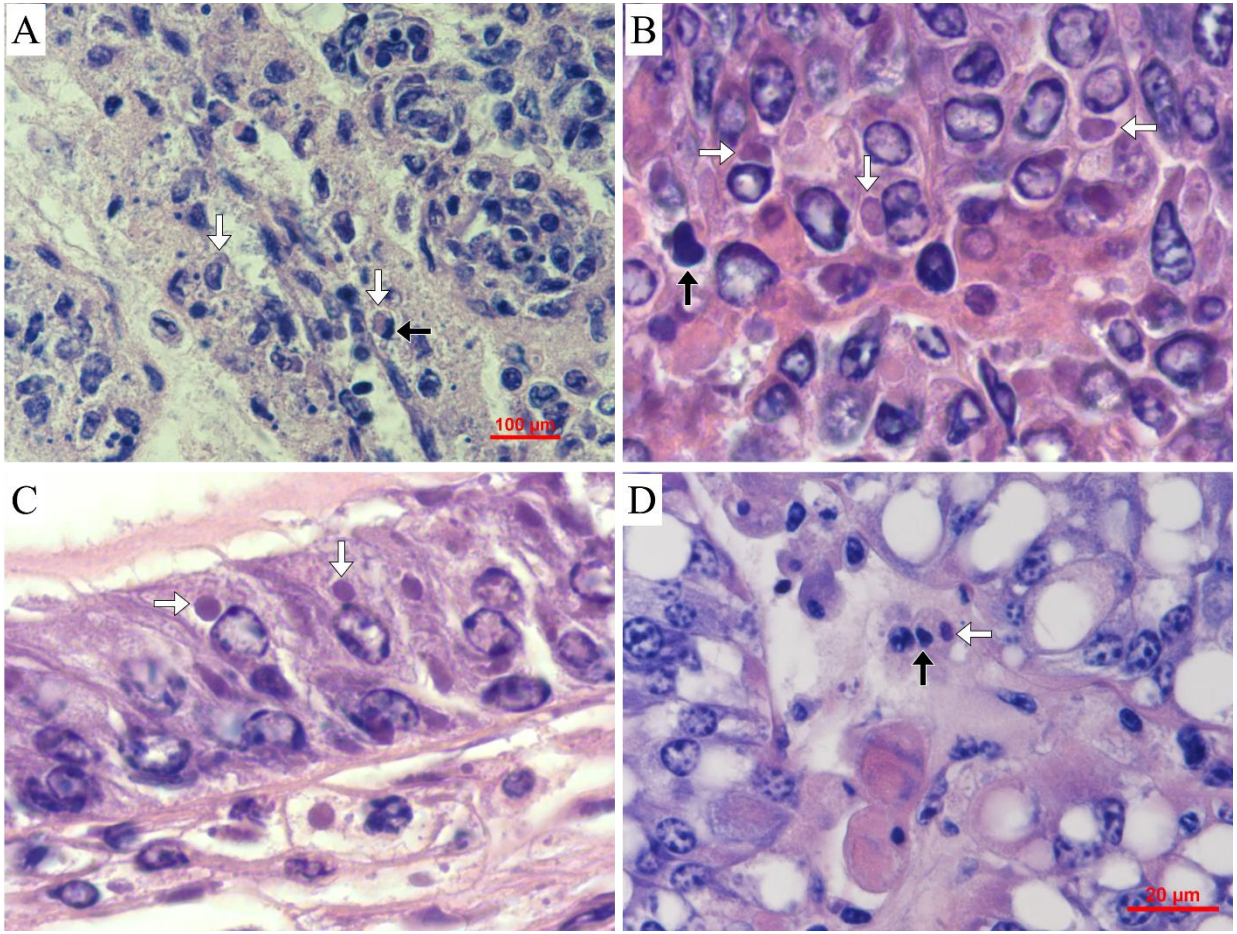
Decapod tissues to be used for DIV1 testing

Animal stage	Tissues to be sampled
Decapod <1 cm	remove the eyestalk, sample the whole individual
Decapod 1-3 cm	remove the eyestalk, sample the whole cephalothorax
Decapod >3 cm	lymphoid organ, hematopoietic tissue, gills, hepatopancreas, appendages, hemolymph

- It would be best to split a tissue sample into 4 parts for using purpose of histological, TEM, PCR and viral isolation assay, respectively.
- 1. Davidson's alcohol-formalin-acetic acid fixative (DAFA) (Bell and Lightner, 1988) for histological evaluation;
- 2. TEM fixative (2% paraformaldehyde, 2.5% glutaraldehyde, 160 mM NaCl, and 4 mM CaCl2 in 200 mM PBS, pH 7.2) for TEM test;
- 3. 95 percent ethanol (or equivalent reagents for DNA preservation purpose) for PCR-based analyses;
- 4. Frozen (at -20 ℃ or -80 ℃) for PCR or artificial infection experiment, etc.

Diagnosis of disease

Histopathology (Level II)



Histopathological characterization of DIV1 infected shrimp, H&E stain. (A) Lymphoid organs; (B) Hematopoietic tissues; (C) Epithelium; (D) hemocytes in the hepatopancreatic sinus. (Black arrows: karyopyknosis; White arrows: eosinophilic inclusions)

Diagnosis of disease

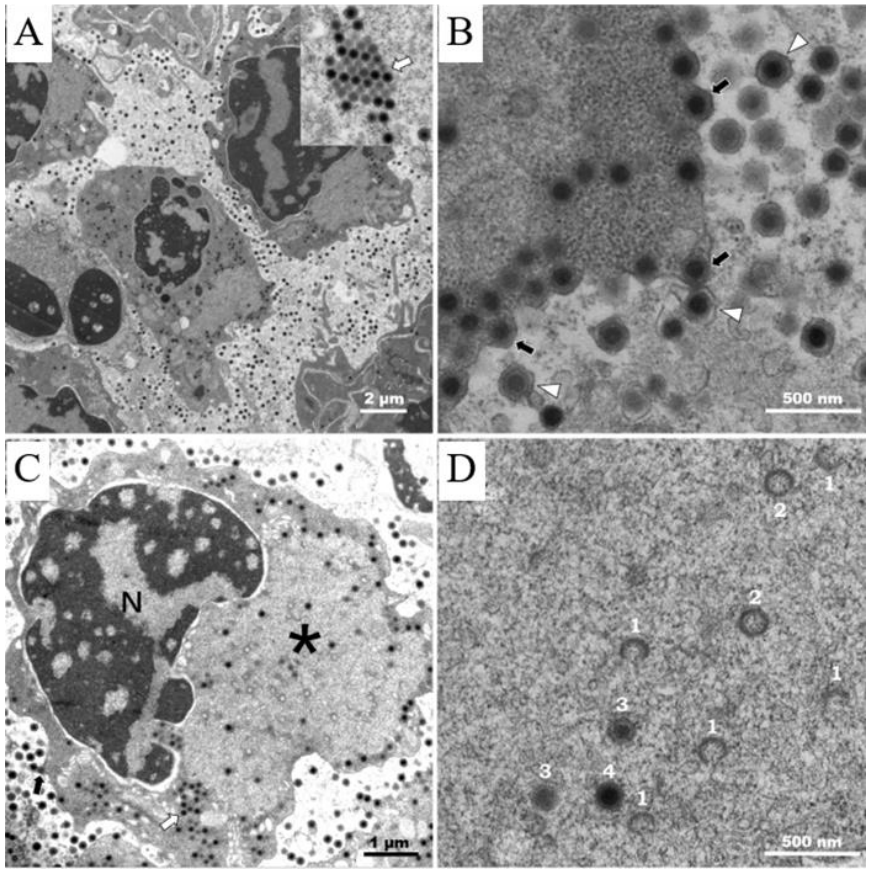
Histopathology (Level II)

- It was recommend that the **hematopoietic tissues**, together with the **lymphoid organs**, be the key tissues to examine while using histological analysis by H&E to diagnose a suspected case of DIV1 disease.
- The **lymphoid organs** can be useful in screening for DIV1 infections by first examining the lymphoid organs for the DIV1-type of pathology using a 10× objective lens and confirming them with a 40× objective lens.
- Then, 40× and 100× objective lenses can be used to examine the **hematopoietic tissues** to confirm pathognomonic DIV1 lesions. (Sanguanrut et al. 2022)
- However, the limitation is that the lymphoid organs are unique to penaeid shrimp, so this approach would not apply to the many other species of crustaceans that are susceptible to DIV1 infection.

Diagnosis of disease

Transmission electron microscopy (TEM) (Level III)

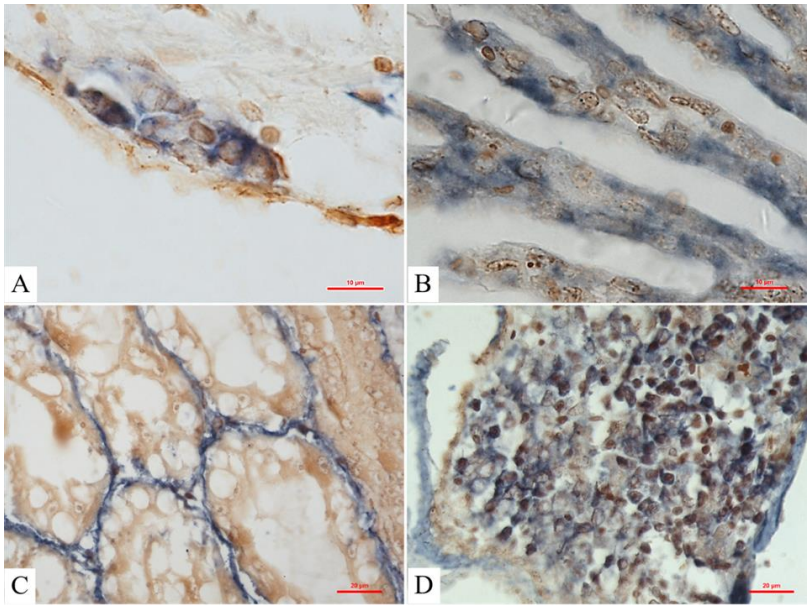
- Assembling and mature icosahedral iridescent virions are observed dispersing within and gathering around the virogenic stroma in the cytoplasm of hematopoietic tissues, hemocytes, and lymphoid organs under TEM.



Diagnosis of disease

Molecular techniques (Level III)

- Nested PCR (Qiu et al., 2017);
- Loop-mediated isothermal amplification (LAMP) (Zou et al., 2020);
- Real-time PCR (Qiu et al., 2020 or other verified methods);
- ISH and ISDL (*in situ* DIG-labelling-loop-mediated DNA amplification)



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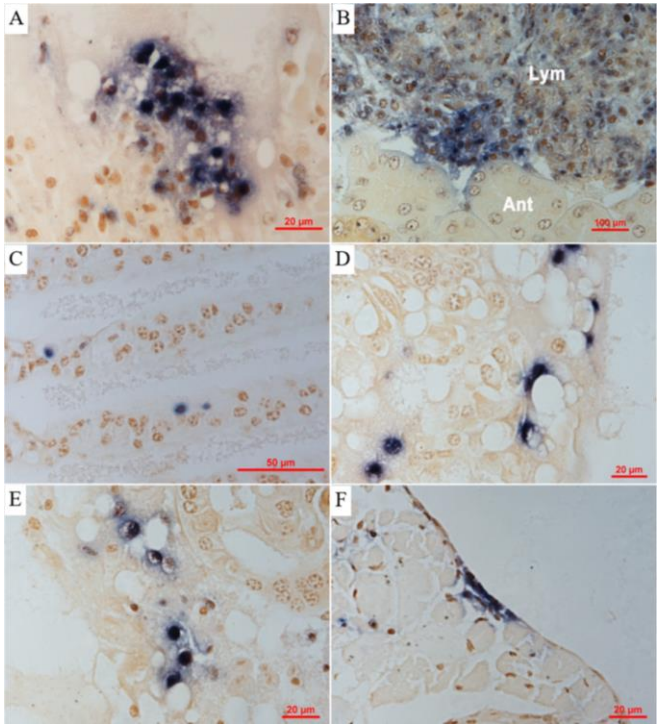

ISDL

Diagnosis of disease

1. **Definition of a suspect case**
   Infection of DIV1 is suspected if at least one of the following criteria is met:
   (1) mortality and clinical signs consistent with iDIV1
   (2) histopathology consistent with iDIV1
   (3) detection by PCR (or qPCR/LAMP)
2. **Definition of a confirmed case**
   Infection of DIV1 is considered to be confirmed if two or more of the following criteria are met:
   (1) clinical signs and histopathology consistent with iDIV1
   (2) assembling and mature DIV1 virions are observed under TEM
   (3) ISH or ISDL positive result in target tissues
   (4) detection by PCR (followed by sequencing)
   (5) detection by TaqMan probe-based real-time PCR

- **iDIV1 is an WOAH notifiable disease, so countries with positive cases should report the information to WOAH's World Animal Health Information System (WAHIS).**

Epidemiology

Persistence in the environment

It is important to know how long DIV1 can survive in the environment.

- DIV1 is likely to survive in freezing for years (unpublished data).
- The ICTV report of *Iridoviridae* family describes that iridovirids are stable in water at 4 ℃ for extended periods and can be inactivated when the temperature is higher than 55 ℃ within 30 min.
- Some ranaviruses remain infectious after desiccation, e.g., Bohle iridovirus (BIV) survives desiccation at temperatures up to 42 ℃ for up to 6 weeks, whereas others are sensitive to drying.
- Iridovirids are inactivated by pH <3.0 and >11.0 and by exposure to UV-irradiation on the order of 10^3 µWs/cm^2 (ICTV, 2020).

Epidemiology

Vectors and reservoir host

- As an important live feed organism for the maturation of shrimp broodstock, the presence of DIV1 in clam worm ***N. succinea*** will cause significant pathogen transmission risk to shrimp offspring.
- Wild organisms such as channeled applesnail ***Po. canaliculata*** and jumping spider ***Pl. paykulli*** may spread DIV1 to different ponds by carrying it on their body surface or the digestive tract (Qiu et al., 2020a).
- It was found that wild crabs (***Hel. tientsinensis*** and ***Hem. penicillatus***) in the drainage ditches of shrimp farms had a high DIV1 detection rate and a relatively high DIV1 load.
- The findings warn that polyculture with shrimps and crabs has a nonnegligible risk of cross-species transmission of pathogens, and wild crabs are very likely to be vectors of DIV1 to an aquaculture system (Qiu et al., 2022).

Epidemiology

Vertical transmission

- Although there is **no definitive evidence**, that DIV1 could be transmitted from shrimp broodstock to offspring through vertical transmission, as the virus has been detected by PCR in postlarvae collected from breeding hatcheries and commercial hatcheries through the National Aquatic Animal Disease Surveillance Program in China (Qiu et al., 2018b, 2019b, 2020b, 2021a).

Epidemiology

Risk factors

Environmental conditions that affect both the pathogen and the shrimp host are important determinants of disease outbreaks.

- The National Aquatic Animal Disease Surveillance Program in China in 2017-2021 detected DIV1 at temperatures from 16 ℃ to 32 ℃. The positive rate decreased significantly at temperatures above 32 ℃.
- The positive rate of samples in freshwater was higher than that in brackish water and seawater.
- Slightly more positives of DIV1 were detected with the pond water at pH7.5~8.4. (Qiu et al., 2018b, 2019b, 2020b, 2021a).

Prevention and elimination

- Crustaceans lack acquired immunity, so there is no proper vaccine for DIV1. Good aquaculture **biosecurity** practices at the national level are essential for the prevention of DIV1.
- The major goals in responding to iDIV1 outbreaks are (1) to eradicate the disease where possible, (2) to prevent the spread of the disease, and (3) to prevent re-emergence.
- At the farm level, DIV1 can be introduced through **five major pathways**: affected PLs, water, fomites, vectors, and fresh feed; prevention measures can be implemented based on this knowledge to potentially mitigate the risk of DIV1 introduction and spread on decapod farms.

Representative published works

Qiu L., Chen X., Gao W., Guo X.M., Xie G.S., Gong M., Yang B., Li C., Zhang Q.L., Huang J., Confirmation of susceptibility of swimming crab to infection with Decapod iridescent virus 1. Aquaculture, 2022, 548: 737607.

Qiu L., Chen, X., Guo X-M., Gao, W., Zhao, R.-H., Zhang, Q.-L., Huang, J. A TaqMan probe based real-time PCR for the detection of Decapod iridescent virus 1, Journal of Invertebrate Pathology, 2020, 173: 107367.

Qiu L, Dong X, Wan X-Y, Zhang Q-L, Huang J, 2021a. Analysis of iridescent viral disease of shrimp (SHID) in 2020. In: Fishery and Fishery Administration Bureau under the Ministry of Agriculture and Rural Affairs, National Fishery Technical Extension Center (Ed.), 2021 Analysis of Important Diseases of Aquatic Animals in China. China Agriculture Press, Beijing, pp. 182–196 (in Chinese).

Qiu L, Chen X, Gao W, Li C, Guo X-M, Zhang Q-L, Yang B, Huang J. 2020. Molecular epidemiology and histopathological study of a natural infection with decapod iridescent virus 1 in farmed white leg shrimp, *Penaeus vannamei*. Aquaculture, 533: 736105.doi: 10.1016/j.aquaculture.2020.736105.

Qiu L, Dong X, Wan X-Y, Zhang Q-L, Huang J, 2020b. Analysis of iridescent viral disease of shrimp (SHID) in 2019. In: Fishery and Fishery Administration Bureau under the Ministry of Agriculture and Rural Affairs, National Fishery Technical Extension Center (Ed.), 2020 Analysis of Important Diseases of Aquatic Animals in China. China Agriculture Press, Beijing, pp. 185–200 (in Chinese).

Zou Y, Guo X-M, Wan X-Y, **Qiu L**, Zhang Q-L. 2020. Establishment and application of the LAMP detection method for decapod iridescent virus 1 (DIV1). Progress in Fishery Sciences, 41(6): 156-164 (in Chinese).

Qiu L, Chen X, Zhao R-H, Li C, Gao W, Zhang Q-L, Huang J. 2019. Description of a natural infection with decapod iridescent virus 1 in farmed giant freshwater prawn, *Macrobrachium rosenbergii*. Viruses 11: 354. doi: 10.3390/v11040354.

Chen X, **Qiu L**, Wang H-L, Zou P-Z, Dong X, Li F-H, Huang J. 2019. Susceptibility of *Exopalaemon carinicauda* to the infection with shrimp hemocyte iridescent virus (SHIV 20141215), a strain of decapod iridescent virus 1 (DIV1). Viruses 11 (4): 387. doi: 10.3390/v11040387.

Qiu L, Dong X, Wan X-Y, Huang J, 2019b. Analysis of iridescent viral disease of shrimp (SHID) in 2018. In: Fishery and Fishery Administration Bureau under the Ministry of Agriculture and Rural Affairs, National Fishery Technical Extension Center (Ed.), 2019 Analysis of Important Diseases of Aquatic Animals in China. China Agriculture Press, Beijing, pp. 189–207 (in Chinese).

Qiu L., Chen, M.-M., Wan, X.-Y., Zhang, Q.-L., Li, C., Dong, X., Yang, B., Huang, J. Detection and quantification of shrimp hemocyte iridescent virus by TaqMan probe based real-time PCR, Journal of Invertebrate Pathology, 2018, 154: 95-101.

Qiu L, Chen M-M, Wang R-Y, Wan X-Y, Li C, Zhang Q-L, Dong X, Yang B, Xiang J-H, Huang J. 2018. Complete genome sequence of shrimp hemocyte iridescent virus (SHIV) isolated from white leg shrimp, *Litopenaeus vannamei*. Arch. Virol. 163 (3): 781–785. doi: 10.1007/s00705-017-3642-4.

Qiu L, Dong X, Wan X-Y, Huang J, 2018b. Analysis of iridescent viral disease of shrimp (SHID) in 2017. In: Fishery and Fishery Administration Bureau under the Ministry of Agriculture and Rural Affairs, National Fishery Technical Extension Center (Eds.), Analysis of Important Diseases of Aquatic Animals in China in 2017. China Agriculture Press, Beijing, pp. 187–204 (in Chinese).

Qiu L, Chen M-M, Wan X-Y, Li C, Zhang Q-L, Wang R-Y, Cheng D-Y, Dong X, Yang B, Wang X-H, Xiang J-H, Huang J. 2017. Characterization of a new member of *Iridoviridae*, shrimp hemocyte iridescent virus (SHIV), found in white leg shrimp (*Litopenaeus vannamei*). Sci. Rep. 7 (1): 11834. doi: 10.1038/s41598-017-10738-8.

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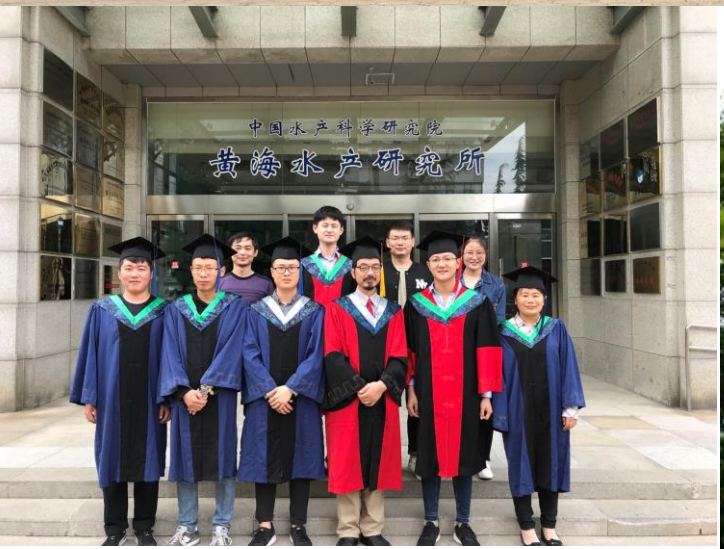

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