

**Research Assessment Exercise 2020**  
**Impact Case Study**

**University:** City University of Hong Kong |  
**Unit of Assessment (UoA):** 9 Chemistry |

**Title of case study:** Marine medaka fish and horseshoe crab as models for environmental and ecosystem health |

**(1) Summary of the impact**

Human disturbances have great impacts on the integrity and functioning of the marine ecosystem as well as survival of individual species. **Richard Kong's group** has successfully developed the marine medaka (*Oryzias melastigma*) as an elegant fish model for marine pollution assessment and ecotoxicology, especially in relation to transgenerational effects in reproduction linked to hypoxia (and impact on fish sustainability) in the marine environment. **SG Cheung's group** has adopted an integrated approach through population assessment, artificial breeding, education outreach and the formation of the IUCN Horseshoe Crab Specialist Group in the conservation of horseshoe crabs at the global level. |

**(2) Underpinning research**

**Kong's** research team has carried out internationally-recognized research on the endocrine-disruptive effects of hypoxia on fish and successfully established the marine medaka (*Oryzias melastigma*) as a useful marine fish model for molecular ecotoxicological studies and toxicity testing. His group has been investigating the novel proteins/mechanisms/pathways that may be associated with hypoxia-induced endocrine disruption in fish. Studies by his team have demonstrated unequivocally that hypoxia impairs testicular and ovarian development in male and female fish, respectively, at the molecular, cellular, biochemical, organismal and population level. Importantly, the hypoxia-induced reproductive impairments (retarded gonad development) in F0 were transgenerationally and epigenetically transmitted to the F1 and F2 offsprings despite these progenies having never been exposed to hypoxia [1]. Additionally, marine medaka is a more appropriate fish model than freshwater fish (e.g. zebrafish and Japanese medaka) for marine pollution and ecotoxicological studies because the toxicity of some chemicals are known to be potentiated by salinity, and hence the adverse effects will be masked in studies using freshwater fish models. The team has successfully established a variety of genetic resources for the marine medaka which include a number of *de novo* assembled miRNA [2] and mRNA [3] transcriptomes (e.g., brain, liver, testes and ovary) for male and female fish to facilitate its utility for monitoring ecosystem health.

Horseshoe crabs are living fossils with the earliest records dated 475 million years ago. There are four extant species with two of them being found in Hong Kong. All the species are facing the risk of extinction. **Cheung's** integrated approach to the conservation of horseshoe crabs started in 2004 and included field population assessment, innovative laboratory rearing techniques and education outreach. The population assessment has provided solid data reflecting a rapid decline of horseshoe crabs in Hong Kong [4] and the diet analysis has clarified the significance of seagrasses, the protected species in Hong Kong, to the nutrition of juvenile horseshoe crabs. Since oyster culture and juvenile horseshoe crabs utilize similar habitats on the mudflat, field manipulation experiments and home range studies have confirmed the impact of oyster clutches on the foraging behaviour, population distribution and home range of the juveniles [5]. Conservation of the species can be achieved through population enhancement in the laboratory. Cheung's pioneering works on laboratory rearing of this animal include artificial breeding, formulation of optimal diet and an understanding of environmental stresses (including metals and hypoxic stress) on the health of juvenile horseshoe crabs using blood quality as a proxy [6]. The message of the significance of conserving the species was delivered to school kids and the public through an education outreach program in which school kids were provided

with juvenile horseshoe crabs from his research laboratory. The juveniles were taken care by the school kids for half a year and a group wild release was organized in summer holiday. The program has been running since 2009. |

### (3) References to the research

- [1] Wang, S.Y., Lau, K., Lai, K.P., Zhang, J.W., Tse, A.C.K., Li, J.W., Tong, Y., Chan, T.F., Wong, C.K.C., Chiu, J.M.Y., Au, D.W.T., Wong, A.S.T., **Kong, R.Y.C.** and Wu, R.S.S. 2016. Hypoxia causes transgenerational impairments in reproduction of fish. *Nature Communications* **7**: 12114.
- [2] Lau, K., Lai, K.P., Bao, J., Zhang, N., Tse, A., Tong, A., Li, J.W., Lok, S., **Kong, R.Y.C.**, Lui, W.Y., Wong, A. and Wu, R.S.S. 2014. Identification and expression profiling of microRNAs in the brain, liver and gonads of marine medaka (*Oryzias melastigma*) and in response to hypoxia. *PLoS One* **9(10)**: e110698.
- [3] Lai, K.P., Li, J.W., Wang, S.Y., Chiu, J.M.Y., Tse, A., Lau, K., Lok, S., Au, D.W.T., Tse, W.K.F., Wong, C.K.C., Chan, T.F., **Kong, R.Y.C.** and Wu, R.S.S. 2015. Tissue-specific transcriptome assemblies of the marine medaka *Oryzias melastigma* and comparative analysis with the freshwater medaka *Oryzias latipes*. *BMC Genomics* **16**: 135.
- [4] Kwan, B.K.Y., Hsieh, H.L., **Cheung, S.G.**, Shin, P.K.S. 2016. Present population and habitat status of potentially threatened Asian horseshoe crabs *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* in Hong Kong: a proposal for marine protected areas. *Biodiversity and Conservation* **25(4)**: 673-692.
- [5] Kwan, B.K.Y., Chan, H.K., **Cheung, S.G.** 2018. Habitat use of globally threatened juvenile Chinese horseshoe crab, *Tachypleus tridentatus* under the influence of simulated intertidal oyster culture structures in Hong Kong. *Aquatic Conservation* **28(1)**: 124-132.
- [6] Kwan, B.K.Y., Chan, A.K.Y., **Cheung, S.G.**, Shin, P.K.S. 2014. Hemolymph quality as indicator of health status in juvenile Chinese horseshoe crab *Tachypleus tridentatus* (Xiphosura) under laboratory culture. *Journal of Experimental Marine Biology and Ecology* **457**: 135-142. |

### (4) Details of the impact

**Kong's team** has been the pioneer in successfully **establishing** the marine medaka at the City University of Hong Kong as a marine fish model for ecotoxicology and ecotoxicogenomics to support ecological risk assessment and research, as evidenced by its widespread use by researchers at other local institutions such as the HKUST (e.g., Prof. Wenxiong Wang's team) and HKU (e.g., Prof. Kenneth Leung's team) for these purposes. Moreover, publications by other researchers in HK [A], Korea [B] and mainland China [C] and over 110 publications on marine medaka since 2006 are strong testimony of its increasing usage as a marine fish model. Notably, a major impact is in the adoption of the marine medaka for monitoring marine ecosystem health by the Environmental Protection Department (EPD) and Drainage Services Department (DSD) of the Hong Kong SAR Government who have officially designated the use of the marine medaka as one of the fish models as a standard test for WETT (Whole Effluent Toxicity Test) in EIA (Environment Impact Assessment) in Hong Kong. (*Supporting Letter from the EPD of HKSAR Government is available at <https://bit.ly/2LfsO9u>*).

Studies by **Kong's team** on hypoxia and medaka have also attracted worldwide attention - the transgenerational effects of hypoxia show that the risk and impact of hypoxia have been grossly underestimated and may pose a significant and long lasting threat to the sustainability of natural fish populations worldwide; and the data could be used to inform government policies in future. For example, a study by members of our group [D] has highlighted the masculinization effects of hypoxia on female medaka fish and has been used as evidence to inform development of government policies in the EU [E]. An upcoming article will be published in *Science for Environment Policy* by the

European Commission's Environment Directorate-General which is based on a 2019 publication in Environmental Science & Technology by Kong's team on the study "*Hypoxia Causes Transgenerational Impairment of Ovarian Development and Hatching Success in Fish*" (<https://bit.ly/2Lj7FIi>).

**Cheung** has raised the conservation profile of horseshoe crabs to a world leading level by organizing an international workshop on the science and conservation of horseshoe crabs in Hong Kong in 2011. The papers presented in the international workshop in Hong Kong were published in 2015 by Springer in a book of which Cheung was one of the editors. [F] Cheung also helped with the establishment of the Horseshoe Crab Specialist Group (HCSG) under the International Union for Conservation of Nature (IUCN) to assess the conservation status of all the horseshoe crab species. Cheung was a founding member of the specialist group and is now a steering committee member. [G] The assessment report on the tri-spine horseshoe crab submitted by the specialist group was approved by the IUCN which renewed the status of the species from "data deficient" to "endangered" in March 2019 [H]. The Horseshoe Crab Conservation Union was established among China, Hong Kong and Taiwan in 2014 and SG Cheung was a founding member of the union. He was also invited as a keynote speaker to deliver a talk in the International Conference on the Conservation of Horseshoe Crabs in August 2017 in Bangkok, Thailand.

Another contribution by SG Cheung is to initiate an outreach program "Horseshoe Crab Rearing Program" with the Ocean Park Conservation Foundation in 2009. Until 2019, the program has attracted more than 2,700 secondary students who reared juvenile horseshoe crabs in schools for half a year and then released the crabs in summer. The students have acquired the knowledge on the horseshoe crabs, developed a correct attitude towards wildlife and helped with the conservation. [I] His work was extensively interviewed by media. [J] Recently, the government has agreed that horseshoe crabs require immediate conservation and the government will develop action plans for their conservation. In Feb 2018, he was appointed by the government as a member of the Expert Group on Ecological Conservation of Pui O, Shui Hau and Tai O. Shui Hau is well-known as the only habitat of Chinese horseshoe crabs in the southern part of Lantau Island, Hong Kong. The government is now reviewing the conservation status of horseshoe crabs in Hong Kong following the criteria developed by IUCN and he was appointed as reviewer to help review the submission. |

## (5) Sources to corroborate the impact

### For Richard Kong's work:

- [A] Ye, R.R., Peterson, D.R., Seemann, F. and Au, D.W.T. 2017. Immune competence assessment in marine medaka (*Oryzias melastigma*) – a holistic approach for immunotoxicology. Environmental Science and Pollution Research 24(36) Special Issue: SI 27687-27701.
- [B] Lee, D.H., Jo, Y.J., Eom, H.J. et al. 2018. Nonylphenol induces mortality and reduces hatching rate through increase of oxidative stress and dysfunction of antioxidant defense system in marine medaka embryo. Molecular and Cellular Toxicology 14(4), 437-444.
- [C] Chen, L., Lam, J.C.W., Hu, C. et al. 2018. Perfluorobutanesulfonate exposure causes durable and transgenerational dysbiosis of gut microbiota in marine medaka. Environmental Science and Technology 5(12), 731-738.
- [D] Cheung, C.H.Y., Chiu, J.M.Y. and Wu, R.S.S. 2014. Hypoxia turns genotypic female medaka fish into phenotypic males. Ecotoxicology 23(7), 1260-9.
- [E] Science for Environmental Policy: Female fish swap sex in polluted, low-oxygen water, European Commission DG Environment News Alert Service. 30 October 2014 Issue 391.

### For SG Cheung's work:

- [F] As co-editor of a book published by Springer in 2015: Changing Global Perspectives on Biology, Conservation and Management of Horseshoe Crabs, Springer, pp. 599. (<https://www.springer.com/gp/book/9783319195414>)
- [G] IUCN Horseshoe Crab Specialist Group (<https://www.iucn.org/commissions/ssc-groups/invertebrates/horseshoe-crab>)
- [H] Laurie, K., Chen, C.-P., Cheung, S.G., Do, V., Hsieh, H., John, A., Mohamad, F., Seino, S., Nishida, S., Shin, P. & Yang, M. 2019. *Tachypleus tridentatus*. *The IUCN Red List of Threatened Species* 2019: e.T21309A149768986. (03 October 2019) (<https://www.iucnredlist.org/species/21309/149768986>)
- [I] The Ocean Park Conservation Foundation Horseshoe Crabs Rearing Program (<https://www.opcf.org.hk/en/community-education/juvenile-horseshoe-crab-rearing-programme>)
- [J] Media interview (in Cantonese) on horseshoe crabs on 21 June 2017 by the World Green Organization (WGO) online program. (<https://youtu.be/PvMp3buiVfk>) |