附件四

秘書處專用 申請編號:SFDF-0031

漁業持續發展基金

Sustainable Fisheries Development Fund

期終報告書

Final Report

請 填 妥 這 份 期 終 報 告 書 · 並 於 項 目 完 成 後 的 四 個 月 內 送 交 漁 業 持 續 發 展 基 金 秘 書 處 。 請 注 意 · 這 份 報 告 書 將 會 上 載 至 漁 護 署 網 頁 供 公 眾 查 閱 。

項目名稱:Improving Fish Health and Production in Hong Kong (SFDF-0031)

申請機構: City University of Hong Kong

批准日期: 3 September 2018

資助款額:Capped at \$4,940,069.00

報告期間:1 September 2019至 to 31 December 2020

注意

凡故意在本報告書中作失實陳述或漏報資料,有關項目可被中止發放資助 款項,而受資助者被發現有虛報資料,亦可遭檢控。受資助者須注意,以 欺詐手段取得金錢利益,屬刑事罪行。

聲明

本人證實所夾附的期終報告書和經審計帳目,以及所提交的額外資料和證 明文件均正確無誤,所購買的物料(包括資產)和獲取的服務,是本項目 內的活動所必要的,而且價格公道合理。

> 機構負責人姓名: Project-in-charge

簽署並機構蓋印 Signature & Co. Chop Prof Sophie Natasha ST-HILARIRE

香港身分證號碼: HKID No.:

1

日期: Date: April 23,2021

1. 項目時間表 Project Timeline

開始日期 Proj	ect Start Date	完成日期 Project End Date			
原定 <i>(根據協</i>	實際 Actual	原定 <i>(根據協</i>	實際 Actual		
議 <i>所示)</i> Planned		議所示)Planned			
1 September	1 September	31 August 2020	31 December		
2018	2018		2020		

2. 項目目的撮要 Project objectives summary

• Identify major health constraints to aquaculture productivity in Hong Kong

• Augment veterinary field services for the AFCD Fish Health Inspection Programme

• Establish a pharmacy for aquatic animal veterinary products

• Investigate treatment options for parasitic and bacterial

problems in the Hong Kong aquaculture industry

• Develop specific best practice guidelines for intervention strategies for producers and fish health professionals, including veterinarians

• Increase the aquatic animal vetiernary capacity in Hong Kong

項目目的和 / 或範圍的更改 (如有,請註明有何更改以及所持理由)
 Any Change of Project objectives / scope (If yes, please specify the changes and justifications)

No Changes

4. 報告期間的項目推行撮要

(請簡述主要活動,並按適當情況列明日期、地點及受惠人數)

Project Summary/ Progress during the reporting period (Please briefly describe the major activities, and list the date(s), venue(s) and the number of beneficiaries from the activities)

See Appendix A and B.

5. 參加者估計數目與實際數目的比較

Comparison of estimated number of partipants with actual number

日期	地 點	活 動	預計受惠	實際受惠人	漁業界受惠
Date	Venue	Activities	人數	數	人數 Number
			Estimated	Actual	of
			number of	number of	beneficiaries
			beneficiari	beneficiaries	in the Fishery

			es		field
Sept.	Aqua	Veterinary	60	67	67
2018 to	farms in	Farm visits			
Dec. 30	Hong				
2020					
		R	esearch	L	
March	Agua	Emambectin	4	5	100+ salt
– Mav	farms in	Benzoate trial		-	water farmers
2020	Hong				
	Kong				
Dec	Aqua	Mebendozole	3	1	Failed
2019	farms in	trial	-	_	treatment so
	Hong				discontinued
	Kong				
March	Fresh	Summer	2	2	Potentially
– Sept	water	water quality	_	_	50+ Fresh
2020	Адца	mullet project			water farms
2020	farms in	marree project			in Hong Kong
	Hong				
	Kong				
	nong	0	utreach		
Octobe	Адца	Nanobubble	12	12	Unknown
r = 1 - 20 - 18	Farmers	technology			e inkilo w il
1 1 20 10	and	seminar			
	researche	(CityLI)			
	rs				
June 5	Aqua	Antibiotic	20	16	16 farmers
2019	Farmers	use in	20	10	ro runners
2017	in Hong	aquaculture			
	Kong	(Ping Shan agriculture farm	ners association buildi	ng)	
June 5	Veterina	Basic fish	30	50	50 vets
2019	rians in	health for your	50	50	50 0005
2017	Hogn	nractice			
	Kong	(CityU)			
Oct 22	Fish	Water quality	30	5	5 fish health
2019	health	(zoom due to	50	5	staff on
2017	team	issues with			project
	team	nrotests on			project
		campus)			
Dec 3	Veterina	Water	30	~ 31	30
2020	rians in	Quality for			veterinarians
2020	Hong	vets			, ctormarrans
	Kong	(The HKFYG Leadership In	stitute)		
Dec 17	Farmers	Sea lice	30	~ 34	30 fish
2020	in Hong	treatment	50		farmers
	Kong	(Online)			14111015
1	ixong		1	1	

6. 項目的成效評估(根據申請計劃的成效指標)

Project effectiveness evaluation (according to the effectiveness indicators of the application form)

See Appendix K

7.項目延期(與原定時間表比較,並須交待原因)
 Project Delay (Compared to the planned schedule and please provide justification.)

We were delayed in several aspects of this project due to protests at CityU and COVID 19 in Hong Kong but other than one CPD we were able to accomplish all our service and outreach goals. We did not conduct as many research experiments as we wanted due to the difficulty in going out to farms during the COVID 19 stay-at-home work orders. Because of this we return much of the funding associated with this aspect of the project to AFCD.

8. 在報告期間所遇的問題(如有)

Problems/ difficulties encountered during the reporting period (if any)

We had issues visiting farms for research purposes due to COVID 19 restrictions. We were able to retain our service for emergency purposes. We took additional precautions by wearing PPE.

9. 為解決問題所採取的補救措施及這些措施的成效(如有) Remedial measures taken to solve the abovementioned problems and the effectiveness (if any)

We increased our PPE when we went on farms and reduced our team size to less than 3 people to comply with the COVID 19 regulations.

- 10. 列出項目的出品(報告書、唯讀光碟等)、已製備的宣傳物料或印刷品
 - (如有)

(請註明種類和數目,並各提供兩個副本)

Project productions (report, CD-ROM,etc), promotional materials or printed materials (if any)

(Please specify the type(s) and number and provide two copies of each)

Manuscript for emambectin benzoate treatment of sea lice

11. 財務報告(請根據協議內訂明<u>另外</u>提供經審計賬目,可參考夾附的文件清 單及樣本)

Financial Report (Please provide financial statement/audited accounts per stated in the agreement. Refer to the list of documents and specimen enclosed)

a) 整體狀況

截 至	至 1.9.2019		的開始結餘:		742,546.67 元	
加上朝		: 由	1.9.2019	至	31.12.2020	

共___

3,149,733.57 元

(金額與「收入分項數字詳情」的總和一致)

 減去報告期間的支出:
 2,792,957.29元

 (金額與「收入分項數字詳情」的總和一致)

 截至
 31.12.2020

 的結餘:
 1,099,322.95元

b) <u>收入分項數字詳情(包括項目利息)Details of income (including</u> <u>interest)</u>

細項	用途	金額(港元)
Transaction items	Use	Amount
2 nd instalment		900,000.00
3 rd instalment		1,300,000.0
		0
4 th instalment		925,000.00
Interest		24,733.57
	Total	3,149,733.5
		7

c)]	支出分	項數字	詳情	Details	of	expenses
------	-----	-----	----	---------	----	----------

	批准	宦 妪	超 計	數量	由
	1儿 /出	貝际		刻 里	中明 日 川 早 豚 /
Iransaction items	<u> </u>	開 文	闬 文	Quantity	収除編號
	Budget	Expenses	Est.		Invoice/ Receipt
(請根據資助協議	(請根據資	(請保留所有	expens	(請根據	no.
的「獲批項目」填	助協議的	收據正本)	es	資助協議	
	「獲壯項日		(預計	的「獲批	
, , , , , , , , , , , , , , , , , , ,	<u>贤</u> 加侯口 <u>問士</u> 疳		下一胡	<i>百日, 宿</i>	
	<i>'''''' ''' '' '' '' '' '' '' '' '' '' '</i>				
	易。如月獲			為1	
	批的預算調		<i>開文</i>)		
	撥,則填寫				
	經調整後的				
	最新全額)				
G1 Personal	31 776 00	12 122 10		2 hatches	441089
	51,770.00	12,122.10		2 batches	OHRP 191127035
					R1
equipment					RH 200330005 R1
					OHRP 200810048
					R1
					\$13028604
					PH 201028020 R1
					PH 201103014 R1
					PH 201102015 P1
					PH 201103013 R1
					PH 201123092 R1
					PH 201123093 K1
					PH 201124013 K1
					PH 201217014 R1
					PH 201224015 KI
	4 6 9 6 5 7 9 9	70 200 00		100 1	5121144277
G2. PM/nisto	160,657.00	70,290.00		400 times	AN 193811K
					19000957
					19000955
					19000958
					19000960
					19000956
					19001302
					19001301
					19001070
					19001071
					19001184
					19001186
					20000223
					20000224
					20000236
					20000470
					20000471
					20000597
					20000846
					20000847
					20000848
					20000863
					20000864

			г		
					20001260
					20001261
					20001262
					20001127
					20001128
					20001403
					20001404
					20001405
					20001406
G3. PM/PCR	190,070.00	83,521.78		400 times	IN1910034
					IN1910029
					MFI1910037
					20225867
					5121131316
					IN20-007414
					IN20-007326
					IN20-007742
					\$10/10122755
					IN2004815
					95046684
					95046848
					IN20-008615
					20071506
					20231148
					20071506TT
					22200-0002872
					22200 0002872
					\$10/10125196
					20242247
					20242347
					311100102012312
					201200127
					201200129
					201200135
					201200142
					201200163
					PO617969
					PO617969TT
					9881LT
G4. PM/	120,005.00	7,200.00		400 times	19000958
bacteriology					19001070
culture					19001071
					19001184
					19001186
					20000224
					20000236
					20000471
					20000481
					20000597
					9181LP
G5. Sensitivity	93,470.00	23,030.00		400 times	19000960
analysis/ MIC	,	,			19000956
analysis					19000957
					19001070
					19001184

				20000223
				20000236
				20000470
				20000471
				20000481
				20000864
				20001260
				20001262
				20001127
				20001128
				20001403
				20001404
				20001405
				20001406
CC Water quality	404 421 00	201 220 4	 90 timos	\$120200022
Go. Water quality	404,451.00	201,229.4	ou times	11202000023
tests		9		112A71E42
				112A71343
				112A/1/38
				112A72066
				112A/2161
				\$1202000593
				112A73206
				112A73242
				112A71841
				112A72258
				112A72534
				112A72551
				112A72627
				112A72857
				112A72858
				112A73558
				112A73759
				112A74070
				112A71420
				112074407
				112/07/1537
				112074855
				112A74055
				112A74558
				112A75240
				112A/5410
				112A/0U45
				13/32/
				0013/327TT
				112A77321
				112A77432
				11669
				112A76191
				112A76476
				112A76612
				112A76995
				112A77019
				112A77102
				SI200921001
				\$1202000996

				112473995
				112/07/8/2
				112A77842
				112A77845
				112A77933
				51202001261
				112A75583
				112A/6334
				112A77400
				112A77407
G7. Parasitology tests	51,350.00	26,192.98	400 times	4145046126
G8. Miscellaneous	161,965.41	16,162.73	2 times	441089
expenses with				441089
pharmacy				PH 191111024 R1
, , , , , , , , , , , , ,				PH 191111026 R1
				PH 191111028 R1
				OHRP 191204011
				R1
				9208148123
				PH 191231039 R1
				PH 200414021 R1
				PH 200414022 R1
				120200503
				PH 200923031 B1
				OHRP 210113020
				D1
	450 500 00			
G9. HPLC analysis	158,520.00	83,000.00	240 times	DN-CTS-200082
oftissues				DN-CTS-200081
				DN-CIS-200108
				DN-CIS-200155
				DN-CTS-200163
				DN-CTS-200217
				DN-CTS-200218
				DN-CTS-200237
				DN-CTS-200262
				DN-CTS-200288
				DN-CTS-200317
				DN-CTS-200332
				DN-CTS-200342
				DN-CTS-200371
				DN-CTS-200442
				DN-CTS-200426
				DN-CTS-200427
				DN-CTS-200539
G10. Monthly use	121,482,00	11.616 46	12 months	PH 191107024 R1
of tanks and care	,.02.00	,00.10		MFI1910037
of fich				PH 200317031 R1
				PH 200317032 R1
				PH 200317034 R1
				PH 200327012 P1
G11	120 011 00	20 120 10	2 hotohoo	
	120,011.90	20,138.10	2 batches	120200503
Pharmaceuticals				120200503
G12. Cost of fish	126,700.00	17,000.00	600 fishes	20190527/CITYU
				20190603/CITYU
				PH 191010015 R1

				PH 200514044 R1 PH 200514045 R1 PH 200602020 R1 PH 200624021 R1
G13. 3 continuing education sessions for veterinarians	46,555.93	7,413.24	60 people	PH230420 PH230420A PH230420ATT 1343 1343TT
G14. 2 workshops for industry	61,769.10	0.00	80 people	
G15. Printing materials	31,291.10	1,239.10	2 times	1179102 PH 191111023 R1 851010 OHRP 200327013 R1
G16. Consultant travel cost	20,070.00	0.00	5 times	
G17. Consultant subsistence cost	0.00	0.00	20 days	
G18. Consumables (slides, stains, etc)	98,291.90	52,491.16	2 years	4861KJG PH 190926015 R1 PH 190919034 R1 PH 190919033 R1 4554KLGPE PH 191017018 R1 PH 191204014 R1 PH 191231044 R1 PH 191231046 R1 PH 200115046 R1 PH 200327009 R1 OP/I965609 SI202000377 SI202000377 SI202000377 SI202000377 PH 200422006 R1 PH 200514047 R1 7340LD 7636LE PH 200601007 R1 PH 200601007 R1 PH 200602018 R1 IN20-007998 PH 200706031 R1 PH 200706033 R1 PH 200706033 R1 PH 200706033 R1 PH 200812007 R1 PH 200812007 R1 PH 200812007 R1 PH 20092026 R1 PH 20092026 R1 PH 201016009 R1 PH 201028019 R1 PH 201217017 R1

				9208177567
				9208177568
				20243520
				20210027915
				920210027515
				IN20-012720
				206271
G19 Car rental	196 / 69 38	72 909 40	1/0 times	PH 191003014 B1
(including	190,409.90	72,303.40	140 times	PH 191205040 B1
				PH 191231042 R1
insurance)				PH 200514042 R1
G20 Potrol	51 120 //7	25 706 47	 140 timos	PH 190911012 R1
G20. Fettol	51,159.47	33,700.47	140 times	PH 190926020 B1
				PH 190926020 R1
				PH 190926019 R1
				PH 190926017 R1
				PH 191010014 P1
				PH 191010014 KI
				PH 191010013 R1
				PH 191010012 R1
				PH 191104019 RI
				PH 191107022 RI
				PH 191107021 RI
				H000659098
				H000671034
				H0006//0/8
				PH 191204016 R1
				PH 191205041 R1
				PH 191231038 R1
				PH 191231037 R1
				PH 191231036 R1
				PH 200115042 R1
				PH 200115044 R1
				PH 200115043 R1
				H000689098
				PH 200317033 R1
				PH 200325008 R1
				PH 200325009 R1
				PH 200325010 R1
				PH 200327011 R1
				PH 200414023 R1
				PH 200414024 R1
				H000716087
				PH 200511014 R1
				PH 200508023 R1
				H000726518
				H000731491
				PH 200601009 R1
				PH 200602016 R1
				PH 200514038 R1
				PH 200514039 R1
				PH 200514040 R1
				PH 200514041 R1
				PH 200514049 R1
				PH 200624018 R1
				PH 200624019 R1

				PH 200624020 R1
				H000751545
				PH 200706028 R1
				PH 200706029 R1
				PH 200706030 R1
				PH 200804013 R1
				PH 200804014 R1
				PH 200804015 B1
				PH 200812008 KI
				PH 200812009 RI
				H000760854
				PH 200818003 R1
				PH 200818004 R1
				PH 200818005 R1
				PH 200818006 R1
				PH 200902025 R1
				PH 200902028 R1
				PH 200902029 R1
				PH 200902030 R1
				PH 200928051 B1
				PH 200928052 R1
				PH 200929047 R1
				PH 201012035 R1
				PH 201012036 R1
				H000769649
				PH 201028022 R1
				H000778386
				H000778386
				H000791737
				H000778386
				PH 201201067 R1
				PH 201201068 R1
				PH 201217016 R1
G21 Boat	71 700 00	20 037 00	30 times	PH 190919032 R1
	71,700.00	29,937.00	50 times	PH 191107023 R1
transportation				PH 101204012 P1
				PH 191204012 R1
				PH 191204013 RI
				PH 191204017 R1
				PH 191204015 R1
				PH 191231045 R1
				PH 191231043 R1
				PH 191231041 R1
				PH 191231040 R1
				PH 200115045 R1
				PH 200325007 R1
				PH 200327010 R1
				PH 200422005 R1
				PH 200514042 P1
				PH 200514045 N1
				PH 200/06032 R1
				PH 200818007 R1
				PH 201201066 R1
				PH 201217015 R1

M1. 2 veterinarians	1,763,900.58	1,172,597.	24	M1-01-01
(part-time)		28	months	M1-01-02
				M1-01-03
				M1-01-04
				M1-01-05
				M1-01-06
				M1-01-07
				M1-01-08
				M1-01-09
				M1-01-10
				M1-01-11
				$M_{1-01-12}$
				M1-01-15
				M1-01-16
				M1-02-01
				M1-02-02
				M1-02-03
				M1-02-04
				M1-03-01
				M1-03-02
				M1-03-03
				M1-03-04
				M1-03-05
				M1-03-06
				M1-03-07
				M1-03-08
				M1 02 00
				M1-03-09
				M1-03-10
M2. 1 technician	376,103.23	340,200.0	24	M2-01-01
for veterinary		0	months	M2-01-02
(full-time)				M2-01-03
				M2-01-04
				M2-01-05
				M2-01-06
				M2-01-07
				M2-01-08
				M2-01-09
				M2-01-10
				M2-01-11
				M2-01-12
				M2-01-13
				M2-01-14
				$M_{2-01-15}$
				M2 01 16
	220 5 40 00	222 475 0		M2-01-10
NI3. 1 technician	330,540.00	329,1/5.0	24	WI3-01-01
for research		0	months	M3-01-02
(part-time)				M3-01-03
				M3-01-04
				M3-01-05
				M3-01-06
				M3-01-07
				M3-01-08
				M3-01-09

				M3-01-10
				M3-01-11
				M3-01-12
				M3-01-13
				M3-01-14
				M3-01-15
				M3-01-16
M4. 1	95,800.00	85,785.00	5 times	M4-01-01
administrative				M4-01-02
assistant for				M4-01-03
outreach				M4-01-04
(part-time)				
	0.00	0.00	F b ¹ m n n	
G22. Expert	0.00	0.00	5 times	
consultant for				
outreach				
(honorarium)				
G23. External audit	56,000.00	14,000.00	2 years	47002810367

12. 申請發放最後一筆資助款項的金額(如有) Amount requested for next installment (if any)

NONE

13. 須歸還政府的餘款數目(如有)The amount of unspent balance to be returned to the government (if any)

\$ 1,099,322.95

<完 END>

註 1:實際開支中[\$0]已於報告期內支付。餘下[\$5000]元按權責發生制原則計提,屬 本報告期的應付未付費用,並於下一份報告書內繳付。

	人 件 冱 単			
		是	否 (<i>請於</i> 備提供)	有關文件 /備註
1	已 根 據 資 助 協 議 要 求,另外 提 交 財 務 報 表 / 經 審 計 賬 目。	V		
	(財務報表包括財務狀況表(資產負債表)及收入 支出表;經審計賬目包括核數師報告(包括證 明受資助者按照批撥條件使用資助額的聲 明)、財務狀況表(資產負債表)、收支表、現金			
	流動表及其他附註解釋資料。) 收支表須清楚 列明各收入及支出細項(參考協議 Schedule 11 內 的獲批預算),有關收入及支出細項應為經審計 帳目內的一部分,受資助者有責任通知核數師			
	有關要求。)			

2	確認另外提交的財務報表/經審計賬目與進 度/年度報告書的財務資料一致。 如資料不一致,請另外說明。		
3	就「項目收入」,除政府資助金額外,確認已 提交其他收入的證明及內容。	V	
4	除利息收入、政府資助及已於申請書明的預計 收入外,報告期没有其收入。 如有非申請書聲明的其他收入,請說明相關性 質及提供證明。		
5	就員工開支,確認已提交「薪金簽收單」及各 員工的對應「強積金供款確認書」。		
6	確認項目所有員工於項目期間只進行項目的相關工作。		
7	如「獲批項目開支」與資助協議內的原金額不同,確認所有經調整的「獲批項目開支」已預 先向秘書處申請並獲得批准。		

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Appendix A

Review of City University Aquatic Veterinary Ambulatory Service

Appendix A

Review of City University Aquatic Veterinary Ambulatory Service 2018/2019/2020

Background:

The City University Aquatic Veterinary Ambulatory Service (the service) started operating in October 2018. The service caters to local aquaculture fish farmers and related industries. In December 2020 it employed 2 part-time veterinarians, 1 full time technician, and 1 part-time technician. We also had two CityU faculty members to cover calls, conduct research projects that stem from health issues identified during our site visits, and manage the overall programme. Appendix B contains a table with the list of the proposed activities and our achievements. The following is an overview of our programme. Please note we have had to reduce our veterinary service to emergency visits only during the protests in November 2019 and when the government implemented restrictions to control COVID-19 outbreaks in Hong Kong.

<u>Set Up:</u>

- Hired two part-time veterinarians from Hong Kong
- Hired one full time technician and one-part time technician
- Set up a service for receiving and tracking cases
 - o Dedicated Phone line
 - o Dedicated software system
 - Basic equipment
 - o Dedicated vehicle
 - o Collaboration with VDL
 - Pharmacy with vet clinic

Service:

During the 2.5 years of operation, our veterinary service has:

- Responded to 161 cases (21 cases in 2018; 62 cases in 2019; 78 cases in 2020;)from 67 clients involving 408 site visits (See Appendix C for details on our cases, and Appendix D for a list of our clients (beneficiaries) with contact information). We also include a soft copy of the excel spreadsheet in Appendix C. Thirty-four of our cases have required prescription medication for treatment. We include a letter to AFCD from one of our clients describing our service provided during the first year (See Appendix E)
 - Our farm visits generally consist of: water quality assessment and collection of fish specimens for diagnostic work-up. Laboratory work-ups includes post-mortems, wet mounts for parasites, tissue smears for pathogen idenfication, bacteriology/sensitivity, and histology as required. For our viral testing we have been submitting samples to AFCD.
 - o Diseases/pathogens identified and treated to date include:
 - Bacterial: Vibrio spp, Photobacterium spp, Mycobacterium spp, Aeronomas spp., Streptococcus iniae, Chryseobacterium spp, Edwardsiella spp, Enterobacter spp, Plesiomonas spp, Staphylococcus spp

- Parasite: Crytocaryon irritant, monogeneans, scuticociliates, sea lice, Sanginicola sp., Ichthyophthirius multifiliis, Trichodina, Oodinium, Amyloodinium, Chilodonella, brooklynella
- Viral: Iridovirus (ISKNV and RSIV), VNN
- Water Quality issues: High DO and ammonia, as well as PH fluctuations issues during the summer in earthen ponds. Ammonia, Nitrite, Nitrate issues in recirculation systems.
- Pharmacy
 - We have developed a functional pharmacy for fish pharmaceutical products. We have proposed for project budget change to subsidize the drug costs by 50% to the farmers to reduce the illegal purchases of antibiotics (i.e. drugs purchased by farmers without the proper paperwork), in which approval was received from the SFDF Secretariat on 11 October 2019. The current prices of the two antibiotics we carry are:
 - \$26 USD/kg OTC (1000 kg of fish would require 1.4 kg which would cost \$38 USD)
 - \$60 USD/ Kg of Florfenicol (1000 kg of fish would need 300 g approximately \$20 USD).
 - Given our preliminary findings on Emamectin Benzoate for the treatment of sea lice (see Appendix F) we will be sourcing this drug for our clients.
- Location of ambulatory service
 - We have moved our services to Trinity Tower in Sham Shui Po. We have had access to the Veterinary Diagnostic Laboratory at CityU to conduct fish necropsies.
- Limitations identified

We had political protests at our University in November 2019, and the government has implemented work-from-home health regulations that have lasted several weeks to control COVID19 since January 2020. This has reduced our ability to carry out our veterinary service and research initiatives. During these periods of time we could only operate a an emergency service. Despite these set-backs we have managed to attract 67 clients to our service.

Research:

- We have been tracking AMR in all our bacterial isolates (see Appendix G excel file)
- We have published our sea lice emamectin trial (See Appendix F).
- We have assigned a graduate student to start on biosecurity plans for farmers in this time when site visits are difficult to do due to COVID-19.
- We are completed our preliminary assessment of water quality on mullet farms in the summer
 - Results indicate water quality is an issue on farms but that the summer of 2020 was not terribly problematic for farmers
 - We have a short report (Appendix H) on our findings
 - We will be conducting a water quality workshop in May 2021 for fish farmers

Outreach:

• Due to the political situation in Hong Kong it was impossible to invite guest speakers in Autumn 2019 so we scheduled a skype seminar for our veterinarians and technicians to assist us in developing a water quality service (See appendix I for skype call participants).

- Due to COVID 19 we were unable to conduct any face-to- face workshops for the farmers or veterinarians so we had zoom meetings for both these groups in December 2020 (Appendix I).
- Dr. **Dr. Construction** has also been providing educational information to farmers during organic farmer meetings organized by Baptist U and PolyU in December 2019, Sept. 2020, and October 2020 (Appendix I).

Appendix B

Table of Achievements

Appendix B. Activities under the AFCD SFDF grant on Improving Fish Health and Production in Hong Kong Sept 2018 to December 2020

<u>Proposed Activity</u> (including Planning, <u>Recruitment, etc.)</u>	Date of completion	Anticipated No. of Participants	Comments
 Hiring of personnel Veterinarian 1 (part time) Veterinarian 2 (part time) Technician full-time Technician part-time 	 October 2018 February 2019 September 2019 October 2019 	 4 people benefited from these hires. We also trained two additional technicians in fish health that moved on to graduate school or permanent positions at CityU. These trainees continue to work in some capacity with fish. 	 We hired 2 part-time veterinarians instead of 1 full-time veterinarian because we could not find anyone who wanted to work full-time as a fish veterinarian. We hired a full time technician Prior to this hire we trained two other individuals in fish health (1990) We hired a retired AFCD technician (1990) We hired a retired AFCD technician (1990)
 SERVICE: Provide veterinary services to aquaculture clients Established a veterinary service that farmers can access for fish health management advice Establish a pharmacy which stocks fish medicines 	 See Appendix C for date of site visits and information on cases March 2019 	 We have provided veterinary advice to 67 aquaculture clients during the project Our pharmacy is stocked with two antibiotics(oxytetracycline and florfenicol) and two antiparasitics (emamectin benzoate and praziquantel). We will subsidize the cost of these products to encourage farmers not to purchase drugs over the boarder or on line without a prescription. 	 We overestimated the number of farmers that would use our service, but we underestimated the number of cases. We partner with the AFCD fish health programme on cases We set up preventative visits in the last 6 months of the project We delivered additional farmer workshops to improve compliance of fish health measures
RESEARCH			

Research: Aquatic animal health assessment	April 2020	•	Existing 38 clients at the time of the report	•	We outlined the cases we investigated in Appendix C and we provide a soft copy of the excel spreadsheet. We have also outlined the common issues we have observed over the last year in Appendix J. We focused our service and research projects on mitigating the common issues identify in Appendix J
Research: Evaluation of emamectin benzoate (EMB) for salt water ectoparasites	February 2021	•	We published our findings from our trial on emamectin benzoate	•	We have recently successfully treated sea lice on Grouper. We published our results in February 2021 (Appendix F). • Farmers are pleased with the results
Research: Mullet Summer Mortality Syndrome	April 2020 to August 2020	•	We investigated water quality in Earthen ponds with Mullet in Hong Kong over the summer months	•	Project was slightly delayed due to COVID-19 but we found the water temperature was very high for mullet and for several days we had issues with DO, Ammonia, and fluctuations in pH (Appendix H) Despite these water quality issues we did not observe as high fish mortality as reported in the past. Further all mortality were preceded by management events or storms.
Research: Biosecurity plans for farmers	Initiated in August 2020	•	21 salt water farmers have been surveyed about their management practices	•	We are starting this project as part of a graduate student project. Given our inability to conduct field research due to Covid-19 this was a good alternative project to work on.
OUTREACH					
Train veterinarians in aquatic animal medicine through	December 2020	•	We trained one veterinarian (Dr. K. Chan) We are training one additional	•	Dr. came to us with limited fish experience in January 2019. She trained with us for 12 months.

internship programme (1 year			veterinarian (Dr. Kwok Zu Lim)	•	Dr. has been working with our veterinary team
each)		•	We trained two technicians		since March 2020.
			(Jennifer Lam and Sandy Cheng)	•	Ms. worked for us from October 2018
					to October 2019
				•	December 2020
Develop and administer 2 fish health courses for undergraduate curriculum at CityU	1/9/18 to 15/12/18 ; 1/1/19 to 1/05/19 ; & 02/09/2019 to 14/12/2019; 07/01/2020	•	We have prepared and delivered three undergraduate courses and delivered these to 53 BVM students. We have taken 8 students out on one week practical field service internships.	•	One of our courses is in fresh water aquaculture and fish health and the other is in salt water aquaculture and fish health. Since the end of January 2020 we have been offering our fish health course on line. We have had to stop all field trips and practical laboratories but we have adapted the course accordingly. (see
Internships for practical					Appendix J)
veterinary experience (1 week	January 2020; May			•	The ambulatory internships were cut short due to
long)	2020				COVID but we were still able to bring 8 students (2
Develop and deliver 3					We have not been able to deliver our second
veterinary continuing		•	~50 veterinarians sat in on the		veterinary training course due to the political unrest
education courses in aquatic			didactic lectures and 35		in Hong Kong and the COVID-19 pandemic. Our
animal veterinary medicine			participated in the laboratory		guest lecturer cancelled in November and has not
Basic aquatic animal	a Juna E 2010		This was attended by our team only		been able to reschedule.
veterinary medicine	 June 5 2019 Oct 22 2019 	•	We had approximately 25		
Pond managementWater Quality workshop	 Dec. 3, 2020 		veterinarians online for the water quality seminar		
(postponed)					
Deliver 2 industry workshop				•	Our October 2020 meeting was cancelled because
on fish health issues and		•	12 farmers and researchers		our guest lecturer cancelled due to political unrest
treatment options			workshop		train the staff on this project on pond water quality
Nanobubbler workshop	October 2018				issues. From this skype workshop we have been
with representative from		•	16 farmers attended the		able to set up our water quality laboratory.
Singapore (James Chow		1	Workshop on antibiotic use	•	Our March workshop on water quality has also been
AquaPro)	• June 5 th 2010		«1E populo attended our on line		postponed we anticipate being able to give this
 Prudent use of Antibiotics Sea lice on line workshop 	 Dec. 17, 2020 		sea lice seminar		
sea nee on nine workshop					

•	Organic farmers fish health workshops Water Quality workshop (Postponed until May 2021)	•	Dec 19 2019 and Sep 16 2020 for organic farmers association Sep 25 2020 and Oct 2 2020 for PolyU.	•	Fish health talks by Dr.	•	Two talks for the Organic Farmers Association Two talks for Poly U fish certification programme
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Appendix C

List of Cases

Date	Case number	Client ID	General location	Fish species	Photos	Disease (s) diagnosed	Recommendation(s) including the usage of	Dosage	Rx number
20/6/2018	2018-12	2	Tai Po	Hybrid grouper					
20/6/2018	2018-13	#N/A	Tai Po	Hybrid grouper					
28/7/2018	2018-14	18	Sam Mun Tsai	Pompano					
28/7/2018	2018-29	18	Sam Mun Tsai	Sea bream					
28/7/2018	2018-35	18	Sam Mun Tsai	Muddy					
19/9/2018	2018-16	6	Lau Fau Shan	Grouper		H2O2 burns, Brown blood syndrome			
02/10/2018	2018-17	1	Sai Kung	Pompano		Parasitic infection - Cryptocaryon	Continue with formalin bath treatments		
5/10/2018	2018-18	2	Tai Po	Pompano		Bacterial infection - mixed vibrio sp. (not systemic)			
5/10/2018	2018-19	3	Sai Kung	Pompano		Bacterial infection - Streptococcus iniae (systemic)	Freshwater bath	20 mg/kg*10 days	RX C2018-05
9/10/2018	2018-20	3	Sai Kung	Grouper	<u>2018-20</u>	Wild fish has positive for photobacterium sp.	-None because fish are close to market size -Florfenicol	20 mg/kg*10 days	RX C2018-06
15/10/2018	2018-21	3	Sai Kung	Rabbit fish		Suspect bacterial infection	Florfenicol, Increase supplement vit C		
16/10/2018	2018-23	3	Sai Kung	Japanese Eels		Parasitic infection - trichodina (mild)	Improve water quality add biofilter, Provide increased water exchanged and circulation		
20/10/2018	2018-19	3	Sai Kung	Pompano		Bacterial infection - Streptococcus iniae (systemic)	Florfenicol and increased the oxygen in the water	75 mg/kg*10 days	RX C2018-10
20/10/2018	2018-36	3	Sai Kung	Grouper		Parasitic infection - Sea lice	Emamectin TRIAL ONLY		
22/10/2018	2018-19	3	Sai Kung	Pompano		Bacterial infection - Streptococcus iniae (systemic)	Florfenicol and increased the oxygen in the water	100 mg/kg*10 days	RX C2018-12
22/10/2018	2018-23	3	Sai Kung	Japanese Eels		Parasitic infection - trichodina (mild)	Improve water quality add biofilter, Provide increased water exchanged and circulation		
22/10/2018	2018-24	3	Sai Kung	Pompano (medium size)		Parasitic infection - Moderate to severe	Oxytetracycline and formalin bath	20 mg/kg*7 days	RX C2018-13
22/10/2018	2018-25	3	Sai Kung	Whitemouth croalcer		Parasitic infection - Gill clip: Moderate cryptocaryon irritans observed on primary filament.	Formalin bath and increase air		
29/10/2018	2018-26	66	Kowloon Tong	Horseshoe crabs from school					
30/10/2018	2018-24	3	Sai Kung	Pompano (medium size)		Parasitic infection - Moderate to severe	Oxytetracycline and formalin bath		
30/10/2018	2018-19	3	Sai Kung	Pompano		Bacterial infection - Streptococcus iniae (systemic)	Florfenicol and increased the oxygen in the water	20 mg/kg*7 days	RX C2018-08
19/11/2018	2018-27	4	Lamma Island	Sabah hybrid grouper			Florfenicol	20 mg/kg*7 days	RX C2018-15
29/11/2018	2018-28	5	Yim Tin Jai	Hybrid grouper	<u>2018-28</u>	Parasitic infection - Sea lice	Emamectin TRIAL ONLY	50 ug/kg/day*7 days	RX C2018-16
17/12/2018	2018-28	5	Yim Tin Jai	Hybrid grouper		Parasitic infection - Sea lice	Emamectin TRIAL ONLY		
17/12/2018	2018-30	3	Sai Kung	Pompano	<u>2018-30</u>	Mixed infection - Parasites (monogeneans) and	Oxytetracycline	100 mg/kg/day*6 days	RX C2018-17
			-			bacteria		100 mg/kg/day*7 days	RX C2018-18

Appendix C. List of cases from June 2018 to December 2020.

17/12/2018	2018-31	3	Sai Kung	Pompano	<u>2018-31</u>	Moderate Trichodina, Other small worm like parasite	Oxytetracycline		
						(Moderate), Amyloodinium? And bacterial disease			
47/40/0040	0040.00		Cailling	Democra	0040.00		Outstaansking		
17/12/2018	2018-32	3	Sai Kung	Pompano	2018-32	parasite	Oxytetracycline		
4/2/2019	2019-03	6	Lau Fau Shan	Giant Grouper		Parasitic infection - trichodina	Formalin bath		
28/2/2019	2019-04	7	Sam Mun Tsai	Grouper	2019-04	Parasitic infection - sea lice	Oxytetracycline, formalin bath	110 mg/kg/day*7 days	RX C2019-01
4/3/2019	2019-05	8	Fanling	Jewel Perch, Tilipa	2019-05	Routine visit	None		
6/3/2019	2019-06	67	Mai Po	Koi/Carp					
11/3/2019	2019-07	9	Yung Shue O	Chicken grunter	<u>2019-07</u>	Sudden mortality	None		
12/3/2019	2019-08	13	Yuen Long	Pearl Perch	2019-08	Routine visit	None		
2/4/2019	2019-10	6	Lau Fau Shan	Giant Grouper	<u>2019-10</u>	Skin ulcers			
12/4/2019	2019-11	5	Yim Tin Jai	Hybrid grouper	<u>2019-11</u>		-Florfenicol (2020/04/17)	15 mg/kg/day*10 days	RX C2019-06
							-Suggested isolated infected fish with other fish.		
							-Stop formalin bath because mucus on fish is		
						Sea lice, monogeneans and bacterial infection	reduced possibly from over treatment		
13/4/2019	2019-12	3	Sai Kung	Pompano		Parasitic infection - Cryptocaryon	Continue and monitor formalin treatment effect		
15/4/2019	2019-13	6	Lau Fau Shan	Grouper		Bacterial infection	Monitor as fish are off feed		
7/5/2019	2019-14	3	Sai Kung	Pompano		Mixed infection - Bacterial infection, cryptocaryon			
14/5/2019	2019-15	18	Sam Mun Tsai	Pompano		General visit for nanobubbler			
22/05/2019	2019-17	20	Cheung Sha Wan	Croacker	<u>2019-17</u>	Bacterial infection (mixed)	Medical intervention not offered as they are to be sent to market		
27/05/2019	2019-18	22	Au Tau	Shrimp					
21/6/2019	2019-21	19	Tai Tau Chau	Hybrid grouper	2019-22	Bacterial disease	OTC prescribed	100 mg/kg/day*7 days	RX C2019-11
24/6/2019	2019-22	3	Sai Kung	White Croacker	<u>2019-23</u>	Environmental issue, nutritional issue	Care with transport - acclimatise properly		
24/6/2019	2019-23	3	Sai Kung	Pompano	<u>2019-24</u>	Parasitic issue			
27/06/2019	2019-24	10	Yim Tin Jai	Grouper		Bacterial infection and water quality issue			
10/7/2019	2019-25	11	Mai Po	Grass carp		Water quality?			
11/7/2019	2019-26	17	Mai Po	Mullet		Water quality	Increase aeration - turn on pumps (client concern - cost for electricity)		
16/7/2019	2019-27	12	Hok Tau / Fanling	Jewel Perch	<u>2019-27</u>	Buoyancy disorder	None - one off conidtion with perforation of swim bladder		
17/07/2019	2019-28	13	Yuen Long	Jade perch		Red spots on body			
22/7/2019	2019-29	6	Lau Fau Shan	Grouper		Bacterial infection			T
23/07/2019	2019-22	3	Sai Kung	White croaker		Environmental issue, nutritional issue	Care with transport - acclimatise properly		

25/07/2019	2019-31	13	Yuen Long	Jade perch	<u>2019-31</u>	Suspect bacterial infection	OTC prescribed		
31/07/2019	2019-32	13	Yuen Long	Jade perch	<u>2019-32</u>	Water quality / bacterial issue	OTC prescribed	100 mg/kg/day*10 days	RX C2019-12
2/8/2019	2019-33	12	Hok Tau / Fanling	Tilapia	<u>2019-33</u>	Enviromental issue, bacterial infection (secondary)	Correct environment - lower stocking density, increase water exchange, decrease feeding		
7/8/2019	2019-32	13	Yuen Long	Jade perch	<u>2019-32</u>		OTC prescribed	15 mg/kg/day*10 days	RX C2019-13
8/8/2019	2019-35	13	Yuen Long	Jade perch	<u>2019-35</u>	Sore in the tail, white eye, parasitic	-None yet - pending culture and sensitivity results. -Reduce feeding, correct water quality issue. -Praziquantel (2020/08/09)	35 mg/kg/day*3 days	RX C2019-14
12/08/2019	2019-37	12	Hok Tau / Fanling	Tilapia/jade perch	<u>2019-37</u>	Water quality check up	Correct water quality issue		
13/08/2019	2019-38	12	Hok Tau / Fanling	Tilapia	<u>2019-38</u>	Bilateral exophthalmos - retrobulbar abscess	Monitor other fish		
19/08/2019	2019-32	13	Yuen Long	Jade perch		Suspect bacterial issue	OTC prescribed		
20/08/2019	2019-26	17	Mai Po	Mullet	<u>2019-26</u>	Water quality	Increase aeration - turn on pumps (client concern -		
21/08/2019	2019-39	2	Tai Po	Bat fish	<u>2019-39</u>	Water quality / sepsis	None yet as culture and sensitivity result were conclusive		
26/8/2019	2019-40	13	Yuen Long	Jade perch	<u>2019-40</u>	Water quality / bacterial	Follow up		
27/8/2019	2019-39	2	Tai Po	Batfish	<u>2019-39</u>	Water quality / sepsis	None yet as culture and sensitivity result were conclusive		
28/8/2019	2019-41	16	Mai Po	Mullet	<u>2019-41</u>	Suspect water quality issue	Non - need confirmation of water quality issue		
29/8/2019	2019-39	2	Tai Po	Bat fish	<u>2019-39</u>	Water quality / sepsis	-None yet as culture and sensitivity result were conclusive. -Oxytetracycline(2020/08/28)	100 mg/kg/day*10 days	RX C2019-15
04/09/2019	2019-26	17	Mai Po		<u>2019-26</u>	Water quality	Increase aeration - turn on pumps (client concern - cost for electricity)		
04/09/2019	2019-42	16	Mai Po	Mullet	<u>2019-42</u>	Water quality issue, secondary problem	Correct water quality issue		
05/09/2019	2019-43	15	Lamma Island	Cobia	<u>2019-43</u>	Suspect parasitic problem	None - farmer moved sea pen to water with higher current flow, and consider problem in control		
9/9/2019	2019-44	3	Sai Kung	Batfish	<u>2019-44</u>	Suspect water quality issue / potential viral infection	Correct water quality issue		
13/9/2019	2019-45	6	Lau Fau Shan	Grouper		High mortalities and morbidity	Correct water quality issue		
16/9/2019	2019-46	21	San Tin	Jade perch	<u>2019-46</u>	Mortality	Correct water quality issue		
17/9/2019	2019-46	21	San Tin	Jade perch	<u>2019-46</u>	Mortality	Correct water quality issue		
20/9/2019	2019-47	13	Yuen Long	Jade perch	<u>2019-47</u>	Mortality and red eye	Correct water quality issue		
23/9/2019	2019-48	18	Sam Mun Tsai	Batfish	<u>2019-48</u>	Mortality	Correct water quality issue		
24/9/2019	2019-47	13	Yuen Long	Jade perch	<u>2019-47</u>	Mortality and red eye	Correct water quality issue		

24/9/2019	2019-49	13	Yuen Long	Jade perch	<u>2019-49</u>	Parasite	Praziquantel	35 mg/kg/day*3 days	RX C2019-16
27/9/2019	2019-51	22	Au Tau	Tilapia	<u>2019-51</u>	Lesion on the skin	Correct water quality issue		
27/9/2019	2019-49	13	Yuen Long	Jade perch		Parasite	Antibiotic prescribed		
30/09/2019	2019-52	12	Hok Tau / Fanling	Tilapia and jade perch		Water quality, parasite (trichodina)	Correct water quality issue		
02/10/2019	2019-52	12	Hok Tau / Fanling	Tilapia		Water quality, parasite (trichodina)	Correct water quality issue		
04/10/2019	2019-53	13	Yuen Long	Managuense	<u>2019-53</u>	New batch arrived			
16/10/2019	2019-54	13	Yuen Long	Managuense	2019-54	New batch arrived			
16/10/2019	2019-55	13	Yuen Long	Jade perch	<u>2019-55</u>	Lesion on the skin	Correct water quality issue		
18/10/2019	2019-56	23	Tin Shui Wai	Marbled eel	<u>2019-56</u>	Do water quality test	Correct water quality issue		
21/10/2019	2019-54	13	Yuen Long	Managuense	<u>2019-54</u>	New batch arrived			
21/10/2019	2019-55	13	Yuen Long	Jade perch	<u>2019-55</u>	Lesion on the skin	Correct water quality issue		
22/10/2019	2019-57	15	Lamma Island	Batfish	<u>2019-57</u>	Mortality			
22/10/2019	2019-58	15	Lamma Island	Red snapper	<u>2019-58</u>	Mortality			
22/10/2019	2019-59	15	Lamma Island	Grunt	2019-59	Mortality			
24/10/2019	2019-60	23	Tin Shui Wai	Mable Goby	<u>2019-60</u>	New batch arrive			
24/10/2019	2019-54	13	Yuen Long	Managuense	<u>2019-54</u>	New batch arrived			
25/10/2019	2019-61	23	Tin Shui Wai	Grouper	<u>2019-61</u>	New batch arrive			
28/10/2019	2019-62	13	Yuen Long	Jade perch	<u>2019-62</u>	White spot and lesion			
28/10/2019	2019-54	13	Yuen Long	Managuense		Revisit	Follow up		
28/10/2019	2019-55	13	Yuen Long	Jade perch		Revisit	Follow up		
28/10/2019	2019-63	24	Yuen Long	Jade perch	<u>2019-63</u>	Gill fester and red spot on the body			
30/10/2019	2019-64	25	Yuen Long	Mitten crabs	<u>2019-64</u>				
5/11/2019	2019-55	13	Yuen Long	Jade perch	<u>2019-55</u>	Recheck low chronic mortality	Follow up		
5/11/2019	2019-54	13	Yuen Long	Managuense	<u>2019-54</u>	Recheck after formalin treatment			
12/11/2019	2019-65	26	Lamma Island	Grouper	<u>2019-65</u>	Mortality	EMB prescribed	50 ug/kg/day*7 days	RX C2019-17
18/11/2019	2019-65	26	Lamma Island	Grouper	<u>2019-65</u>	Recheck slice treatment and pump			
19/11/2019	2019-66	27	Kau Sai Wan	Star snapper /Grouper	<u>2019-66</u>	Mortality			
25/11/2019	2019-65	26	Lamma Island	Grouper	2019-65	Recheck slice treatment and pump			
26/11/2019	2019-66	27	Kau Sai Wan	Grouper	<u>2019-66</u>	Seac lice treatment and pump	EMB prescribed	50 ug/kg/day*7 days	RX C2019-18
27/11/2019	2019-64	25	Yuen Long	Mitten crabs	<u>2019-64</u>	Revisit	Follow up		
29/11/2019	2019-67	38	Yung Shue Au	Pompano	<u>2019-67</u>	Sea lice			
2/12/2019	2019-66	27	Kau Sai Wan	Grouper	2019-66	Recheck sea lice treatment	Follow up	50 ug/kg/day*7 days	RX C2019-18b

4/12/2019	2019-65	26	Lamma Island	Grouper	2019-65	Recheck sea lice treatment and sample collection	Follow up		
09/12/2019	2019-65	26	Lamma Island	Grouper	2019-65	Recheck sea lice treatment and sample collection	Follow up		
11/12/2019	2019-68	13	Yuen Long	Jade perch	2019-68	Meeting with Bruce	None		
13/12/2019	2019-66	27	Kau Sai Wan	Grouper	2019-66	Recheck sea lice treatment	Follow up		
17/12/2019	2019-66	27	Kau Sai Wan	Grouper	2019-66	Recheck sea lice treatment	Follow up		
17/12/2019	2019-69	33	San Tin	Shrimp	<u>2019-69</u>	Mortality			
18/12/2019	2019-70	2	Tai Po	Pompano	<u>2019-70</u>				
18/12/2019	2019-69	33	San Tin	Shrimp		Revisit			
23/12/2019	2019-66	27	Kau Sai Wan	Grouper	<u>2019-66</u>	Recheck sea lice treatment	Follow up		
27/12/2019	2019-72	28	San Tin	Jade perch	<u>2019-72</u>				
27/12/2019	2019-69	33	San Tin	Shrimp	2019-69	Mortality			
30/12/2019	2019-71	18	Sam Mun Tsai	Pompano	<u>2019-71</u>	Lesion on the gill			
31/12/2019	2019-66	27	Kau Sai Wan	Grouper	<u>2019-66</u>	Recheck sea lice treatment	Follow up		
06/01/2020	2020-01	29	Mui Wo	Jade perch	<u>2020-01</u>	New site visit	None		
06/01/2020	2020-02	30	Pui O	Grass carp	<u>2020-02</u>	New site visit	None		
09/01/2020	2019-66	27	Kau Sai Wan	Grouper		Recheck slice treatment	Emamectin benzoate treatment	50 ug/kg/day*7 days	C2019-18C
10/01/2020	2019-71	18	Sam Mun Tsai	Pompano	<u>2019-71</u>	Recheck parasite	Follow up		
17/01/2020	2020-03	13	Yuen Long	Jade perch	2020-03	White spot parasites	Saltwater bath		
17/01/2020	2020-04	13	Yuen Long	Jade perch	<u>2020-04</u>	New fish egg arrive	Monitor fish egg		
20/01/2020	2020-03	13	Yuen Long	Jade perch	<u>2020-03</u>	White spot parasites	Follow up		
20/01/2020	2020-04	13	Yuen Long	Jade perch	2020-04	New fish egg arrive	Follow up		
21/01/2020	2019-66	27	Kau Sai Wan	Grouper	<u>2019-66</u>	Recheck slice treatment	Follow up		
22/1/2020	2019-69	13	Yuen Long	Shrimp	<u>2019-69</u>	Revisit	Follow up		
17/02/2020	2020-05	6	Lau Fau Shan	Grouper	<u>2020-05</u>	Lesion on the body	Correct water quality issue		
17/02/2020	2020-06	13	Yuen Long	Jade perch fry	<u>2020-06</u>	Check for fry	Follow up		
17/02/2020	2020-07	13	Yuen Long	Jade perch	<u>2020-07</u>	Check water quality	Correct water quality issue		
25/02/2020	2020-06	13	Yuen Long	Jade perch fry	2020-06	Recheck for fry	Follow up		
3/2/2020	2020-06	13	Yuen Long	Jade perch fry	2020-06	Recheck for fry	Follow up		
17/03/2020	2020-08	31	Cheung Sha Wan	Hybrid grouper	2020-08	Lesion on the body and sea lice	Follow up		
17/03/2020	2020-09	32	Cheung Sha Wan	Star snapper	2020-09	Gill parasite	Follow up		
18/03/2020	2020-10	33	San Tin	四大家族	<u>2020-10</u>	Water quality check	Correct water quality issue		

18/03/2020	2020-11	34	Yuen Long	Jade perch	<u>2020-11</u>	Water quality check	Correct water quality issue		
19/03/2020	2019-72	28	San Tin	Jade perch	<u>2019-72</u>		Follow up		
19/03/2020	2020-12	28	San Tin	Jade perch fish fry	<u>2020-12</u>	Water quality check	Correct water quality issue		
19/03/2020	2020-13	28	San Tin	Tilapia	2020-13	Water quality check	Correct water quality issue		
23/3/2020	2020-14	29	Mui Wo	Jade perch	<u>2020-14</u>	Build up the bio filter			
24/3/2020	2020-15	13	Yuen Long	Jade perch		Parasite	Saltwater bath		
25/3/2020	2020-16	35	Nam Sang Wai	Jade perch	<u>2020-16</u>	Water quality and parasite check	Correct water quality issue		
25/3/2020	2020-17	36	Nam Sang Wai	Jade perch	<u>2020-17</u>	Water quality check	Correct water quality issue		
26/3/2020	2019-46	21	San Tin	Jade perch / Mullet	<u>2019-46</u>	Water quality check	Follow up		
30/3/2020	2020-06	13	Yuen Long	Jade perch	<u>2020-06</u>	Water quality check	Correct water quality issue		
30/3/2020	2020-18	6	Lau Fau Shan	Grouper	<u>2020-18</u>	Mortality	Follow up		
4/1/2020	2020-08	31	Cheung Sha Wan	Hybrid grouper	<u>2020-08</u>	Sea lice	EMB treatment	50 ug/kg/day*7 days	C2020-01
4/2/2020	2020-19	37	Tai Tau Chau	Hybrid grouper	<u>2020-19</u>	Sea lice	EMB treatment		
4/6/2020	2020-19	37	Tai Tau Chau	Hybrid grouper	<u>2020-19</u>	Sea lice treatment Day 0	EMB treatment	50 ug/kg/day*7 days	C2020-02
4/7/2020	2020-13	28	San Tin	Tilapia	<u>2020-13</u>	Mortality	Correct water quality issue		
4/8/2020	2020-08	31	Cheung Sha Wan	Hybrid grouper	<u>2020-08</u>	Sea lice treatment Day 7	EMB treatment		
4/8/2020	2020-06	13	Yuen Long	Jade perch	<u>2020-06</u>	Mortality	Correct water quality issue		
4/8/2020	2020-20	6	Lau Fau Shan	Grouper			Follow up		
14/4/2020	2020-19	37	Tai Tau Chau	Hybrid grouper	<u>2020-19</u>	Sea lice treatment Day 7	EMB treatment		
14/4/2020	2020-21	19	Tai Tau Chau	Hybrid grouper	<u>2020-21</u>	Sea lice	EMB treatment		
15/4/2020	2020-08	31	Cheung Sha Wan	Hybrid grouper	<u>2020-08</u>	Sea lice treatment Day 14	EMB treatment		
15/4/2020	2020-06	13	Yuen Long	Jade perch	2020-06	Red spot on the body	OTC treatment	100 mg/kg/day*10 days	C2020-03
20/4/2020	2020-06	13	Yeun Long	Jade perch	<u>2020-06</u>	Mortality	Follow up		
20/4/2020	2020-22	6	Lau Fau Shan	Grouper	2020-22	Lesion	Correct water quality issue		
21/4/2020	2020-19	15	Tai Tau Chau	Hybrid grouper	<u>2020-19</u>	Treatment Day 14	EMB treatment		
21/4/2020	2020-21	16	Tai Tau Chau	Hybrid grouper		Sea lice	EMB treatment	50 ug/kg/day*7 days	C2020-04
21/4/2020	2020-23	17	Tai Tau Chau	Hybrid grouper	<u>2020-23</u>	Sea lice	Collect sea lice sample		
22/4/2020	2020-08	7	Cheung Sha Wan	Hybrid grouper	<u>2020-08</u>	Treatment Day 21	EMB treatment	50 ug/kg/day*7 days	C2020-01B
23/4/2020	2020-24	6	Lau Fau Shan	Grouper		Lesion	Correct water quality issue		
27/4/2020	2020-06(1)	13	Yeun Long	Jade perch		Mortality	Praziquantel treatment	35 mg/kg/day*3 days	C2020-05
27/4/2020	2020-06(2)	13	Yeun Long	Jade perch		Mortality	OTC treatment	100 mg/kg/day*10 days	C2020-06
28/04/2020	2020-19	15	Tai Tau Chau	Hybrid grouper	<u>2020-19</u>	Treatment Day 21	EMB treatment	50 ug/kg/day*7 days	C2020-02B
28/04/2020	2020-21	16	Tai Tau Chau	Hybrid grouper	2020-21	Treatment Day 0	Follow up		

28/4/2020	2020-06(1)	13	Yeun Long	Jade perch		Mortality	Follow up		
28/4/2020	2020-06(2)	13	Yeun Long	Jade perch		Dactylogyrus spp	Follow up		
29/04/2020	2020-11	10	Yuen Long	Jade perch	<u>2020-11</u>	Mortality	Correct water quality issue		
5/4/2020	2020-06(3)	13	Yuen Long	Jade perch fry		Dactylogyrus spp	Praziquantel treatment	50 mg/kg/day*3 days	RX C2020-07
5/4/2020	2020-06(2)	13	Yuen Long	Jade perch fry		Dactylogyrus spp	Praziquantel treatment	50 mg/kg/day*3 days	RX C2020-06B
5/5/2020	2020-21	16	Tai Tau Chau	Hybrid grouper	<u>2020-21</u>	Treatment Day 7	Follow up		
5/6/2020	2020-25	18	San Tin	Jade perch	<u>2020-25</u>	Water quality check	Correct water quality issue		
5/12/2020	2020-21	16	Tai Tau Chau	Hybrid grouper	2020-21	Treatment Day 14	Follow up		
14/5/2020	2020-06(3)	13	Yuen Long	Jade perch fry	<u>2020-06(3)</u>	Dactylogyrus spp	Follow up		
15/05/2020	2020-26	41	So Kwun Wat	Jade perch	<u>2020-26</u>	Ich and trichodina	Salt bath		
18/05/2020	2020-27	16	Mai Po	Mullet	<u>2020-27</u>	Water quality trial			
18/5/2020	2020-06(3)	13	Yuen Long	Jade perch fry	<u>2020-06(3)</u>	Dactylogyrus spp	Follow up		
18/5/2020	2020-15	13	Yuen Long	Jade perch	<u>2020-15</u>	Water quality check	Correct water quality issue		
19/05/2020	2020-19	37	Tai Tau Chau	Hybrid grouper	<u>2020-19</u>	Treatment Day 42	Follow up		
19/05/2020	2020-21	19	Tai Tau Chau	Hybrid grouper	<u>2020-21</u>	Treatment Day 21	Follow up		
20/05/2020	2020-28	42	San Tin	Mullet	<u>2020-28</u>	Water quality trial			
20/05/2020	2020-29	43	Yuen Long	Jade perch	<u>2020-29</u>	Water quality check	Correct water quality issue		
25/05/2020	2020-27	16	Mai Po	Mullet	<u>2020-27</u>	Water quality trial			
26/05/2020	2020-21	19	Tai Tau Chau	Hybrid grouper	<u>2020-21</u>	Treatment Day 28	Follow up		
27/05/2020	2020-28	42	San Tin	Mullet		Water quality trial			
27/05/2020	2020-30	22	Au Tau	Jade perch		Fry mortality	Correct water quality issue		
29/05/2020	2020-26	41	So Kwun Wat	Jade perch	<u>2020-26</u>	Ich and trichodina	Salt bath		
29/05/2020	2020-12	28	San Tin	Jade perch	<u>2020-12</u>	Mortality	Correct water quality issue		
6/1/2020	2020-27	16	Mai Po	Mullet		Water quality trial			
6/1/2020	2020-31	44	Tai Sang Wai	Mullet	<u>2020-31</u>	Mortality	Correct water quality issue		
6/2/2020	2020-12	28	San Tin	Jade perch		Water quality check	Correct water quality issue		
6/3/2020	2020-28	42	San Tin	Mullet		Water quality trial			
6/8/2020	2020-06(3)	13	Yuen Long	Jade perch fry		Dactylogyrus spp	Follow up		
6/9/2020	2020-27	16	Mai Po	Mullet		Water quality trial			
6/9/2020	2020-32	15	Lamma Island	Cobia	2020-32	Water quality issue			
6/10/2020	2020-28	42	San Tin	Mullet		Water quality trial			
6/10/2020	2020-33	34	Yuen Long	Jade perch fry	<u>2020-33</u>	Mortality	Correct water quality issue		

6/10/2020	2020-34	34	Yuen Long	Mud carp	2020-34	Mortality	Correct water quality issue		
15/06/2020	2020-12	28	San Tin	Jade perch	<u>2020-12</u>	Follow up			
15/06/2020	2020-35	28	San Tin	Jade perch fry	2020-35	Water quality check			
15/6/2020	2020-36	45	Tai Po	Jade perch fry	<u>2020-36</u>	Dactylogyrus spp and Ich	Salt bath		
16/06/2020	2020-27	16	Mai Po	Mullet		Water quality trial			
16/06/2020	2020-33	34	Yuen Long	Jade perch fry	<u>2020-33</u>	Follow up			
16/06/2020	2020-34	34	Yuen Long	Mud carp	2020-34	Follow up			
16/06/2020	2020-11	34	Yuen Long	Jade perch	<u>2020-11</u>	Follow up			
17/06/2020	2020-28	42	San Tin	Mullet		Water quality trial			
18/6/2020	2020-06(3)	13	Yuen Long	Jade perch fry	<u>2020-06(3)</u>	Dactylogyrus spp	Praziquantel treatment	50 mg/kg/day*3 days	RX C2020-08
22/06/2020	2020-27	16	Mai Po	Mullet		Water quality trial			
22/6/2020	2020-36	45	Tai Po	Jade perch fry	<u>2020-36</u>	Dactylogyrus spp and Ich	Follow up		
23/06/2020	2020-12	28	San Tin	Jade perch	<u>2020-12</u>	Follow up			
23/06/2020	2020-35	28	San Tin	Jade perch fry	<u>2020-35</u>	Follow up			
23/6/2020	2020-06(3)	13	Yuen Long	Jade perch fry		Dactylogyrus spp	Follow up		
23/6/2020	2020-06(2)	13	Yuen Long	Jade perch fry		Dactylogyrus spp	Follow up		
23/6/2020	2020-37	13	Yuen Long	Jade perch fish fry	<u>2020-37</u>	Dactylogyrus spp	Follow up		
24/06/2020	2020-28	42	San Tin	Mullet		Water quality trial			
24/06/2020	2020-33	34	Yuen Long	Jade perch fry		Follow up			
24/06/2020	2020-34	34	Yuen Long	Mud carp		Follow up			
24/06/2020	2020-11	34	Yuen Long	Jade perch		Follow up			
26/06/2020	2020-38	47	Fanling	Jade Perch	2020-38	Trichodina	Salt bath		
26/06/2020	2020-32	15	Lamma Island	Cobia	2020-32	Water quality issue			
29/06/2020	2020-27	16	Mai Po	Mullet		Water quality trial			
29/6/2020	2020-06(3)	13	Yuen Long	Jade perch fry		Follow up			
29/6/2020	2020-06(2)	13	Yuen Long	Jade perch fry		Follow up			
29/6/2020	2020-37	13	Yuen Long	Jade perch fish fry	<u>2020-37</u>	Follow up			
30/06/2020	2020-28	42	San Tin	Mullet		Water quality trial			
30/06/2020	2020-12	28	San Tin	Jade perch		Follow up			
30/06/2020	2020-35	28	San Tin	Jade perch fry		Follow up			
30/06/2020	2020-39	2	Tai Po	Batfish	<u>2020-39</u>	Cryptocaryon irritans	Formalin bath		
7/2/2020	2020-32	15	Lamma Island	Cobia	2020-32	Mortality			

7/3/2020	2020-40	48	Tai Po	Jede perch	<u>2020-40</u>	Water quality test	Water changing	
7/6/2020	2020-27	16	Mai Po	Mullet		Water quality trial		
7/6/2020	2020-06(3)	13	Yuen Long	Jade perch fry		Follow up		
7/6/2020	2020-06(2)	13	Yuen Long	Jade perch fry		Follow up		
7/6/2020	2020-37	13	Yuen Long	Jade perch fish fry		Follow up		
7/6/2020	2020-41	13	Yuen Long	Jade perch fish fry		Follow up		
7/7/2020	2020-42	49	Sandy Ridge	Tilapia	2020-42	Water quality test	No	
7/7/2020	2020-12	28	San Tin	Jade perch	<u>2020-12</u>	Follow up		
7/7/2020	2020-35	28	San Tin	Jade perch fry	<u>2020-35</u>	Follow up		
7/8/2020	2020-28	42	San Tin	Mullet		Water quality trial		
7/8/2020	2020-40	48	Tai Po	Jade Perch		Follow up		
7/9/2020	2020-43	6	Lau Fau Shan	Grouper	<u>2020-43</u>	Mortality		
7/10/2020	2020-38	47	Fanling	Jade Perch	<u>2020-38</u>	Follow up		
7/10/2020	2020-40	48	Tai Po	Jade Perch	<u>2020-40</u>	Follow up		
13/07/2020	2020-27	16	Mai Po	Mullet		Water quality trial		
13/7/2020	2020-37	13	Yuen Long	Jade perch fish fry		Follow up		
13/7/2020	2020-44	13	Yuen Long	Jade perch fish fry		Fish heath check-moderate levels of ich	-Formalin treatment -Cupramine treatment	
14/07/2020	2020-12	28	San Tin	Jade perch		Follow up		
14/07/2020	2020-35	28	San Tin	Jade perch fry		Follow up		
14/07/2020	2020-45	46	Yuen Long	Mullet	<u>2020-45</u>	Water quality check	Water changing	
15/07/2020	2020-28	42	San Tin	Mullet		Water quality trial		
15/07/2020	2020-40	48	Tai Po	Jade Perch		Follow up		
17/07/2020	2020-46	51	Yung Shue Au	Batfish	<u>2020-46</u>	Fish heath check	Water changing	
20/07/2020	2020-27	16	Mai Po	Mullet		Water quality trial		
20/07/2020	2020-47	33	San Tin	Jade Perch	<u>2020-47</u>	Mortality	Correct water quality issue	
21/07/2020	2020-45	46	Yuen Long	Mullet	<u>2020-45</u>	Follow up		
21/07/2020	2020-12	28	San Tin	Jade Perch	<u>2020-12</u>	Follow up		
21/07/2020	2020-35	28	San Tin	Jade perch fry	<u>2020-35</u>	Follow up		
22/07/2020	2020-28	42	San Tin	Mullet		Water quality trial		
22/7/2020	2020-37	13	Yuen Long	Jade perch fish fry	<u>2020-37</u>	Follow up		
22/7/2020	2020-44	13	Yuen Long	Jade perch fish fry	2020-44	Follow up		

27/07/2020	2020-27	16	Mai Po	Mullet		Water quality trial			
27/07/2020	2020-47	33	San Tin	Jade Perch	<u>2020-47</u>	Mortality	Correct water quality issue		
27/07/2020	2020-48	52	San Tin	Mullet	<u>2020-48</u>	Water quality check	Correct water quality issue		
27/07/2020	2020-49	53	San Tin	Mullet	<u>2020-49</u>	Water quality check	Correct water quality issue		
28/07/2020	2020-45	46	Yuen Long	Mullet		Follow up			
28/07/2020	2020-50	13	Yuen Long	Jade Perch	<u>2020-50</u>	Fish heath check-parasite	-Increase salinity - Formalin treatment - Cupramine treatment - Increase oxygenation support due to parasite load.		
28/07/2020	2020-12	28	San Tin	Jade perch		Follow up			
28/07/2020	2020-35	28	San Tin	Jade perch fry		Follow up			
28/07/2020	2020-13	28	San Tin	Tilapia		Follow up			
29/07/2020	2020-28	42	San Tin	Mullet		Water quality trial			
29/07/2020	2020-51	54	Ma Tso Lung	Mullet	<u>2020-51</u>	Mortality	Correct water quality issue		
31/07/2020	2020-52	55	Ma Tso Lung	Mullet	<u>2020-52</u>	Mortality	Correct water quality issue		
31/07/2020	2020-53	26	Lamma Island	Hybrid grouper	2020-53	Dactylogyrus spp	Praziquantel treatment	35 mg/kg/day*3 days	RX C2020-09
8/3/2020	2020-27	16	Mai Po	Mullet		Water quality trial			
8/3/2020	2020-47	33	San Tin	Jade perch		Follow up			
8/3/2020	2020-54	56	San Tin	Mullet	<u>2020-54</u>	Water quality check	No		
8/4/2020	2020-45	46	Yuen Long	Mullet		Follow up			
8/4/2020	2020-50	13	Yuen Long	Jade perch		Follow up			
8/4/2020	2020-12	28	San Tin	Jade perch		Follow up			
8/4/2020	2020-35	28	San Tin	Jade perch		Follow up			
8/4/2020	2020-13	28	San Tin	Tilapia		Follow up			
8/5/2020	2020-28	42	San Tin	Mullet		Water quality trial			
8/5/2020	2020-51	54	Ma Tso Lung	Mullet		Follow up			
8/7/2020	2020-55	57	Mai Po	Tilapia, grey mullet, bighead	2020-55	Water quality check	On site water quality measurement revealed water		
8/7/2020	2020-56	58	Ngau Tam Mei	Tilapia, grey mullet and jade perch	<u>2020-56</u>	Water quality check	Reduce feed, Water exchange, Increase aeration		
8/7/2020	2020-57	59	Ngau Tam Mei	Tilapia		Water quality check	Water exchange,Reduce feeding,Removal of bottom waste and excess feed from bottom of the pond		
8/10/2020	2020-27	16	Mai Po	Mullet		Water quality trial			
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8/10/2020	2020-47	33	San Tin	Jade perch		Follow up			
8/11/2020	2020-45	46	Yuen Long	Mullet		Follow up			
8/11/2020	2020-58	13	Yuen Long	Jade perch fry	<u>2020-58</u>	Follow up			
8/11/2020	2020-12	28	San Tin	Jade perch		Follow up			
8/11/2020	2020-35	28	San Tin	Jade perch fry		Follow up			
8/11/2020	2020-13	28	San Tin	Tilapia		Follow up			
8/12/2020	2020-28	42	San Tin	Mullet		water quality trial			
8/12/2020	2020-59	60	Yuen Long	Jade perch	<u>2020-59</u>	Water quality check	Increase aeration, reduce feeding and removal bottom waste / remain feed		
17/08/2020	2020-27	16	Mai Po	Mullet		Water quality trial			
17/08/2020	2020-47	33	San Tin	Jade perch		Follow up			
17/08/2020	2020-60	33	San Tin	Shrimp		Water quality check	Prepare pumps to introduce ST050 pond water to help increase water levels at ST053.		
18/08/2020	2020-45	46	Yuen Long	Mullet		Follow up			
18/08/2020	2020-58	13	Yuen Long	Jade perch fry		Follow up			
18/08/2020	2020-12	28	San Tin	Jade perch		Follow up			
18/08/2020	2020-35	28	San Tin	Jade perch fry		Follow up			
18/08/2020	2020-13	28	San Tin	Tilapia		Follow up			
21/08/2020	2020-28	42	San Tin	Mullet		Water quality trial			
21/08/2020	2020-61	17	Mai Po	Mullet	<u>2020-61</u>	Mortality	 Reduce feed Prompt removal of obvious dead fish Increase aeration 10:00 to 16:00 hours and 20:00 hours to 08:00 hours of the following day 		
24/08/2020	2020-27	16	Mai Po	Mullet		Water quality trial			
25/08/2020	2020-12	28	San Tin	Jade perch		Follow up			
25/08/2020	2020-35	28	San Tin	Jade perch fry		Follow up			
25/08/2020	2020-13	28	San Tin	Tilapia		Follow up			
25/08/2020	2020-47	33	San Tin	Jade perch		Follow up			
25/08/2020	2020-60	33	San Tin	Shrimp		Follow up			
26/08/2020	2020-28	42	San Tin	Mullet		Water quality trial			

26/08/2020	2020-62	13	Yuen Long	Jade perch fry	<u>2020-62</u>	Fish heath check			
28/08/2020	2020-47	33	San Tin	Jade perch		Follow up			
28/08/2020	2020-63	23	Tin Shui Wai	Mable Goby	2020-63	Water quality check	On site water quality measurement revealed water		
31/08/2020	2020-27	16	Mai Po	Mullet		Water quality trial			
31/08/2020	2020-64	6	Lau Fau Shan	Grouper	<u>2020-64</u>	Lesion			
9/1/2020	2020-47	33	San Tin	Jade perch		Follow up			
9/1/2020	2020-60	33	San Tin	Shrimp		Follow up			
9/1/2020	2020-65	61	Yuen Long	Mullet		Mortality	Commended farmer for monitoring pH . Advise to continue to do so esp with any weathr change) upcoming rain) Aerator to be turned on more in 11a. Unable to explain acidity in the NSW12 pond. IN face it is the NSW 12 b (further pond) that is more acidic, not the near pond. Farmer to call us for any adverse findings and advised to monitor pH and DO as frequently as he		
9/2/2020	2020-28	42	San Tin	Mullet		Water quality trial	(2)		
9/2/2020	2020-66	13	Yuen Long	Jade perch fry		Fish heath check			
9/7/2020	2020-27	16	Mai Po	Mullet		Water quality trial			
9/7/2020	2020-47	33	San Tin	Jade perch		Follow up			
9/7/2020	2020-60	33	San Tin	Shrimp		Follow up			
9/8/2020	2020-12	28	San Tin	Jade perch		Follow up			
9/8/2020	2020-35	28	San Tin	Jade perch fry		Follow up			
9/8/2020	2020-13	28	San Tin	Tilapia		Follow up			
9/8/2020	2020-66	13	Yuen Long	Jade perch fry		Follow up			
9/8/2020	2020-28	42	San Tin	Mullet		Water quality trial			
9/9/2020	2020-67	33	San Tin	Shrimp		Water quality check			
9/9/2020	2020-68	56	San Tin	Mullet		Water quality check			
9/9/2020	2020-65	61	Yuen Long	Mullet		Follow up			
9/11/2020	2020-58	13	Yuen Long	Jade perch fry		Follow up	Florfenicol treatment	35 mg/kg/day*7 days	RX C2020-10
14/09/2020	2020-27	16	Mai Po	Mullet		Water quality trial			
14/09/2020	2020-58	13	Yuen Long	Jade perch fry	<u>2020-58</u>	Follow up	Oxytetracycline treatment	50 mg/kg/day*10 days	RX C2020-11*3
15/09/2020	2020-12	28	San Tin	Jade perch		Follow up			
						•	· · · · · · · · · · · · · · · · · · ·		

15/09/2020	2020-35	28	San Tin	Jade perch fry		Follow up			
15/09/2020	2020-13	28	San Tin	Tilapia		Follow up			
16/09/2020	2020-28	42	San Tin	Mullet		Water quality trial			
16/09/2020	2020-60	33	San Tin	Shrimp		Follow up			
16/09/2020	2020-69	33	San Tin	Jade perch		Water quality check	 Application of lime Increase aeration Normal feeding 		
16/09/2020	2020-65	61	Yuen Long	Mullet		Follow up			
18/09/2020	2020-70	62	Chai Wan	Jade perch	2020-70	Mortality	Stop feeding until we start medication tomorrow - Antibiotics in feed – Oxytetracycline (OTC 45%) 50 mg/kg sid x 7 days Drug dispensed after accounting for fish death: CF3- 270 fish x 26 g - 7 kg biomass (0.8g/day) CF1 - 37 fish x 120 g - 4.5 kg biomass (0.5g/day) Module B - 60 fish x 150 g - 9 kg biomass (1 g/day)	50 mg/kg/day*10 days	RX C2020-12a-c
21/09/2020	2020-27	16	Mai Po	Mullet		Water quality trial			
21/09/2020	2020-60	33	San Tin	Shrimp		Follow up			
21/09/2020	2020-69	33	San Tin	Jade perch		Follow up			
21/09/2020	2020-58	13	Yuen Long	Jade perch fry		Follow up			
22/09/2020	2020-70	62	Chai Wan	Jade perch		Follow up			
23/09/2020	2020-28	42	San Tin	Mullet		Water quality trial			
25/09/2020	2020-12	28	San Tin	Jade perch		Follow up			
25/09/2020	2020-35	28	San Tin	Jade perch fry		Follow up			
25/09/2020	2020-13	28	San Tin	Tilapia		Follow up			
28/09/2020	2020-58	13	Yuen Long	Jade perch fry		Follow up			
29/09/2020	2020-70	62	Chai Wan	Jade perch		Follow up			

30/09/2020	2020-71	16	Mai Po	Mullet	<u>2020-71</u>	Mortality	Water monitor by farmer. Owner may get more		
							equipment and he is getting probes.		
							Will follow up with lesson.talk on pH, TAN etc		
							important water parameters to monitor		
							Increase aeration advised.		
30/09/2020	2020-72	13	Yuen Long	Jade perch fry	<u>2020-72</u>	Fish heath check	Salinity elevation to 5-6ppt		
30/09/2020	2020-12	28	San Tin	Jade perch		Follow up			
30/09/2020	2020-35	28	San Tin	Jade perch fry		Follow up			
30/09/2020	2020-13	28	San Tin	Tilapia		Follow up			
10/5/2020	2020-70	62	Chai Wan	Jade perch	<u>2020-70</u>	Follow up			
10/6/2020	2020-12	28	San Tin	Jade perch	<u>2020-12</u>	Follow up			
10/6/2020	2020-35	28	San Tin	Jade perch fry	<u>2020-35</u>	Follow up			
10/6/2020	2020-13	28	San Tin	Tilapia	<u>2020-13</u>	Follow up			
10/6/2020	2020-72	13	Yuen Long	Jade perch fry		Follow up			
10/7/2020	2020-71	16	Mai Po	Mullet		Follow up			
10/9/2020	2020-65	61	Yuen Long	Mullet		Follow up			
10/12/2020	2020-12	28	San Tin	Jade perch		Follow up			
10/12/2020	2020-35	28	San Tin	Jade perch fry		Follow up			
10/12/2020	2020-13	28	San Tin	Tilapia		Follow up			
10/12/2020	2020-72	13	Yuen Long	Jade perch fry		Follow up			
14/10/2020	2020-65	61	Yuen Long	Mullet		Follow up			
15/10/2020	2020-73	3	Sai Kung	Grouper	2020-73	Lesion in the mouth -Sea lice	Formalin Bath		
19/10/2020	2020-70	62	Chai Wan	Jade perch		Follow up			
20/10/2020	2020-65	61	Yuen Long	Mullet		Follow up			
21/10/2020	2020-73	3	Sai Kung	Grouper	<u>2020-73</u>	Follow up			
21/10/2020	2020-72	13	Yuen Long	Jade perch fry		Follow up			
23/10/2020	2020-35	28	San Tin	Jade perch fry		Follow up			
23/10/2020	2020-13	28	San Tin	Tilapia		Follow up			
27/10/2020	2020-72	13	Yuen Long	Jade perch fry	<u>2020-72</u>	Follow up	Oxytetracycline treatment	50 mg/kg/day*7 days	RX C2020-13
27/10/2020	2020-60	33	San Tin	Shrimp		Follow up			
27/10/2020	2020-69	33	San Tin	Jade perch		Follow up			
30/10/2020	2020-74	63	Fanling	Tilapia and Jade perch	<u>2020-74</u>	Water quality check			

11/2/2020	2020-72	13	Yuen Long	Jade perch fry	<u>2020-72</u>	2 Follow up Oxytetracycline treatment 10		100 mg/kg/day*7 days	RX C2020-13b
11/6/2020	2020-75	64	Yuen Long	Jade perch	<u>2020-75</u>	Water quality check	1) Reduce stocking density2) Water exchange		
11/9/2020	2020-76	26	Lamma Island	Hybrid grouper	2020-76	Skin lesion	 I)Increase water exchange rate within the nursery system 2)Maintain current feeding rate at 2.5% for another 5-7 days until fish conditions are mostly normal and minimal morbidity and mortality. 3)Removal of sick and moribund fish 4)Future cohort importation can consider acquiring smaller size fry (2-3g size) for easier acclimatization 		
11/10/2020	2020-65	61	Yuen Long	Mullet		Mortality	Advise when weather is ok,(raining these few days), consider moving fish from near side 12.		
11/10/2020	2020-69	33	San Tin	Jade perch		Follow up			
11/11/2020	2020-35	28	San Tin	Jade perch fry		Follow up			
11/11/2020	2020-13	28	San Tin	Tilapia		Follow up			
13/11/2020	2020-65	61	Yuen Long	Mullet		Follow up			
13/11/2020	2020-69	33	San Tin	Jade perch		Follow up			
18/11/2020	2020-65	61	Yuen Long	Mullet		Follow up			
18/11/2020	2020-69	33	San Tin	Jade perch		Follow up			
20/11/2020	2020-65	61	Yuen Long	Mullet		Follow up			
20/11/2020	2020-72	13	Yuen Long	Jade perch fry	<u>2020-72</u>	Mortality			1
23/11/2020	2020-65	61	Yuen Long	Mullet		Follow up			
23/11/2020	2020-40	48	Tai Po	Jade perch		Water quality check			
24/11/2020	2020-72	13	Yuen Long	Jade perch fry	2020-72	Follow up	Oxytetracycline treatment	100 mg/kg/day*7 days	RX C2020-14
25/11/2020	2020-77	26	Lamma Island	Hybrid grouper	2020-77	Skin lesion	Stop feeding		
9/12/2020	2020-78	13	Yuen Long	Jade perch		Check RAS			

Appendix D

List of Clients

Client name	General location	Contact number Type of syste				
AFCD	Au Tau		Pond			
	Chai Wan		RAS(fresh water)			
	Cheung Sha Wan		Marine			
	Cheung Sha Wan		Marine			
	Cheung Sha Wan		Marine			
	Fanling		Pond			
	Fanling		Pond			
	Fanling		Pond			
	Hok Tau / Fanling		RAS(fresh water)			
	Kau Sai Wan		Marine			
	Kowloon Tong		RAS(fresh water)			
	Kowloon Tong		RAS			
	Lamma Island		Marine			
	Lamma Island		Marine			
	Lamma Island		Marine			
	Lau Fau Shan		RAS(salt water)			
	Lau Fau Shan		Pond			
	Ma Tso Lung		Pond			
	Ma Tso Lung		Pond			
	Mai Po		Pond			
	Mai Po		Pond			
	Mai Po		Pond			
	Mai Po		Pond			
	Mai Po	_	RAS(fresh water)			
	Mui Wo		RAS(fresh water)			
	Nam Sang Wai		Pond			
	Nam Sang Wai		Pond			
	Ngau Tam Mei		Pond			
	Ngau Tam Mei	-	Pond			
	Pui O		Pond			
	Sai Kung		Marine			
	Sai Kung		Marine			
	Sam Mun Tsai		Marine			
	Sam Mun Tsai		Marine			
	San Tin		Pond			
	San Tin		Pond			
	San Tin		Pond			
	San Tin		Pond			
	San Tin		Pond			
	San Tin		Pond			
	San Tin		Pond			
	San Tin		Pond			
	Sandy Ridge		Pond			
	So Kwun Wat		RAS(fresh water)			

Tai Po	Marine
Tai Po	Marine
Tai Po	Marine
Tai Po	RAS(fresh water)
Tai Po	Pond
Tai Sang Wai	Pond
Tai Sang Wai	Pond
Tai Tau Chau	Marine
Tai Tau Chau	Marine
Tai Tau Chau	Marine
Tin Shui Wai	RAS
Tung Long Island	Marine
Yim Tin Jai	Marine
Yuen Long	RAS(fresh water)
Yuen Long	 RAS(fresh water)
Yuen Long	RAS
Yuen Long	Pond
Yuen Long	RAS(fresh water)
Yuen Long	Pond
Yuen Long	Pond
Yuen Long	Pond
Yung Shue Au	Marine
Yung Shue Au	Marine

Appendix E

Thank you letter from client

Appendix E

18th October,2019

Mr. Lai Kin-ming Assistant Director (Fisheries) Agriculture, Fisheries and Conservation Department 5/F, Cheung Sha Wan Government Offices 303 Cheung Sha Wan Road Kowloon

Letter of Appreciation

Dear Mr. Lai,

We would like to take this opportunity to send our deep gratitude to Dr. Sophie and her team (of vet service) from the City University of Hong Kong. Their professional advises of preparation, cultivation, monitoring and medication are undoubtedly a big help to us to tackle the problems that we can never solve on our own. Their proactive actions and expertise deserve the merits for sure.

At the time we are growing our fish, we will have the Jade Perch fertilized eggs from Australia in mid-November. Bruce Sambell the father of Jade Perch who will be attending our very first time to experiment hatchery in Shipping Container (**MoVertical Farm - Moveable Vertical Farm**) that we would like to share this experience with interested growers and request the technical support and knowledge from your department (Au Tau office). On the one hand, it would help us to grasp the technique to handle fish eggs, and on the other hand it also helps to promote the local fishery industry as we can provide fish fry in early Spring and continue to supply throughout the season. So growers need not to wait for another season.

As a government department for fishery, we wish to have your practical supports for development which is good to the community.

We look forwards to hearing from you soon. Please feel free to contact me on email:

Thank you very much.



Hong Kong Aquaponic Holdings Limited. (Member of MoVertical Farm)

C.C. Professor Sophie ST-HILAIRE (ssthilai@cityu.edu.hk) Ms Ma (AFCD Office Au Tau) Appendix F

Emamectin Benzoate manuscript





Emamectin Benzoate Treatment of Hybrid Grouper Infected With Sea Lice in Hong Kong

Sophie St-Hilaire^{1*}, Tzu Hsuan Cheng¹, Stephen Chi Ho Chan¹, Chi Fai Leung¹, Ka Man Chan¹, Kwok Zu Lim¹, William Furtado¹ and Giana Bastos Gomes^{1,2}

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Sea lice (Copepoda: Caligidae) are ectoparasites which negatively impact marine aquaculture species around the world. There are a limited number of treatments licensed for use against sea lice in tropical and semi-tropical farmed fish species. Emamectin benzoate (EB) was an effective pharmaceutical drug against sea lice infestations in several salmon industries before resistance to the product developed. This drug has not been extensively tested in marine fish within Asia. The objective of this study was to determine whether this drug could be used to treat oral infections with sea lice in hybrid grouper (Mycteroperca tigris × Epinephelus lanceolatus) cultured in saltwater net-pen sites in Hong Kong. We observed an overall reduction in sea lice infections over time, starting on the last day of the treatment up to the end of our study (i.e., 14 days after the last EB treatment). We also observed a large variation in concentrations of EB in fish on the last day of the treatment, which provides an explanation for the variation in response to the treatment. It also suggests that distribution of the medication to fish in saltwater net-pens is difficult, especially when medication is hand-mixed in the feed and possibly unevenly distributed in the daily rations. Overall, this study provides preliminary evidence that EB could be used to treat sea lice found in Hong Kong and potentially in other regions of SE Asia.

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St-Hilaire S, Cheng TH, Chan SCH, Leung CF, Chan KM, Lim KZ, Furtado W and Bastos Gomes G (2021) Emamectin Benzoate Treatment of Hybrid Grouper Infected With Sea Lice in Hong Kong. Front. Vet. Sci. 8:646652. doi: 10.3389/fvets.2021.646652 Keywords: grouper aquaculture, copepodid parasites, Caligus, parasite control, emamectin benzoate

INTRODUCTION

Sea lice are copeped parasites of economically important marine finfish found around the world. Some sea lice species affect only certain species of fish (i.e., *Lepeophtheirus salmonis*), while others, such as those belonging to the genus *Caligus*, are known to be more generalists (1, 2). *Caligus* species can live in a wide range of environments (1, 2). The larval stages of *Caligus* species can survive up to 8 days without food in temperatures ranging from 19 to 26° C (tropical and semi-tropical regions) in both marine and brackish waters (1, 2). In Asia, several species of *Caligus* sea lice, including *Caligus epidemicus*, *C. chiastos*, *C. punctatus*, *C. multispinosus*, and *C. rotundigenitalis* have been reported to infect farmed grouper (3–5).

In Hong Kong, which has a family-based marine aquaculture industry that produces \sim 1,000 tons of fish per year (https://www.gov.hk/en/about/abouthk/factsheets/docs/agriculture.pdf) there have been anecdotal reports of unknown species of sea lice affecting farmed hybrid grouper

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(*Mycteroperca tigris* \times *Epinephelus lanceolatus*) for several years. Unlike the *Caligus* spp. that infect salmon, the sea lice found in Hong Kong are smaller (i.e., 2–4 mm as adults) and they are predominantly localized in the oral cavity of the grouper (**Figure 1**). Infections caused by the sea lice species found in Hong Kong can be severe, cause fish to go offfeed, and lead to mortality (A. Leung, Agriculture, Fisheries and Conservation Department Hong Kong, 2020, Personal Communication). Formalin baths have been used to control this parasite in saltwater net-pens in Hong Kong on a small scale

(A. Leung, Agriculture, Fisheries and Conservation Department Hong Kong, 2020, Personal Communication). This product is a carcinogen so these treatments are difficult to administer, and like most bath treatments the effect is not long lasting. Further, because the sea lice in grouper from Hong Kong are located inside the oral cavity, bath treatments are not always as effective as in the salmon industry, where sea lice species are predominantly found on the external surface of fish.

Systemic treatments with a long residual effect, such as with emamectin benzoate (EB), and Teflubenzuron, are not widely



FIGURE 1 | Examples of the range of sea lice infections observed on farms during the emamectin trial. Fish (A1–A3) were considered moderate to severe infections (category 2). Fish (B1–B3) were considered mild infections (category 1). Fish (C1–C3) were considered fish that were not infected or recovered from infection. The black arrow (on fish A1) illustrates a chronic lesion associated with sea lice. The red arrow (on fish B3) illustrates sea lice clusters (newer infections) with no tissue response. The blue arrow (on fish C1) illustrates tissue response in fish which was no longer infected with sea lice (post-treatment).

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used to treat sea lice in Hong Kong, despite their frequent use in many salmon industries around the world (6-9). Orally fed EB is distributed in different tissues including the mucus of the fish (10, 11). The drug is taken up by sea lice when it ingests mucus and blood from treated fish. The product disrupts chloride ion movement in nerve cells by blocking glutamategated (GluCl) and y-aminobutyric acid (GABA)-gated (GABA-Cl) chloride channels, which eventually results in the death of the sea lice (12, 13). When EB was first introduced on the market several decades ago the product was very effective against sea lice (Caligus spp. and L. salmonis) infecting salmon; however, in the last 10 years there have been reports of resistance to EB in most salmon industries around the world (14, 15). The objective of this study was to assess the efficacy of EB on local sea lice parasites infecting the oral cavity of hybrid grouper in Hong Kong saltwater net-pen sites. We report results of sea lice treatments on five independently managed farms in bays surrounding Hong Kong.

MATERIALS AND METHODS

Farm Selection

Hybrid grouper farms around several fish farming regions in Hong Kong affected by sea lice were identified through the diagnostic services of the City University of Hong Kong aquatic animal veterinary service. Farmers were asked to participate in an EB field trial. Farmers participating in our study had between 3 and 10 small (3m by 3m by 3m) net-pens of hybrid grouper. The criteria for inclusion in this study were: (1) that fish had sea lice infections (i.e., microscopic evidence of copepodid parasites in the oral cavity) and no bacteria or other parasitic pathogens as determined by our initial diagnostic work-up; (2) fish were not to be harvested for 21 days post treatment; (3) farms had no crustacean aquaculture species on the site with the hybrid grouper; (4) fish were still eating; and (5) the farmer was willing to withhold at least 30 untreated fish in a separate pen as controls for comparison up to 21 days from the start of the treatment. Our initial diagnostic work-up consisted of a gross necropsy, wet mount scraping of the oral cavity lesions and the gills, and a bacterial culture from the kidney of the fish on blood agar, marine agar (2% salt), and tryptic soy Agar. Gills were examined for evidence of other parasites (i.e., ciliated protozoans, monogeneans, etc) and the kidney bacterial culture was to verify that fish did not have co-infections with bacterial pathogens.

Field Trial Emamectin Benzoate (EB)

Once a farmer agreed to participate in our trial and met the inclusion criteria, five fish were collected, using a dip net (outside their regular feeding period) prior to the start of the administration of EB for a baseline sample (day 0 of experiment). Fish were euthanized with an Ikigun (Auckland, New Zealand), weighed, and externally examined for the presence of sea lice. We used a 3-point photographic scale to qualitatively categorize the severity of sea lice infestation (**Figure 1**) because the sea lice infecting grouper were too small to grossly quantify numerically (adults were between 2 and 4 mm) (**Figure 2**). The tissue reaction associated with the sea lice infections also obscured the parasites



FIGURE 2 | Photo of sea lice found on hybrid grouper during our study period.

making it difficult to quantify (**Figure 1**). A fish was considered to have a category 1 level infection if there were three or fewer small (<1 cm) lesions with parasites in its oral cavity. A fish was considered to have a category 2 infection if there were more than three small lesions, or if the lesions in its oral cavity were larger than 1 cm in diameter.

Once a farm was confirmed to only have sea lice infections and no comorbidities (i.e., other parasites and or bacterial infections), we prescribed SLICE[®] (Merck & Co., Inc., Kenilworth, NJ, USA.) (0.2% EB) at the dose recommended for salmon: 50 μ g EB/kg for 7 days. We monitored one pen of treated fish and one pen of untreated fish on each farm included in our study. The control fish (untreated) were usually from the same pen and were maintained in a small enclosure close to the treated animals. The number of fish in the treated pens across five farms ranged between 750 and 1,350, and the weight of the fish included in our study ranged from 0.2 to 2 kg depending on the farm. The untreated pens contained between 30 and 100 fish. The treated fish on each farm were only compared to the control fish on the same farm to control for exposure level. After 21 days post treatment, we offered to treat all control animals with EB.

We calculated the inclusion rate of the medication in the feed based on a 1% feeding rate, the number of fish being treated, and their weight. The EB medication (SLICE[®]) was

hand-mixed in the existing commercial pelleted feed used by the farmer and top coated with canola oil. The veterinarians involved in the study mixed the 1st day of medication with the farmer to demonstrate the procedure. Farmers were asked to keep records of fish mortality in the treated and control fish groups. Water temperature and salinity were recorded on the days of fish sampling.

We randomly sampled five treated fish and five untreated fish on the 7th day of the treatment, and on days 14 and 21. All fish were collected using a dip net (outside of their regular feeding time). We used feed pellets to attract the fish population to the surface to collect our sample. We photographed the oral cavity of all fish and ranked their infection based on a 3-point scale (Figure 1). We calculated the proportion of treated and untreated fish with light infections (category 1) and with moderate to severe (category 2) infections at each sampling time point. Due to limited amount of fish with moderate infections (i.e., there were only 49 category two fish out of 189 fish sampled from five farms), we dichotomized the infection data (combined category 1 and 2) to statistically compare whether the proportions of fish with any infections differed between treated and untreated groups at different time points separately. We assessed whether these proportions differed significantly using a mixed effect logistic regression model with treatment as the fixed effect, and farm as the random effect. All analyses were conducted in STATA 15.0 (Stata Corp LLC, College Station TX USA).

Emamectin Benzoate (EB) Concentration Test

Muscle and skin tissues were collected from the same fish sampled for sea lice assessment on days 0, 7, and 21, on four out of the five farms¹ for EB analysis using liquid chromatography with tandem mass spectrometry (LC-MS-MS) as described in Lehotay (16). The samples were submitted frozen at -80° C to a commercial laboratory (Chemical Testing Services, Faculty of Science, Hong Kong Baptist University, Hong Kong) for analysis. On the days when we sampled for LC-MS-MS we euthanized the fish with an Ikigun (Auckland, New Zealand). Tissue samples were frozen at -20° C and tested for EB at the end of the study. On the two last farms included in this study, we also sampled five treated fish on day 28 after the start of the treatment for EB tissue concentration in case the residue period was longer than expected. We graphically illustrate the EB tissue concentration in treated fish over time.

RESULTS

Water temperature during the treatments on the five study sites ranged from a minimum of 19.7°C to a maximum of 29.1°C (**Figure 3**). Salinity was always above 30‰ on three farms (#2, 4, and 5) and fluctuated between 21 and 32‰ on the other two sites (#1 and 3) (**Figure 3**). Only fish farms with confirmed sea lice infections in the oral cavity and no other pathogens on our



initial health check were included in our trial. Farmers did not report any fish mortality during the trial period.

The proportion of EB treated fish with parasites declined over time on all 5 farms (**Figure 4**). All fish sampled on farms had some level of infection with sea lice prior to starting the treatment. Twenty-one days into the trial the proportion of infected fish on farms that had received SLICE[®], with the exception of the fish on farm 3, was below 40%. Two farms (farm 1 and 2) also had a decline in the proportion of infected fish not treated with EB (**Figure 4**). There were some treated groups that had better overall responses to the medication relative to their untreated counterparts (i.e., farms 1, 2, and 5; **Figure 4**). Overall, the proportion of fish infected with sea lice (category 1 and 2) was statistically lower in the treated groups of fish compared to the non-treated groups of fish, controlling for farm effect, on all days sampled (**Table 1**); however, the most significant difference was observed on the last day of sampling (day 21) (**Table 1**).

The concentration of EB in tissues was below the detectable limit of the LC-MS-MS analysis [2 parts per billion (ppb)] at the start of the trials, and in all fish not treated with $SLICE^{\mathbb{R}}$ throughout the duration of the study, with the exception of one farm (#1) where the average EB concentration in the control fish

¹One farm (farm 2) did not want us to conduct lethal sampling, so we did not submit samples for LC-MS-MS evaluation.



on day 7 was 3.54 ppb (SE mean 0.578). There was a wide range of tissue concentrations on the last day of the treatment (day 7: <2 to 110.4 ppb). The range appeared to cluster by farms, with one farm appearing not to have any of its sampled fish with levels of EB above 2 parts per billion (mg/kg) (Farm #3) (**Figure 5**). All

EB tissue concentrations were below 11 ppb in samples collected 21 days after the start of the first treatment or 14 days post treatment (**Figure 5**), and below 4 ppb (average 3.8, SE mean = 1.2) 21 days after the last treatment (i.e., 28 days from the start of the trials).

DISCUSSION

Our study identified an alternative treatment to bath chemotherapeutants for sea lice in hybrid grouper in Hong Kong. On the five farms in our study, the typical sea lice infections in hybrid grouper were reduced over time with the application of 7 days of $SLICE^{(R)}$, a premixed product containing EB (12), at

TABLE 1 | Summary of mixed effect logistic regression models for day 7, 14, and 21 of our Emamectin benzoate trials.

Term	Coefficient	SE	P-value
DAY 7 MIXED EFFE	ECT LOGISTIC I	REGRESSION	MODEL
Fixed effect			
Treatment	-2.286	1.137	0.044
Random effect			
Farm	0.216	0.785	0.3715
DAY 14 MIXED EFF	ECT LOGISTIC	REGRESSIO	N MODEL
Fixed effect			
Treatment	-2.016	0.955	0.035
Random effect			
Farm	1.970	2.709	0.044
DAY 21 MIXED EFF	ECT LOGISTIC	REGRESSIO	N MODEL
Fixed effect			
Treatment	-2.538	0.845	0.003
Random effect			
Farm	1.540	1.613	0.016

Farms were included as a random effect in the models. The comparison of the treatment was between control and treatment groups. Five fish were included in all samples with the exception of one sampling where two fish photographs were missing. the recommended dose for salmonids (50 μ g/kg/per day). The response to EB may take some time to occur as seen on farms 2 and 4 (**Figure 4**). Despite the fact that the level of EB in tissues of treated fish declined rapidly once the treatment ended (**Figure 5**) the effect of EB on the severity of infection continued to improve. This delayed response has been described in salmonids with *L. salmonis* and *C. rogercresseyi* (12), and is attributed to the mechanism of action of EB on the parasites' nervous system (12).

The response to treatment observed in this study varied by farm and was not as pronounced as what has been described in the early use of this product in the salmon industry (12, 17). Further, we observed sea lice in the oral cavity of several fish post-treatment (**Figure 4**), so we are confident that the EB treatment used did not completely eliminate the sea lice in all the treated fish.

Our inability to treat sea lice on grouper with 100% effectiveness (i.e., a small proportion of fish on all farms except one still had active sea lice infections after treatment) is likely due to several factors. First, the concentration of EB in fish was less than expected, given the dose we used. In fact, on one farm (Farm #3), the fish we sampled had negligible EB concentrations in their tissues on the last day of the treatment (Figure 5), suggesting a failed treatment. In salmonids, the same dose used in this study would result in a large proportion of fish with EB tissue concentrations above 60 parts per billion on the final day of the treatment (day 7) (18). The difference in the concentrations of EB in the fish from this study and salmonids may be multifactorial, and potentially played a role in the efficacy of the treatment. First, grouper and salmonids are different species cultured in very different water temperatures. The latter would have an impact on the metabolism of EB. Further, sea lice infections in grouper from Hong Kong occur in the oral cavity, which may impact the



amount of medication consumed by the fish. Our records suggest a large proportion of the fish on farm 3 had moderate to severe infections prior to the treatment (50% of the fish sampled on day 0 and 7 had category 2 infections on our photographic scale; data not shown), which may explain why there was virtually no EB in the tissues of all the fish sampled on farm 3. It is also possible that the concentration of medication was not evenly distributed in the feed and this resulted in a wide variation of doses to the fish. The EB LC-MS-MS data showed a wide range of tissue concentrations at the end of the treatment both within and between farms (Figure 5) with overall EMB concentrations ranging between not detectable to 110 μ g/kg), suggesting that the medication may have been consumed unevenly by the fish or dispersed on the feed unevenly resulting in the same uneven dosing effect. The actual concentration of drug administered to the fish (50 μ g/kg) relative to the amount of feed was very small, so it was challenging to obtain a homogeneous distribution on the feed. We tried to standardize the protocol by mixing the feed for the farmers, but the size and the oil content of the pellets used on different farms varied and may have impacted the distribution of the medication on the feed during the hand mixing process. In the salmonid industry, all EB treatments would be incorporated into the feed at the feed mills, so the concentration of the medication delivered to the fish would be more consistent.

It should be noted that our sampling of fish may have biased our estimates of EB concentration in fish. We had to hand net fish for our samples, so we may have collected fish that were not feeding and easier to catch at the surface, rather than the healthy population of fish that typically swim deeper in the water column and are more difficult to dip net. We did throw feed in the net-pens to try to reduce this bias, but we noted that the fish swimming rapidly in the net-pen were difficult to catch. Because we potentially sampled fish that did not feed as much as others, we may have underestimated the EB concentration in the population. This underestimation could explain some of the variation on EB concentration in tissue. Further, we only sampled five fish at each time point due to financial constraints. As participants in our study were small farms and infected fish were always over 1 kg with the exception of farm 1, it was cost prohibitive to sample more fish at each time point. This fact limited the accuracy of our population estimates.

In addition to influencing our estimate of EB in fish, our sampling strategy may also have biased our estimate of sea lice on fish. This bias would have occurred in both the control and treated fish groups, and it may have biased our results toward the null (under estimation of the effect). Despite the potential sampling bias, which is inherent in field trials on small farms, all farmers in our trial agreed (personal communication) that the treatment improved grouper feeding response and were pleased with the product, despite the persistence of the mild sea lice infections in the oral cavity of some fish (**Figure 5**). In fact, we only had mild infection (**Figure 1**) in fish 1-week post treatment (day 14) except on farm 3, which still had severe infections after the EB treatment. Farm 3 was also the farm where fish had limited EB in their tissues even on the last day of treatment.

Another explanation for the lower response rate to EB observed in this study, relative to the initial reports of the effect of EB on salmon lice, is that the lice are resistant to the product. Although we were not able to measure EB resistance in our study, this hypothesis is unlikely, given that to the best of our knowledge EB has not been used by the Hong Kong fish farming community. If farmers begin to use this product and are unable to properly mix the medicated feed to deliver an accurate therapeutic dose to their fish, they may observe resistance within a few years, as has been observed in many salmon farming areas around the world (7, 8, 15, 19). Having access to a local feed mill would help reduce the risk of uneven distribution of mediation within the feed and likely improve treatment delivery.

On two of the five participating farms we observed a natural decline in sea lice infections in the control fish (Figure 4). This may have been due to environmental factors such as water temperature and salinity, which are known to impact sea lice survival (20, 21). The salinity measures on farms were all above 30‰, with a few exceptions on two farms (Figure 2). On Farm 3 we observed a salinity of 21‰ but it is unlikely that this led to a natural reduction in sea lice levels as this was the farm with some of the most severe infections. Both farms (#1 and #2), which had a natural decline in sea lice infections over time, had high salinity. Although there may have been some medication drift on farm #1, as the LC-MS-MS data suggested minimal levels of EB in the control fish on day 7, this level was very low and unlikely to be therapeutic. More likely the reduction in sea lice in the control group on these farms was associated with a decline in water temperature. These two farms reported the lowest water temperatures of the study sites (Figure 2) and the decline in sea lice paralleled the decline in water temperature. The reproductive rate of sea lice in salmon industries has been shown to be temperature dependent (20-22). It is possible that water temperature had an influence on the reproduction of the sea lice in this study. Despite the potential natural decline in sea lice on farms 1 and 2, the effect of EB was still apparent in the treatment groups (Figure 4).

One of the limitations of this study was that unlike sea lice species found on salmon, which are easy to enumerate, the species in Hong Kong, are smaller (adults are estimated to be between 2 and 4 mm in length **Figure 2**) and cluster with various life stages inside the mouth of the fish, therefore they are more difficult to count (**Figure 1**). To address this issue, we used a three-point qualitative scale for categorizing the severity of infections and most infected fish in this study had mild infections. This qualitative scale limited our ability to assess the precise reduction in parasite numbers post-treatment. Despite this limitation in counting individual sea lice in the mouth of the fish, we were still able to measure changes in sea lice clusters and lesions.

Within 2 weeks of the last day of treatment, the level of EB in tissues was below 10 ppb. This decline in drug concentrations was steeper than what is reported in salmonids (11, 23–25), and was likely due to the elevated water temperature in our study (range between 19.7 and 29.1°C). By day 28 of our experiment

[21 days after the last treatment the average levels of EB were 3.8 ppb (SE = 1.14)], which suggests the withdrawal period of 21 days recommended for SLICE[®] at a dose of 50 μ g EB/kg of fish per day for 7 days (12) could be used for grouper. The levels of EB in our fish tissue samples on day 21 were well below the 42 ppb maximum residue limit used in some countries (26).

In conclusion, EB appears to be effective for reducing sea lice in the oral cavity of hybrid grouper in Hong Kong. EB has potential to be used within the Asian grouper aquaculture industry with good results if protocols of administration are monitored closely. Response to treatment may be improved by refining the delivery of the product to fish and treating animals early in the infection process to ensure fish are still eating adequately to receive the proper dose of medication. Although we observed a decline in sea lice infestation on most farms after the use of SLICE[®], a small percentage of treated fish still had parasites on all but one farm. The specific dose required for complete elimination of sea lice needs further investigation. This is important because exposing parasites to subtherapeutic levels of EB could exacerbate the risk that resistance develops to this therapeutant (27). Lastly, the environmental impact of using SLICE on farms in Asia should be assessed as it has recently been demonstrated to reduce the abundance of crustaceans in the benthic environment around salmon farms in Scotland (28).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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ETHICS STATEMENT

The animal study was reviewed and approved by City University of Hong Kong Ethics Committee. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

SS-H was the project leader and wrote the first draft of the manuscript. TC, SC, KC, CL, and KL were the technical team who conducted the trails on the farms. WF was the graduate student who assisted with the writing of the manuscript and the data analysis. GBG assisted with the parasite identification and quantification. GBG contribute with article editing. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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AMR Table (excel spreadsheet soft copy only)

Appendix G Summary of antibiotic sensitivity tests on fish pathogens between June 2018 and December 2020

Client ID	Geographic al Region (culture zone for marine)	Bacterial species	Fish Species	Sample d tissue	Fresh or Salt water (+/-RAS)	Sample collection date	Erythro mycin	Florfeni col	Gentam icin	Kanamy cin	Lincomy cin	Neomyc in	Oxytetr acycline	Penicilli n	Spectin omycin	Oxolinic acid	Strepto mycin	Tetracy cline
13	Yuen Long	Aeromonas hydrophila	Jade Perch	Kidney	Fresh(RAS	01/08/19	I	S	S	S	R	S	R	R	S	R	S	NA
13	Yuen Long	Aeromonas hvdrophila	Jade Perch	Eve	Fresh(RAS	01/08/19		R	S	R	R	s	R	R	s	R	S	NA
12	Hok Tau/Fanl	Aeromonas spp	Tilapia	Skin	Fresh	02/08/19	S	S	S	S	R	S	R	S	S	S	S	NA
13	Yuen Long	Aeromonas spp	Jade Perch	Kidney	Fresh(RAS	07/08/19	R	S	S	S	R	S	R	R	S	R	S	NA
13	Yuen Long	Aeromonas spp	Jade Perch	Skin	Fresh(RAS	08/08/19	I.	S	I	I	R	1	S	R	S	R	R	NA
13	Yuen Long	Aeromonas spp	Jade Perch	Kidney	Fresh(RAS	08/08/19	S	S	S	-	R	S	S	R	S	R	R	NA
12	Hok Tau/Fanl	Aeromonas spp	Tilapia	Eye	Fresh	13/8/2019	R	S	S	S	R	S	S	S	S	S	S	NA
13	Yuen Long	Aeromonas spp	Jade Perch	Kidney	Fresh(RAS	26/8/2019	R	S	S	S	R	S	S	R	S	R	S	NA
22	Yuen Long	Aeromonas spp	Tilapia	Spleen	Fresh	27/9/2019	l I	S	S	S	R	S	S	R	S	S	R	NA
13	Yuen Long	Aeromonas hydrophila	Jade Perch	Kidney	Fresh(RAS	15/4/2020	R	S	S	S	R	S	S	R	S	S	S	NA
13	Yuen Long	Aeromonas hydrophila	Jade Perch	Kidney	Fresh(RAS	27/4/2020	R	R	S	S	R	s	R	R	R	R	R	NA
13	Yuen Long	Aeremonas hydrophila	Jade Perch	Kidney	Fresh(RAS	14/5/2020	S	s	S	S	R	S	s	R	S	S	S	NA
33	San Tin	jandaei	Jade Perch	Kidney	Fresh	27/7/2020	R	S	I	R	R	I	S	R	S	S	R	NA
54	Ma Tso Lung	hvdronhila	Grev Mulle	Ascitic fl	Fresh	29/7/2020	R	s	s	S	R	s	s	R	s	s	s	NA
54	Ma Tso Lung	Aeromonas sp	Grev Mulle	Kidnev	Fresh	29/7/2020	R	R	S	s	R	S	1	R	S	S	S	NA
		Aeromonas	0.0)	r ddino y		20/1/2020										-		
13	Yuen Long	caviae complex	Jade Perch	Kidney	Fresh(RAS	08/09/20	R	S	S	S	R	S	R	R	S	NA	NA	NA
60		Aeromonas																
02	Chai Wan	hydrophila	Jade Perch	kidney	Fresh(RAS	18/9/2020	R	S	S	S	R	NA	S	R	S	R	S	NA
62		Aeromonas																
02	Chai Wan	jandaei	Jade Perch	kidney	Fresh(RAS	18/9/2020	R	S	S	S	R	NA	S	R	S	R	S	NA
13	Yuen Long	Aeromonas sp	Jade Perch	Gills	Fresh(RAS	21/9/2020	NA	S	S	S	R	NA	S	R	S	R		NA
16	Mai Po	Aeromonas sp	Grey Mulle	Kidney	Fresn	30/9/2020	R	5	5	5	ĸ	NA	5	ĸ	5	ĸ	5	NA
16	Mai Po	hydrophila	Grey Mulle	Kidney	Fresh	07/10/20	R	S	S	I	R	S	S	R	S	R	S	NA
16	Mai Po	hvdrophila	Grev Mulle	Kidnev	Fresh	07/10/20	1.1	s	S	S	R	s	s	R	S	s	R	NA
13	Yuen Long	Aeromonas sp	Jade Perch	Ascitic fl	Fresh(RAS	20/11/202	R	R	S	S	R	S	R	R	S	R	R	NA
13	Yuen Long	Aeromonas sp	Jade Perch	Kidney	Fresh(RAS	24/11/202	S	S	S	S	R	S	S	R	S	S	S	NA
13	Yuen Long	Aeromonas sp	Jade Perch	Kidney	Fresh(RAS	24/11/202	S	S	S		R	S	R	R	S	R	S	NA
13	Yuen Long	Aeromonas sp	Jade Perch	Eye	Fresh(RAS	24/11/202	S	S	S	S	R	S	R	R	S	R	S	NA
12	Hok Tau/Fanl	Chryseobacteriu m spp	Tilapia	Kidney	Fresh	02/08/19	S	s	R	R	R	S	s	R	s	s	I	NA
12	Hok Tau/Fanl	Chryseobacteriu m spp	Tilapia	Skin	Fresh	02/08/19	R	s	S	S	R	S	s	R	S	S	R	NA
12	Hok Tau/Fanl	Edwardsiella tarda	Tilapia	Eye	Fresh	13/8/2019	R	S	S	S	R	S	s	S	S	s	S	NA
13	Yuen Long	Edwardsiella tarda	Jade Perch	Kidney	Fresh(RAS	26/8/2019	R	R	S	S	R	S	R	R	s	s	R	NA
33	San Tin	Edwardsiella tarda	Jade Perch	Kidnev	Fresh	27/7/2020	R	S	s	S	R		R	R	s	s	s	NA
16	Mai Po	Edwardsiella tarda	Grev Mulle	Kidnev	Fresh	30/9/2020		s	S	s	R	NA	s	R	S	s	S	NA
11	Mai Po	Enterobacter	Carp	Skin	Freeb	10/07/10	P	6	9	1	P	9	D	D	s	6	P	P
	Iviai FU	Photobacterium	Carp	SKIII	116311	10/07/19		5	5	-		5	IX	IX	5	5	IX III	IX.
3		damselae ssp							-	-								
	Yung Shue A	damselae	NA	Kidney	Salt	09/10/18	S	S	S	S	R	S	S	S	S	S	S	NA
5	V: T - T	Photobacterium damselae ssp			0	0.414/0040						_					_	N 10
	TIM IN ISAI	piscida Photobactorium	Hybrid Gro	ruaney	Sait	24/4/2019	ĸ	5	5		ĸ	5	R	R	5	5	ĸ	NA
		damsolao sen																
10	Yim Tin Tsai	damselae Photobacterium	Grouper	Flank (sł	Salt	27/6/2019	S	S	S	S	R	S	S	S	S	S	S	NA
6	l au Fau Shai	damselae ssp	Giant Grou	Kidnev	Salt(RAS)	17/2/2020	S	R	S	I	R	S	R	R	S	S	R	NΔ
13		Plesiomonas				00/0/2020		IX I	0		IX I	0		IX I				
62	r uen Long	spp Plesiomonas	Jade Perch	ridney	resh(RAS	26/8/2019		R	5	R	R	5	R	R	5	R	R	NA
	Chai Wan	shigelloides Staphylococcus	Jade Perch	Kidney	Fresh(RAS	18/9/2020	S	S	S	S	R	NA	S	R	S	S		NA
3	Yung Shue A	aureus	Hybrid Gro	Skin	Salt	16/10/201	S	S	S	S	R	S	S	R	S	R	S	NA

Client ID	Geographic al Region (culture zone for marine)	Bacterial species	Fish Species	Sample d tissue	Fresh or Salt water (+/-RAS)	Sample collection date	Erythro mycin	Florfeni col	Gentam icin	Kanamy cin	Lincomy cin	Neomyc in	Oxytetr acycline	Penicilli n	Spectin omycin	Oxolinic acid	Strepto mycin	Tetracy cline
3	Yung Shue A	Streptococcus iniae	Pompano	Kidney	Salt	05/10/18	R	S	S	R	S	R	S	S	S	R	R	NA
2	Viene Chies A	Streptococcus	Damagna	1/	0-14	05/40/40		<u> </u>			_			<u> </u>			_	NIA
3	Yung Shue A	Inlae	Pompano	Kidney	Salt	05/10/18	ĸ	5	5	ĸ	5	ĸ	5	5	5	ĸ	ĸ	NA
3	Yung Shue A	iniae	Pompano	Kidney	Salt	05/10/18	R	S	S	R	R	R	S	S	S	R	R	NA
3	Yung Shue A	Streptococcus iniae	Pompano	Kidney	Salt	05/10/18	R	S	S	R	S	R	S	S	S	R	R	NA
6	Lau Fau Shar	Vibrio vulnificus	Giant Grou	Kidney	Salt(RAS)	19/9/2018	S	S	S	s	R	R	R	S	S	s	S	S
6	Lau Fau Shar	Vibrio alginolyticus	Giant Grou	Skin	Salt(RAS)	19/9/2018	S	S	S	S	R	R	R	R	S	R	S	R
2	Yim Tin Tsai	Vibrio harveyi	Pompano	Kidney	Salt	05/10/18	R	S	S	I	R	S	R	R	S	R	S	NA
3	Yung Shue A	Vibrio harveyi	Hybrid Gro	Skin	Salt	16/10/201	S	S	S	1	R	S	S	R	S	R	S	NA
3	Yung Shue A	Vibrio harveyi	Pompano	NA	Salt	30/10/201	1	S	S	S	R	S	S	R	S	R	R	NA
3	Yung Shue A	Vibrio parahaemolytic us	Pompano	NA	Salt	30/10/201	S	I	S	I	R	S	R	R	S	R	R	NA
3	Vuna Shua A	alginolyticus	Pompano	ΝΔ	Salt	30/10/201		1	S		R	S	R	R	S	R	R	ΝΔ
4	Lo Tik Wan (Vibrio harvevi	Hybrid Gro	Kidnev	Salt	20/11/201	S	S	S	S	R	S	R	R	S	R	S	NA
4	Lo Tik Wan (Vibrio harvevi	Hybrid Gro	Kidnev	Salt	20/11/201	S	S	S	S	R	S	R	R	S	R	S	NA
5	Yim Tin Tsai	Vibrio harvevi	Hybrid Gro	Kidnev	Salt	29/11/201	R	S	S	R	R	S	R	R	S	R	S	NA
3	Yuna Shue A	Vibrio harvevi	Pompano	NA	Salt	18/12/201	R	S	S	S	R	S	S	R	S	R	R	NA
3	Yung Shue A	Vibrio harveyi	Pompano	NA	Salt	18/12/201	1	S	S	I	R	S	S	R	S	R	I	NA
3	Yung Shue A	Vibrio alginolyticus	Pompano	NA	Salt	18/12/201		s	S		R	s	S	R	s	R	I	NA
7	Yim Tin Tsai	Vibrio spp	Hvbrid Gro	Kidnev	Salt	28/2/2019	R	S	S	S	R	S	S	R	S	R	R	NA
7	Yim Tin Tsai	Vibrio harvevi	Hybrid Gro	Kidnev	Salt	05/03/19	S	NA	S	S	S	S	S	S	S	NA	S	NA
5	Yim Tin Tsai	Vibrio harvevi	Hybrid Gro	Kidnev	Salt	12/04/19	S	NA	S	S	R	R	S	R	S	NA	S	S
5	Yim Tin Tsai	Vibrio harvevi	Hvbrid Gro	Kidnev	Salt	12/04/19	S	NA	S	R	R	R	S	R	S	NA	S	S
6	Lau Fau Shar	Vibrio harveyi	Giant Grou	Kidney	Salt(RAS)	15/4/2019	R	S	S	S	R	R	R	R	S	S	R	R
3	Yung Shue A	Vibrio harveyi	Pompano	Kidney	Salt	15/4/2019	S	S	S	S	R	R	S	R	S	S	S	S
3	Yung Shue A	Vibrio spp	Pompano	Kidney	Salt	29/4/2019	1	S	S	R	R	S	I	R	S	1	R	NA
3	Yung Shue A	Vibrio harveyi	Pompano	Kidney	Salt	29/4/2019	S	S	S	I	R	S	S	R	S	S	R	NA
3	Yung Shue A	Vibrio harveyi	Pompano	Kidney	Salt	07/05/19	S	S	S	R	R	S	S	S	S	S	R	NA
3	Yung Shue A	Vibrio spp	Pompano	Kidney	Salt	07/05/19	1	S	S	R	R	S	1	R	S	S	R	NA
3	Yung Shue A	Vibrio harveyi	Pompano	Kidney	Salt	03/06/19	S	S	S	1	R	S	R	R	S	R	1	NA
19	Tai Tau Chau	Vibrio spp	Grouper	Tail (Sk	Salt	21/6/2019	1	S	S	S	R	S	S	R	S	S	S	NA
19	Tai Tau Chau	Vibrio alginolyticus	Grouper	Kidney	Salt	21/6/2019	I.	S	S	S	R	S	S	R	S	S	S	NA
6	Lau Fau Shar	Vibrio vulnificus	Giant Grou	Skin	Salt(RAS)	22/7/2019	R	R	S	I.	R	S	R	R	S	I.	R	NA
6	Lau Fau Shar	Vibrio alginolyticus	Giant Grou	Kidney	Salt(RAS)	22/7/2019	I	R	S	R	R	S	R	R	R	S	R	NA
6	Lau Fau Shar	Vibrio vulnificus	Giant Grou	Skin	Salt(RAS)	22/7/2019	R	S	s	1	R	s	R	S	s	s	R	NA
6	Lau Fau Shar	Vibrio harvevi	Giant Grou	Kidnev	Salt(RAS)	17/2/2020	S	S	S	S	R	S	R	R	S	S	R	NA
6	Lau Fau Shar	Vibrio vulnificus	Giant Grou	Kidney	Salt(RAS)	17/2/2020	S	S	S	S	R	s	R	R	S	S	R	NA
19	Tai Tau Chau	Vibrio	Hybird Gro	Kidney	Salt	28/4/2020	R	S	S	S	R	S	S	R	S	S	S	NΔ
4	l o Tik Wan (Vibrio harvevi	Hybrid Gro	Kidnev	Salt	31/7/2020	S	S	S	S	R	S	S	R	S	S	S	NA
6	Lau Fau Shar	Vibrio vulnificus	Giant Grou	Ascitic fl		31/8/2020			6				6	i,			6	
16	Mai Pa	Vibrio on	Grov Mullo	Kidnov	Sall(KAS)	07/10/20	R	5	5		R		<u> </u>	5	5	5	S	NA NA
4	Lo Tik Wan (Vibrio sp	Giant Grou	Kidnev	Salt	09/11/20	S	1	5	5	R	5	S	R	5	5	S	NΔ
4	L o Tik Wan (Vibrio sp	Giant Grou	Kidnev	Salt	25/11/202	S	S	S	1	R	S	R	R	S	S	R	NΔ
<u> </u>	1-2 Hour (-0, . 1, 202		-										

Appendix H

Summer Grey Mullet Investigation

Report on the Investigation of Grey Mullet (*Mugil cephalus*) "Summer Mortality" in Hong Kong

City University of Hong Kong, Aquaculture Ambulatory Team

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SUMMARY

An investigation into factors that may contribute to summer mortality of grey mullet (*Mugil cephalus*) in Hong Kong was conducted on four ponds from two farms between late May and September of 2020. Farmers reported mortality events, and various water parameters and rainfall patterns were examined weekly. We also noted management events reported by the farmers on a weekly basis. Each pond was unique, but many of the mortality events were preceded by management events or a cyclone. How these events resulted in mortality is not fully known. In some cases, the events may have led to water quality issues; however, the frequency of monitoring in our study was not sufficient to confirm cause and effect. Also, different events may have led to different water quality issues and there were some underlying issues with water quality that likely exacerbated the events (i.e. stressed fish).

In brief, during some of our sampling periods we detected low Dissolved oxygen, low pH, high total ammonia, high nitrite, and high water temperatures. We also observed some indications of stratification in ponds, which could be problematic under certain weather conditions (i.e. hot calm weather followed by cyclones or heavy winds). Although our study did not precisely pinpoint the event(s) that lead to mortality, we identified several issues that may have, in combination, led to fish mortality. We recommend that farmers monitor their water quality, especially before and after changes in management or hot weather events, to mitigate issues early and minimize losses. Interestingly, we also detected low levels of *Streptococcus* spp. throughout the summer, suggesting that fish may be able to tolerate these bacteria at low levels without mass mortality, and that other underlying events may be required to initiate significant mortality losses associated with these pathogens.

1. INTRODUCTION

Grey mullet (*Mugil cephalus*) has been a prominent species in Hong Kong aquaculture for decades, and the species has been successfully reared by open pond aquaculture system over the years using traditional semi-intensive methods. Mortality in summer has contributed to severe loss of stocks among the local farms in recent years. Agriculture, Fish and Conservation Department (AFCD) recorded up to 80% losses during mortality events on some farms between 2010 and 2019. Since the induction of the City University Aquaculture Ambulatory service, we have observed severe mortality of *M. cephalus* on multiple farms in the New Territory area during the 2018 and 2019 summer and early autumn periods.

Mass mortality events were usually acute in nature, with most deceased fish lacking external symptoms, although a small percentage of fish had visible signs such as exophthalmos, as well as diffused inflammation on the fin and body. Based on AFCD's records, they isolated *Streptococcus iniae* from the internal organs (liver, spleen and kidney) of symptomatic fish in a few mortality cases, indicating systemic bacterial infections and raising speculations that the cause of summer mortality events were of bacterial origin. Records from AFCD also indicated that water quality (i.e. high water temperatures were associated with several fish kills) may have preceded bacterial outbreaks in aquaculture production systems. The purpose of this study was to investigate summer mortality on mullet farms to determine the predisposing factors.

2. MATERIALS AND METHODS

We informed our fish clients that we were interested in investigating fish mortality events and to call us early in the disease process to improve the outcome. Further, we conducted detailed water quality monitoring on two farms with a history of summer mortality events in the New Territories, Hong Kong.

2.1 Pond selection and pond profile

Four ponds on two separate farms were selected for this study, based on farmers' willingness to cooperate and the occurrence of previous events of *M. cephalus* mortality during the summer months. Table 1 summarises the profiles of the four ponds:

Pond	1	2	3	4
¹ Pond Size (square metre)	15010	15451	10083	7373
¹ Depth of Pond (metre)	1.7-2.3	1.8-2.5	1.3-3.0	1.1-2.6
² Fish species and population at the start of study	Mugil cephalus(20000) ³ Ctenopharyngodon idella (ua) ³ Hypophthalmuchthys nobilis (ua) ³ Oreochromis spp (ua)	Mugil cephalus(20000) Ctenopharyngodon idella(ua) Hypopththalmichthys nobilis (ua) Oreochromis sp(ua)	Mugil cephalus (25000) ³ Scortum barcoo(30000) Oreochromis spp(ua) ***	Mugil cephalus (20000) Hypophthalmichthys nobilis (2000) Oreochromis spp (3000) ** 2400 kg total biomass of stock addition on 17 June 2020 of above species and Ctenopharyngodon idella
² Stocking rate (kg/square metre)	0.6	0.58	2.7	10
² Stock date	March 2019	March 2019	June 2019	June 2019
² Feed used	**** By-products and commercially formulated pellets	By-products and commercially formulated pellets	Pellets (formulated by farmer)	Pellets (formulated by farmer)
² Amount of feed/day (kg)	240	240	400	120
² Last drained and dredged	2018	2018	May 2019	May 2019
*Number of 3 Aerators		3 (2 more added on 9 Sep 2020)	5	1

Table 1. Stocking rate, species of fish, feeding practices and brief history of the four ponds included in the study Notes:

1. Information on pond size was provided by AFCD; pond depths were obtained from measurement during study period.

2. Information on stocking rates, species, feeding and husbandry was verbally provided by farmers.

- * With exceptions, usually only one aerator was switched on at a time, for 10-20 hours in total per day.
- ** Fish stock was increased in pond on mid-June 2020, harvest end of August 2020

*** Partial harvest early August 2020

**** By-products were processed human foods eg. bread, instant noodles

- ua population number unavailable
- 3. Ctenopharyngodon idella (Grass carp) Hypophthalmuchthys nobilis (Big-head carp)
 - Oreochromis spp (Nile or hybrid tilapia)
 - Scortum barcoo (Jade perch)

2.2 Water sampling and analysis

Four water samples, two from the surface and two close to the bottom of the pond at the periphery and the center of the pond, were collected once per week for analysis. Samples were collected between 10 am and 12 pm, at approximately seven day intervals, from the last week of May 2020 until the third week of September, 2020. The 4 water sampling sites on each pond were kept consistent throughout the study.

The dissolved oxygen (DO), water temperature, pH, total ammonia nitrogen (TAN), nitrite, carbonate hardness (KH), Secchi disc readings for the deep samples, and depth of pond were measured using a HACH SL1000 PPA system, a pH probe PHC20101 (for TAN, nitrite, KH, pH), a smart Sensor pH 818 pH meter (from May to Mid-August 2020), and a YSI Professional Optical Dissolved Oxygen (YSI ODO). Water samples were diluted five times before using the HACH kit, due to the low detection range of TAN for this system and the high levels in our ponds. Deep water samples from the centre of ponds were also sent to a HOKLAS (Hong Kong Laboratory Accreditation Scheme) accredited laboratory for heterotrophic bacterial plate count (HPC) and faecal *Streptococci* counts (FSC).

2.3 Records of events and recommendations to farmers during the investigation period

Husbandry changes, harvests, mortality, and morbidity events on the day of, or in the week preceding our visit and water sampling were recorded. These were based on the farm hand or farm owners observations. Whenever we detected unfavourable water quality parameters that might adversely affect fish health the farmers were informed and advised on corrective measures, to prevent or minimise fish mortality.

2.4 Weather records

Weather conditions and barometric pressure readings from the YSI were noted on sampling days. Rainfall data (i.e. cumulative seven-day rainfall preceding the visit) was extracted from the Hong Kong Observatory Website. A seven-day cumulative rainfall preceding each data collection day was calculated for each pond and plotted with various parameters and estimated mortality

3. RESULTS

We did not have any fish clients that called with mass mortality events in their mullet ponds during our study period so no investigations were performed. The following are the results of our intensive water quality surveillance on two farms.

3.1 Mortality, water quality, and events

Chronic low-grade mortality was detected across all four ponds, with the earliest detectable mortality in Pond 2 on July 6, 2020. Daily mortality rates were not recorded accurately, but were recorded based on anecdotal reports from the farm operators. In general, mortality was reported to be was less than 0.1% (around 10 fish per day). There were a few larger mortality events (between 30 and 40 fish per day) throughout the summer. The mortality and husbandry events are summarised in Table 2a (for Farm 1, Pond 1 and 2) and Table 2b (for Farm 2, Pond 3 and 4):

Date	Pond 1	Pond 2				
22 June	Pond fertilisation	Pond fertilisation				
		M2* <i>Mugil cephalus</i> and				
6 July	Receiving water from Pond 2	Hypophthalamuchthys nobilis; water				
		pumped into from pond 1				
12 July		M2 Mugil cephalus and				
15 July	-	Hypophthalamuchthys nobilis				
20 1010		M2 Mugil cephalus and				
20 July	-	Hypophthalamuchthys nobilis				
27 July	M1 Mugil cephalus	M1 Mugil cephalus				
3 August	M1 Mugil cephalus	M1 Mugil cephalus				
10 August	M1 Mugil cephalus	M2 Mugil cephalus				
17 August	M2 Mugil cephalus	-				
19 August	Tropical cyclone	Tropical cyclone				
24 August	M3 reported by farmer (no mention of	M2 various species				
24 August	species)					
31 August	-	M2 various species				
7 Sontombor	M2 Mugil conhalus	M3 mainly Hypophthalamuchthys nobilis, 2				
7 September	Wiz Wayn Cephalas	extra aerators added				
14	_	M2 mainly Hypophthalamuchthys pobilis				
September	-					
21	_	M1 various species, three aerators switched				
September	-	on				

Table 2a. Mortality and events in Pond 1 and 2 (information obtained verbally from farmers and approximate cursory counts)

Table 2b. Mortality and events in Pond 3 and 4 (information obtained verbally from farmers and approximate cursory counts)

Date	Pond 3	Pond 4
3 to 10 Jun	-	Farmer reported poor appetite in fish, water change (low pH detected)
17 June	-	2400 kg biomass of fish of various species added post-harvest, water pumped in
24 June	-	M4 Oreochromis sp
30 June	Added lime past week, Increase feed rate	Added lime past week, Increase feed rate
8 July	Partial harvest	-

15 July	Increase feed rate	Increase feed rate
29 July	M3 Mugil cephalus and Oreochromis sp	M2 Mugil cephalus, Hypophthalamuchthys
		nobilis and Oreochromis sp
5 Aug	M1 Mugil cephalus	M1 Mugil cephalus
12 August	M1 Mugil cephalus	M1 Mugil cephalus
19 August	Tropical cyclone	Tropical cyclone
26 August	-	M3 Hypophthalamuchthys nobilis. Post-
		harvest, stop production cycle
2 September		M3 Hypophthalamuchthys nobilis. Stop
		production
8 September	-	M2 Hypophthalamuchthys nobilis
23 September	M1 Mugil cephalus	M1 various species

Notes for table 2a and 2b: *Arbitrary mortality grade: M1 (very minor fish mortality, <10 fish) M2 (minor fish mortality, 10 to 30 fish) M3 (moderate fish mortality, 30 to 40 fish) M4 (moderate to significant fish mortality, >40) Bold and Italics – notable events

Mortality patterns differed between ponds, with Ponds 2 and 4 experiencing more mortality events, from June to September, whereas the mortality cluster of Pond 1 occurred in August (section 3.2 and 3.3). Pond 3 experienced mortality spikes in late July (corresponding to increased feeding rate) and mid-August, in association with a cyclone. The cyclone was associated with an increase in mortality in all pens. Pen 4 also had an increase in mortality associated with increased feeding rates.

Deceased fish reportedly exhibited minimal to no external symptoms, which is typical of acute mortality associated with water quality issues. Fish carcasses were usually bloated in appearance in various degree of decomposition (likely deceased for more than 24-36 hours before gas distension caused carcasses to float). No live fish or fresh dead fish were available for postmortem or sampling.

A brief episode of poor appetite (3-10 June) reported in Pond 4 corresponded with low pH, but these signs resolved with liming of the ponds. Almost all increases in mortality can be explained by events such as over stocking, over feeding, or tropical cyclones (fig 1).



Figure 1. Mortality, events, Oxygen and rainfall in four ponds.

Water Quality

3.2 Water quality and events

Mean water parameter values for the surface and deep samples for each pond were calculated weekly and graphed with mortality and rainfall (see section 3.3 for details on rainfall). Dissolved oxygen (DO), pH, total ammonia nitrogen (TAN), and unionized ammonia (UIA) were compared directly across four ponds. Weather events, fish mortality, and rainfall were plotted with various parameters to determine correlations.

Pond 1 appeared to have a higher mean DO (6.03mg/L) than the other three ponds (4.16 mg/L, 4.21 mg/L, 4.65 mg/L for Pond 2, 3 and 4 respectively) (fig. 2). DO gradually increased in Ponds 1, 2, and 3, while it decreased in Pond 4 over the course of the study. On three occasions the DO at 10 am was below 2 mg/L and the DO was only above 7 mg/L in one pond once. It is possible that the DO in ponds at certain times of the year was lower at sunrise as there were reports of heavy algal growth in some ponds.

Overall comment: Although DO may have been low on some days, as we measured it at 10 am, it did not seem low enough to result in mortality. Pond 4 may have been an exception. In this pond in mid-August the DO appeared to crash and they also reported relatively high mortality.



Figure 2. Mean Dissolved Oxygen (DO) comparison of four ponds

Water *temperature* in all ponds rose to over 30°C after mid-June, and this high water temperature was largely sustained until early September, with dips below 30°C in early to mid-August (3 August in Ponds 1 and 2, 5 August in Ponds 3 and 4) (Fig 3). The optimal temperature range for mullet is between 20 and 26°C. Mullet may be able to tolerate these temperatures if the water temperature is increased slowly, but the fish may still be stressed by these high water temperatures, especially if other water quality parameters are not optimal. It was difficult to interpret the interactions between water temperature and other water quality parameters in this study.



Figure 3. Water temperature, mortality, and rainfall in ponds 1,2,3, and 4.

The mean *pH* throughout the study period for Ponds 1, 2, 3, and 4 were 7.93, 7.75, 6.56, and 6.39 respectively (Fig. 4). The pH was stable in Ponds 1 and 2, falling within the range of 7 to 8.5. The pH of Ponds 3 and 4 tended toward acidity (< 6.9), with a sharp dip to nearly 5 in Pond 4 in mid-June. The farmer added lime on June 30th to stabilize the system. There was no correlation between pH and mortality or rainfall (fig.4).



Figure 4. The pH, mortality and rainfall comparisons in four ponds

It is important to recognize that measuring pH once per day does not provide a clear picture of the potential daily fluctuation that can happen with this parameter, and which can stress fish. We ran



Figure 5. pH over a 5 day interval at Au Tau Fisheries .

a probe at a different site, which measured pH continuously, and which highlights the issue that can occur when pond water is not well buffered and algal growth is high (i.e. high CO2 at night drives the pH down) (Fig5).

Water hardness is important for buffering the pH of ponds. In our study, the ponds with the lowest pH at 10 am were the ponds with the lowest KH (Fig. 6). The farmer of ponds 3 and 4 limed the ponds to

improve the buffering capacity and increase the pH on June 30th. This management appeared to have a slight effect on the KH two weeks after but the pH did not change significantly, especially in pond 3. It is important that mitigative strategies are monitored to ensure they are effective.

Total ammonia nitrogen (TAN) in Ponds 1 and 2 were between 0.8 to 1.8 mg/L, respectively. Ponds 3 and 4 had higher TAN, which hovered largely between 2 to 3 mg/L and peaked at 5 mg/L in both



Figure 6. KH and pH over time in four ponds.





ponds (Fig. 7). However, because the pH in ponds 3 and 4 was low, the unionized ammonia in these ponds was much lower than in ponds 1 and 2 (Fig. 8).

Unionized ammonia (UIA) was above 0.05mg/L (toxic level for some species) in May and early July in Ponds 1 and 2, and in Pond 2, UIA was sustained at 0.05 mg/L for the entire study (Fig.9). UIA never exceeded 0.05 mg/L (toxic levels) in Ponds 3 and 4 (Fig. 7). The start of the sustained, low-grade mortality in Pond 2 occurred two weeks after UIA peaked, and continued throughout the course of the study. This pond also had high mortality after July; however, several other events coincided with the mortality spikes. Pond 1 also had increase in mortality during the study, but did not have associated mortality when UIA was high in June; mortality only started in August, in fact, after the UIA decreased. There was a positive correlation between mortality and UIA in Ponds 3 and 4, but UIA in those ponds did not exceed toxic levels and thus, any correlation was unlikely meaningful.

The *nitrite* levels were very low in Pond 1 (< 0.1 mg/L) and Pond 2 (< 0.14 mg/L). However, in Ponds 3 and 4, earlier months saw nitrite rise to 1.2 mg/L in Pond 3 and > 2 mg/L in Pond 4 (Fig. 10). Nitrite in Pond 3 remained above 0.2 mg/L throughout the study period. Whether this was correlated with mortality is unknown, but biologically it could add a stressor for the fish.



Figure 8. TAN, pH, and unionized ammonia in four ponds.


Figure 9. Correlations between Total Ammonia Nitrogen (TAN), Mortality and Rainfall of the four ponds



Figure 10. Nitrite, Mortality and Rainfall of the four ponds of the four ponds

There were *heterotrophic plate count (HPC)* peaks in the earlier months of the study for Ponds 1 (May and June), 2 (June and July), and 4 (early June). These coincided with pond fertilization in Ponds 1 and 2 and an introduction of biomass in Pond 4. Pond 4 also experienced a small peak near the end of July after increasing feeding. For Pond 3, there was a peak in mid-July associated with increased feeding (Fig. 11). The HPC peaks appeared to precede mortality in Ponds 3 and 4 by two weeks. In Pond 2, the initial HPC peak preceded the mortality peak by two weeks, and then another, subsequent peak coincided with a period of sustained mortality; but when HPC declined,

eventually, mortality continued to persist. It is possible that increasing HPC is a direct result of events such as pond fertilization, increase feeding, and increasing the biomass, which subsequently resulted in fish mortality.



Figure 11. Heterotrophic Plate Count (HPC), Mortality and Rainfall of the four ponds

Faecal Streptococci count (FSC) was relatively high in Ponds 1 (>1000 cfu/ml) and 2 (>1000 cfu/ml) at the start of the study (Fig. 12). Interestingly, the fecal count went down after fertilization in these two ponds. The FSC in Pond 4 was also high (i.e. > 200 cfu/ml) at the start of the study, but subsequently fluctuated at levels below 50 cfu/ml. FSC was generally low in Pond 3 throughout the study (< 35 cfu/ml). These findings suggest that *Streptococcus* is present in the ponds without



Figure 12. Streptococcus spp. counts in different ponds.

significant mortality. It also suggests that the spikes in HPC were not necessarily *Streptococcus,* which could explain why they may not have corresponded to high fish mortalities.

3.2 Weather and rainfall in relation to mortality and water parameters

Overall, rainfall levels were markedly lower than in the previous two years (2018/2019). There was a peak of rainfall at the end of June followed by a dry July, and steadier rainfall which started in August (see figures in section 3.2).

The rainfall and temperature in 2020 differed significantly from the historical norms for the month of July. Hong Kong Observatory noted in their monthly summary that "July 2020 was the hottest month in Hong Kong since record keeping began in 1884," with a long spell of sunny weather. The month was also much drier than usual. The total monthly rainfall was only 125.4 millimetres, about 33 percent of the normal figure of 376.5 millimetres. The accumulated rainfall for the first seven months of the year was 1088.8 millimetres, about 26 percent below the normal figure of 1473.3 millimetres.

Even for the month of June, it was noted by the Hong Kong Observatory that "despite the heavy rain episode on 6-8 June, the monthly total rainfall was only 397.2 millimetres, about 13 percent below the normal figure of 456.1 millimetres. The accumulated rainfall for the first half of the year of 963.4 millimetres was about 12 percent below the normal figure of 1096.9 millimetres."

There was a negative correlation between rainfall and *temperature* (Fig. 3). In July, when there was sparse rain, the temperature rose above 31°C and more or less persisted at that level until midearly August. Rain returned in August, with a drop in temperature.

Stratification of the water column can occur in ponds when there is a long period of warm weather without any wind or rain to mix the surface and bottom water layers. When there is a wind event, the water layers in the pond can be suddenly turned over and cause water quality issues (i.e. low DO). In our study, we evaluated water quality in the centre of the pond at different levels to assess whether this stratification of water occurred (Fig. 13). There was some evidence that deep water samples had lower DO than the surface samples. We did not measure wind in this study, which may explain some of the mixing of the water and convergence of the DO on some dates. It is also possible that the farmers ran the aerators more aggressively during the day to prevent stratification on very hot days. We were unable to detect a difference between the temperature of water in the deep and surface samples, so if stratification occurred it was minimal in our study.



Figure 13. Dissolved Oxygen at surface and deep samples collected in the centre of the ponds

4. DISCUSSION

4.1 Mortality correlation with various water parameters and events

Based on farmers' reports, mortality in earthen ponds in the summer of 2020 were not as high as reported in previous years. Despite this, farmers still reported a few episodes of elevated morality and, in general, these events were preceded by activities such as fertilization of ponds, increased feeding of fish, increasing the stocking density, and tropical cyclones (Fig. 1). The mechanism by which these events may have led to mortality was likely a change in water quality, but we were not able to precisely measure this with our sampling strategy. Many of the events should have led to increased bacterial levels, and /or a drop in oxygen or change in ammonia, but this was not clear from our weekly water quality data. We did observe some problematic water quality parameters during our sampling, but they were not always associated with fish mortality.

Several issues with water quality were identified during our study that could be problematic for mullet health. First, the high water temperature in the summer likely stressed fish as it was always at least 4 to 6 degrees Celsius above the optimal level for mullet. Second, total ammonia nitrogen (TAN) was often high. Based on our pH reading, the unionized component of the ammonia was not high (high pH) but fluctuations in pH could not be determined in our study because we only surveyed ponds between 10 and 12 am on a weekly basis. The lowest pH would occur earlier in the day when the algae were using cellular respiration and producing CO2. We did observe a large fluctuation in pH in a similar pond at a different site which had a continuous monitoring probe. The lowest pH water was in the two ponds with the lowest buffering capacity (ponds 4 and 5 Fig. 6). A 24-hour probe is ideal for monitoring the diurnal rise and fall of pH, temperature and DO due to presence of sunlight and algal activity.

We cannot for certain conclude that nitrite led to fish mortality in this study, but suffice to say that nitrite levels were at the toxic level in at least one pond (#4). With concurrent unfavourable conditions, nitrite can be toxic at 1 mg/L. Long-term (over 6 months) exposure to even very low nitrite levels (0.015 – 0.060 mg/L) can result in mild methemoglobinemia in some fish; this was not observed in fish in our ponds but it was difficult to assess dead fish in this study.

Dissolved oxygen (DO) level is a prime consideration in any aquaculture system. Many factors affect DO – biological oxygen demand (BOD) from biomass (stocking rate) and bacteria population, zooplankton and phytoplankton bloom and die-offs, algal photosynthesis, and temperature. Dissolved oxygen in our study sometimes dipped too low for optimal mullet health and because we did not measure DO before sunset it may have been lower than what we reported in this study.

We observed a slight stratification in the ponds with lower DO in the deeper water. AFCD records have suggested a correlation between severe mortality in *M. cephalus* and high rainfall during the summers. Summer fish kills are often attributed to the phenomenon of pond turnover. Over the course of the summer, large amounts of organic matter can accumulate in the deeper areas of stratified ponds. Premature mixing of these stratified layers can occur after a period of very warm weather followed by sudden heavy rains. Oxygen-deficient bottom layers mix with upper layers, resulting in critically low oxygen levels throughout the water column, resulting in possible acute mass fish mortality. Sediments may also release unfavourable toxic compounds such as hydrogen sulphide into the water column. In summers with higher temperature, demand for oxygen in a pond ecosystem is higher than would be in colder months, accentuating the ill-effects of such events. Heavy rain events can also flush chemicals into the water from the soil of banks and surrounding land, exacerbating the negative impact of rain.

Interestingly, *Streptococcus* spp. were detected in all ponds in this study but were not associated with high levels of mortality, suggesting the identification of streptococcus in the water at low concentrations does not necessarily warrant antibiotic treatment and that this pathogen is ubiquitous in the area. It is possible that if fish are stressed the *Streptococcus* spp. could infect the fish and initiate a fish kill, as has been reported in the past. Maintaining low stress environments may be the key to preventing streptococcus infections. In most reported increases in mortality in this study, there was an event that preceded the mortality.

We suspect the causes of low-and medium grade mortality in this study were multi-factorial, and each pond has its own unique issues. Factors such as expanding biomass, sudden increase in feeding, high water temperatures, fluctuating pH, pond turnover after the cyclone, and or water temperatures over the tolerable limits for *M. cephalus* had negative impacts on fish health. These factors are frequently encountered in semi-intensive open pond aquaculture in Hong Kong.

4.2 The influence of market pressures on pond management and water parameters

Fish productions and sales heavily influence production management and, in turn, influence feeding strategies within a production cycle. Sales in Hong Kong for *M. cephalus* (and other fish species) usually peak around autumn to meet demands for fish products during certain festivals (Mid-Autumn festival, National holiday, Chong Yeung Festival). Therefore, farms will intensify production to target maximal fish growth between July and early September. The increased feeding and stock additions to target this market during the hottest period of the year can affect water parameters. Thus, it is not surprising that this stage of the production cycle, which coincides with unstable summer weather and fluctuating temperatures and rainfall, could trigger massive mortality, especially when conditions for a pond turnover are present. This is especially so if ponds are not closely monitored, or no extra mitigating measures are taken to offset the increased production demands.

4.3 Limitations of the study

There were information gaps in this study. As mentioned, we did not have good farm records and therefore, feeding rates, stocking densities, daily mortality rate, chemical usage, water exchange, and the aerator(s) operational time and placement variation could only be estimated. This lack of precision made it impossible to conduct statistical trend analyses on these farms and may have biased our findings. Another limitation was that there were large variations between the study ponds in terms of size, shape, and stocking density, making it difficult to compare ponds. Lastly, mitigating measures carried out by the farmers upon our advice, when we detected unfavourable conditions (i.e. increase aeration or water exchange), might have, to some degree, altered pond water characteristics and masked the factors which resulted in major grey mullet mortality events in previous summers.

5. Conclusions and recommendations

Although there were no massive fish kills in the ponds that we monitored over the summer of 2020 there were some water quality issues identified and management strategies that likely caused some mortality in *M. cephalus*. The following are recommendations to reduce the impact of some of these events on fish mortality.

- 1) When adding fish (biomass) to a pond:
 - a. ensure the water quality can support the increase in biomass and the additional feeding that occurs with the increased biomass
 - b. Ensure fish introduced to the pond are healthy so pathogens are not introduced into the system
- 2) When increasing feeding:
 - a. Ensure it is done in a gradual manner so the water quality is not compromised
 - b. Ensure the ammonia in the pond is not too high
 - c. Ensure the DO in the pond can support the extra ammonia produced by the increased feed rate
- 3) To reduce the potential for pond turn-over during a cyclone:
 - a. Aerate the pond when the weather is very hot and there is no wind, to reduce the likelihood of stratification
- 4) Fluctuations in DO and pH:
 - a. Reduce the algae by changing the water, copper treatment, apply nanobubbles, adding algae-eating fish, reduce the sunlight to the pond
 - b. Increase the buffering capacity of the pond by increasing KH, but only if the TAN is not too high (otherwise TAN will shift to more toxic form of ammonia).
 - i. Prepare the ponds before fish enter to make sure the bottom is not anoxic and the soil is well buffered (limed)
- 5) To increase the DO:
 - a. If the problem is only in the early morning,
 - i. Control the algae (see 4.a.)
 - b. If the problem is throughout the day
 - i. Increase the algae
 - ii. Increase the aeration in the pond, especially in the evening. To avoid stratification, aerate during the hottest part of the day
- 6) To reduce ammonia:

- a. Reduce feeding
- b. Increase heterotrophic bacteria
- c. Increase algae
- d. Reduce stocking density
- e. Change water
- f. Clean the ponds periodically to reduce organic material on the bottom
- 7) To reduce nitrite:
 - a. Change the salinity of the water (add salt water if avaible. Chloride competitively inhibits uptake of nitrite through the gills)
 - b. Increase nitrification bacteria
 - c. Reduce feeding

Appendix I

Outreach events

Appendix H

From:Prof. Sophie ST-HILAIRETo:Prof. Sophie ST-HILAIRECc:Subject:Subject:Re: Flyer for farmersDate:Tuesday, 2 October 2018 6:56:04 AMAttachments:Flyer.pdf

Hi Prof Sophie, please see attached flyer . Hope this help and thank you for the support.

Best regards

On Monday, October 1, 2018 1:58 PM, Prof. Sophie ST-HILAIRE <ssthilai@cityu.edu.hk> wrote:



Thanks for this.

I will print some copies off for the talks but can you send me a one pager (with a photo and a few words plus your logo info) that I can translate and send to the aquaculture association on Tues. I want to get the farmers to come to your presentation... I don't think they will read 8 pages until after they have heard your talk!

Cheers,

Sophie

From: Sent: Monday, 1 October 2018 1:27 PM To: Prof. Sophie ST-HILAIRE <ssthilai@cityu.edu.hk> Subject: Re: Flyer for farmers

Hi Prof Sophie , please find attached flyer and brochure for shrimp farm. please feel free to comments

Best regards

On Monday, October 1, 2018 12:09:32 PM +08,

wrote:

Hi prof sophie , the arrange looks good. i will revert by this evening on the material.

Best regards

÷.

Sent from Yahoo Mail on Android

On Mon, 1 Oct 2018 at 9:42, Prof. Sophie ST-HILAIRE <<u>ssthilai@cityu.edu.hk</u>> wrote:

Hi James

Can you design this flyer for the farmers here in HK for Friday. Sorry about the short notice I just really thought of this idea over the weekend given I could not find a farm to take you to! I will try to recruit as many farmers as I can to come and hear what nanobubble technology can do for aquaculture. I would focus your presentation on where it is applied already with some personal experiences in Indonesia...you can also mention that we are working to test different applications with you but I would not promise them that it will get rid of all their diseases yet!!

Do you speak Cantonese? If so it would be better to do the presentation in Cantonese but if not I will have my technician translate That means the talk will be a bit slower! . If you can make your slides in Chinese that is best but if not please send us the slides before thurs and we can translate some of them. I would put more pictures than words on them... they are easier to translate!!

I will set up the meeting from 10 to 11 am on Friday.

Sophie



Department of Infectious Diseases and Public Health

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Virus-free. <u>www.avast.com</u>



NanoBubble Technology & Farming





1) Stay in the water for long time. Microorganism can be activated due to the oxygen inside these bubbles





2) It has Negative charge that can attract positive molecules and enable the bubble to stay in the water for long time 3) When it Collapse it decomposed Organic Foulants and produce OH Radicals

Benefits

Water Quality Management

NB can increased the DO of the pond and maintain stable pH throughout. Hence, reduced used of

paddle wheel resulted in energy saving

NB can reduced NH3 and NO2 hence minimised water changed energy saving

NB can exterminate bacteria due to free radical effect

Disease Outbreak

NB will minimized disease outbreak Low Survival rate

NB will increased Survival rate to 90%.

Model : aQua+110M



Description	Specification
Dimensions	635 (W) X 300 (D) X 400 (H) mm
Weight	Approx. 30Kg
Inlet/Outlet connector	32mm(1 1/8") /25 mm (1') Dia
Power Supply	Single phase* 220V*50HZ
Power consumption	1.5hp (1.1KW)
Principle of Bubble Generation	Pressurized Dissolutions Method (PDM)
Water temperature	5 to 60 deg C
Bubble Size	50nm – 250nm (80%)
Water type	Fresh Water n Marine water
Auto BackWash With Self recovery	Yes
Output*	4.0 m3/hr

AquaPro Solutions Pro-

Senting Lane #07-03 Genting Building S349568. www.AquaPro-solutions.com



Jockey Club College of Veterinary Medicine and Life Sciences

> 香港城市大學 City University of Hong Kong in collaboration with Cornell University

水產養殖業工作坊 如何在水產養殖中謹慎使用抗生素

了解怎樣及何時使用抗生素來降低魚類的死亡率。 如何在飼料中混合抗生素以及如何監測魚類對這些產品 的反應,以提高治療的成功率。

- 日期: 2019年6月5日(星期三)
- 下午1:30至4:30 時間:
- 元朗屏山孖峰嶺香港農業聯合會辦事處 地點:
- **如有興趣,請於6月2日前致電或whatsapp(電話:5596-0777), 以便安排坐位。謝謝!

香港城市大學水產流動獸醫服務





元朗屏山孖峰嶺香港農業聯合會辦事處









Appendix K list of farmers participating in AMU workshop

5/6/2019 水產養殖工作坊

貴賓名單 Guest List

姓名 Name	公司/機構 Company/Organisation	電話 phone number	簽名 Signature
	香港創新環保漁業		
	香港新界養魚協進會		
	香港漁民團體協會		
	香港漁民團體協會		
	川上農莊		
	香港錦鯉會		
	浸會大學		
	浸會大學		

雲峰園



5/6/2019 水產養殖工作坊

貴賓名單 Guest List

姓名 Name	公司/機構 Company/Organisation	電話 phone number	簽名 Signature
	AFCD		
	AFCD		
	AFCD		





Jockey Club College of Veterinary Medicine and Life Sciences

> 香港城市大學 City University of Hong Kong in collaboration with Cornell University

BASIC FISH MEDICINE FOR YOUR PRACTICE



Dr Richmond Loh

DipProjMgt, BSc, BVMS, MPhil (Pathology), MANZCVS (Aquatics& Pathobiology), CertAqV, Fellow WAVMA

Dr Richmond Loh is based in Perth, is an aquatic veterinarian & fish pathologist, consulting as The Fish Vet, servicing all aspects of ornamental pet fish owners including hobbyists, public aquaria, retailers, wholesalers and fish farmers since 2002. His is famously known in veterinary circles for "Fish Vetting Essentials" book, and in fish hobbyists circles featuring in "The Fish Doctor" YouTube Channel. He served as the 2014 President of the World Aquatic Veterinary Medical Association (WAVMA), and Secretary of the Aquatic Animal Health Chapter of the ANZCVS. Dr Loh graduated from Murdoch University and is an Adjunct Senior Lecturer, has a Masters in veterinary pathology, Memberships in Aquatic Animal Health and Pathobiology, is a Certified Aquatic Veterinarian and a Distinguished Fellow of the WAVMA.

\approx Topic covered \approx

- >>> common diagnostics and diseases of koi fish
- practical exercise on diagnostics used in fish medicine (i.e. gill biopsies, skin scrapes, &water testing)
- case studies including common treatment strategies for parasitic, bacterial, & water quality problems.

Date: 5 June 2019, Wednesday

Time: 7:00 pm to 10:00 pm (Light Dinner will be available at 6:30pm)

Venue: CP211, CityU School of Continuing and Professional Education

ChinaChem Golden Plaza, UG2 Floor

77 Mody Road, Tsim Sha Tsui East

Fee: HKD180

Registration: http://www.cityu.edu.hk/jcc/links/20190605CPE.asp

Enquiries:

Tel : (852) 3442 6138 El

Email: cvmls.cpe@cityu.edu.hk



Appendix I





Jockey Club College of Veterinary Medicine and Life Sciences 香港城市大學 City University of Hong Kong

in collaboration with Cornell University



Online Seminar Water Quality Class for Vets

Speaker: Dr Richmond LohDate:3 December 2020, ThursdayTime:7:00 pm to 9:00 pm

Registration: https://www.cityu.edu.hk/jcc/20201203CPE

Good water quality is critical for healthy fish. Poor water quality can lead to primary pathology, as well as secondary disease issues in fish. Water quality can also influence your treatment choices. As part of an aquatic animal veterinary service it is essential that veterinarians and technicians know how to assess water quality, and that they are aware of the normal ranges for key water parameters important to sustain good fish health. This webinar will review the relevant water quality parameters to monitor as part of a fish veterinary practice, and the clinical signs you may see in fish associated with different common water quality issues. Dr. Loh will also discuss strategies used to mitigate water quality problems in veterinary practice.



Dr Richmond Loh

BSc, **BVMS**, **MPhil** (Pathology), **MANZCVS** (Aquatics& Pathobiology), CertAqV, Fellow WAVMA Graduating from Murdoch University in 2001, Dr Loh followed his passion of fish to take his first post as a fish pathologist intern at DPIPWE in Tasmania. His aquatic work encompassed salmonids, oyster and abalone health monitoring and pathology. There, Dr Loh began consulting with pet fishes as "The Fish Vet". Now based in Perth (Western Australia), he is joined by a team spread across Australia to provide veterinary, and pathology services for a range of clients including pet fish, display aquaria, retailers and fish farmers. He has been admitted to the Australian and New Zealand College of Veterinary Scientists by examinations in both the subjects of Pathobiology, and in Medicine & Management of Aquaculture Species. He is a Certified Aquatic Veterinarian and has been awarded the George Alexander International Fellowship by the International Specialised Skills Institute. He has also been inducted as a Fellow of the World Aquatic Veterinary Medical Association. He promotes aquatic veterinary medicine by producing educational materials (http://tinyurl.com/thefishdoctor).



Enquiries: Tel : (852) 3442 6138 Email: cvmls.cpe@cityu.edu.hk



Screen captures of the participants for the veterinarian workshop n=31



Screen captures for the farmer workshop n=34





Appendix J

Annual Fish Health internal summary up to April 2020

Appendix J

Outline of Fish health issues (internal report)

April 2020

•

The following are the common causes of disease that our team has observed over the last 12 months.

- Salt water mortality cases (50 cases)
 - o Bacterial infections
 - Streptococcus iniae
 - Vibrio spp.
 - o Parasite infections
 - Cryptocaryon irritans
 - Tricodina spp.
 - Uronema spp.
 - Caligus spp. (sea lice)
 - Monogenean parasites
 - o Viral infections (irrido virus)
 - Water quality (salinity or nitrogenous waste issues in recirculation systems)
 - Fresh water mortality cases (22 cases)
 - o Water quality issues
 - Knowledge of water quality and mitigation strategies for different issues is low in the industry. Common issues facing farmers in Hong Kong include:
 - Fluctuation pH
 - Low alkalinity
 - Anoxic Turnover
 - High Total Ammonia
 - o Bacterial diseases
 - Aeromonas hydrophila
 - Edwardsiella Tarda
 - others
 - o Parasite diseases
 - Ichthyophonis multiphilis
 - Oodinium spp.
 - Tricodina
 - Monogenean parasites

A common problem to both fresh and salt water farming in Hong Kong is issue around the removal of dead fish from the aquatic environment. This is likely exacerbating infectious disease spread within and between farms.

Appendix K

Expected Outcomes and Impact of the Project

Expected Outcomes and Impact of the Project (Examples: sustainability of the project and its outcomes/impact, promotional value and potentials of the project outcomes, contributions to the local fisheries community and the entire fisheries industry)

Outcomes

- Annual list of the disease priorities ranked by impact for the Hong Kong aquaculture industry
- Establishment of a veterinary service through CityU to complement AFCD's Fish Health Inspection Programme
- Summary of annual veterinary services provided through the proposed programme including sales of pharmaceutical products for aquatic animal use sold through the CityU veterinary pharmacy
- Manual for the use of emamectin benzoate against ectoparasites of salt water fish found in Hong Kong
- Scientific paper of research on emamectin benzoate outlining efficacy and withdrawal periods for minimum residue levels
- Manual and scientific paper on intervention strategy for at least one other infectious disease affecting the aquaculture industry in Hong Kong
- Two trained aquatic animal veterinarians
- Two undergraduate fish health courses for the Bachelor in Veterinary Medicine Programme at CityU
- Three CPD courses with at least 20 participants each
- Two industry fish health workshops attended by 20 to 50 individuals
- Final evaluation of programme with recommendations on how to address future veterinary needs of the aquaculture industry in Hong Kong

Impacts

- Improved capacity and access to aquatic animal veterinary service and diagnostics
- Reduction in the overall mortality associated with disease outbreaks through earlier detection and improved treatment efficacy
- Improved fish health, welfare and productivity, and environmental sustainability of the aquaculture industry in Hong Kong
- Improved food safety from evidence-based disease intervention strategies
- Improved consumer confidence in fish products from Hong Kong

Methods in Sharing Project Outcomes/Experience

- Individual veterinarian-farmer communication during farm visits
- Hands-on training of veterinarians to provide clinical service
- CPD courses for veterinarians
- Fish health courses incorporated into the BVM curriculum to reach all new veterinary graduates
- Farmer workshops to promote their role in fish health
- Aquatic animal health manuals on intervention strategies (i.e. emamectin benzoate use for ectoparasites) for Hong Kong veterinary practitioners and aquaculture producers (accessible on the AFCD website and distributed during workshops and by veterinarians and feed sales representatives)
- Presentations at local conferences
- Communication about project in the local media
- Submission of manuscripts to peer-reviewed scientific journals

Outcome Ind measurable (Examples: 1 who will par contribution output quan	dicators (indicators must be specific, and achievable) the number of beneficiaries (count by heads) ticipate in the key activities, expected as to the agricultural industry, changes in tity/quality)	Method of Data Collection	Accomplishment
Key	At least 60 of aquaculture farmers will utilise	□ Registered Data of the	We have been able to recruit
Indicator 1	the aquatic animal veterinary service by the end	Participants	67 clients to our service
for service	of the 2 year programme	\Box Evaluation Question Set	(Appendix D).
component		☑ Follow-up Survey or Interview	
		□ Other Methods	
		Please	
		specify:	
Key		□ Registered Data of the	• We provide AFCD with
Indicator 2		Participants	a spreadsheet every

for research component	Fish health reports every 12 months identifying research needs and priorities	 Evaluation Question Set Follow-up Survey or Interview Other Methods Please specify: Analysis of AFCD fish health database and veterinary service database 	 month with a tally of all the cases and the medications that we prescribe. These are summarized in Appendix C. We also update them on any antibiotic resistance testing we do every month (summarized in Appendix G). We also conducted a review of the literature on aquatic animal diseases found Hong
			Kong (Appendix L)
Key Indicator 3 for research component	Development of intervention strategies for at least 2 fish health issues	 Registered Data of the Participants Evaluation Question Set Follow-up Survey or Interview Other Methods Please specify: laboratory and/or field research conducted to assess intervention strategy 	 We successfully treated sea lice and developed a protocol for the use of emamectin benzoate in grouper We tried to treat ciliated protozoans with mebendazole but this was not successful.
Key Indicator 4	One peer-reviewed manuscript and one	☐ Registered Data of the	We published on the
for research	evaluated. These materials will be distributed via	Evaluation Ouestion Set	lice with emamectin
component	the AFCD website and as part of our outreach	□ Follow-up Survey or Interview	benzoate (Appendix F).

	CPD courses and workshops	☑ Other Methods	
		Please specify: Quantifiable	
		products.	
Key	70% of the aquaculture farmers that utilise the	□ Registered Data of the	Farmers were pleased with
Indicator 5	new intervention strategies developed through	Participants	the new treatment for sea
for research	this research programme will report an	\Box Evaluation Question Set	lice but the cost of the
component	improvement in their fish health (i.e. reduction	☑ Follow-up Survey or Interview	product is quite high. We
phase 3	in mortality by ¹ / ₂). We will assess the success of	□ Other Methods	interviewed our clients
	our treatments with a follow-up visit to the	Please	during site visits.
	farms.	specify:	
Key	Two trained veterinarians in Aquatic animal	☑ Registered Data of the	Done
Indicator 6	veterinary medicine	Participants	
outreach		\Box Evaluation Question Set	
component		□ Follow-up Survey or Interview	
		\Box Other Methods	
		Please	
		specify:	
Key	Three CPD courses with at least 20 veterinary	☑ Registered Data of the	We were only able to
Indicator 7	participants in each course	Participants	deliver 2 CPD courses due
outreach		\Box Evaluation Question Set	to the COVID issues
component		□ Follow-up Survey or Interview	(Appendix I)
		\Box Other Methods	
		Please	
		specify:	
Key	Two industry workshops with 20 to 50	☑ Registered Data of the	We delivered 4 industry
Indicator 8	participants at each event	Participants	workshops (Appendix I)
outreach		\Box Evaluation Question Set	
component		□ Follow-up Survey or Interview	
		\Box Other Methods	
		Please	
		specify:	

Key	Two fish health courses delivered to the BVM	☑ Registered Data of the	We delivered 3 courses in
Indicator 9	class at City U. We have 12 students in the	Participants	the BVM programme (n=
outreach	programme and anticipate 20 next year.	\Box Evaluation Question Set	53 students)
component		□ Follow-up Survey or Interview	
		\Box Other Methods	
		Please	
		specify:	
Key		□ Registered Data of the	We have submitted another
Indicator 10	Programme assessment report including	Participants	proposal to SFDF_0042
for	recommendations for the future of the	□ Evaluation Question Set	with the recommendations
Assessment	programme	□ Follow-up Survey or Interview	for the next three years.
of		☑ Other Methods	
programme		Please specify: Analysis of our	
		costs and benefits (i.e. key indictor	
		performance)	

Appendix L

Hong Kong Marine Fish disease survey (data summarized from AFCD)

Table 1. Pathogens/diseases reported from marine net-pen fish farms in Hong Kong. The data are from two different sources, the Agriculture, Fisheries, and Conservation Department of the Hong Kong Specieal Administrative Region, SAR (AFCD) and Fish Health Ambulatory Service of the City University of Hong Kong (FHAS).

Pathogen	Report date	Fish culture zone or general location ¹	Affected fish species	Source of data
Viruses				
Iridovirus	May 24, 2011	Yim Tin Tsai (East)	Green grouper	AFCD
Iridovirus	August 30, 2011	Yung Shue Au	Green grouper	AFCD
Iridovirus	June 28, 2012	Lo Tik Wan	Sweetlip	AFCD
Iridovirus	July 5, 2012	Yim Tin Tsai	Potato cod	AFCD
Iridovirus	August 16, 2013	Kau Sai	Hybrid grouper	AFCD
Iridovirus	December 8, 2014	Tai Tau Chau	Hybrid grouper	AFCD
Nervous necrosis virus (NNV)	November 26, 2013	Yung Shue Au	Hybrid grouper	AFCD
Nervous necrosis virus (NNV)	December 8, 2014	Tai Tau Chau	Hybrid grouper	AFCD
Nervous necrosis virus (NNV)	June 5, 2015	O Pui Tong	Giant grouper	AFCD
Nervous necrosis virus (NNV)	June 5, 2015	O Pui Tong	Green grouper	AFCD
Nervous necrosis virus (NNV)	June 8, 2015	Yung Shue Au	Hybrid grouper	AFCD
Nervous necrosis virus (NNV)	July 15, 2015	Yung Shue Au	Hybrid grouper	AFCD
Nervous necrosis virus (NNV)	May 27, 2016	Tai Tau Chau	Hybrid grouper	AFCD
Nervous necrosis virus (NNV)	November 29, 2017	Kau Sai	Hybrid grouper	AFCD
Read sea bream iridovirus (RSIV)	May 11, 2015	Tai Tau Chau	Hybrid grouper	AFCD
Read sea bream iridovirus (RSIV)	March 8, 2016	Kau Sai	Hybrid grouper	AFCD
Read sea bream iridovirus (RSIV)	March 30, 2016	O Pui Tong	Green grouper	AFCD
Read sea bream iridovirus (RSIV)	September 7, 2016	Yim Tin Tsai	Hybrid grouper	AFCD
Read sea bream iridovirus (RSIV)	September 18, 2017	Sok Kwu Wan	Pompano	AFCD
Read sea bream iridovirus (RSIV)	November 29, 2017	Kau Sai	Hybrid grouper	AFCD
Unknown (suspected viral infection)	September 3, 2019	Tai Po	Batfish	FHAS
Unknown (potential viral infection)	September 9, 2019	Sai Kung	Batfish	FHAS
Bacteria				
Aeromonas salmonicida	October 31, 2013	Sok Kwu Wan	Black croaker	AFCD
Aeromonas salmonicida	October 31, 2013	Sok Kwu Wan	Cobia	AFCD
Aeromonas salmonicida	October 31, 2013	Sok Kwu Wan	Red drum	AFCD
Photobacterium damselae	December 17, 2015	Yim Tin Tsai	Hybrid grouper	AFCD
Photobacterium damselae	January 13, 2016	Yung Shue Au	Hybrid grouper	AFCD
Photobacterium damselae	December 28, 2016	Yim Tin Tsai	Pompano	AFCD
Photobacterium damselae	January 5, 2017	Cheung Sha Wan	Pompano	AFCD
Photobacterium damselae	April 23, 2017	Tap Mun	Rabbit fish	AFCD
Photobacterium damselae	June 22, 2017	Cheung Sha Wan	Blackspotted croaker	AFCD
Photobacterium damselae	June 22, 2017	Cheung Sha Wan	Pompano	AFCD
Photobacterium damselae	June 29, 2017	Sok Kwu Wan	Pompano	AFCD

Photobacterium damselae	August 7, 2017	Sok Kwu Wan	Pompano	AFCD
Photobacterium damselae	September 18, 2017	Sok Kwu Wan	Pompano	AFCD
Photobacterium damselae	October 13, 2017	Sok Kwu Wan	Pompano	AFCD
Streptococcus iniae	April 23, 2017	Tap Mun	Rabbit fish	AFCD
Streptococcus iniae	June 29, 2017	Sok Kwu Wan	Pompano	AFCD
Streptococcus iniae	October 30, 2017	Sok Kwu Wan	Crescent sweetlips	AFCD
Streptococcus iniae	October 5, 2018	Sai Kung	Pompano	FHAS
Tenacibaculum maritimus	April 5, 2016	Cheung Sha Wan	Star snapper	AFCD
Vibrio alginolyticus	November 8, 2011	Leung Shuen Wan	Green grouper	AFCD
Vibrio alginolyticus	November 8, 2011	Leung Shuen Wan	Mangrove snapper	AFCD
Vibrio alginolyticus	May 22, 2012	Sok Kwu Wan	Sweetlip	AFCD
Vibrio alginolyticus	June 6, 2012	Lo Fu Wat	Giant grouper	AFCD
Vibrio alginolyticus	June 11, 2012	Sok Kwu Wan	Duskytail grouper	AFCD
Vibrio alginolyticus	June 28, 2012	Lo Tik Wan	Sweetlip	AFCD
Vibrio alginolyticus	August 8, 2012	Po Toi	Mangrove snapper	AFCD
Vibrio alginolyticus	August 8, 2012	Cheung Sha Wan	Cobia	AFCD
Vibrio alginolyticus	August 8, 2012	Cheung Sha Wan	Red drum	AFCD
Vibrio alginolyticus	August 8, 2012	Cheung Sha Wan	Greater amberjack	AFCD
Vibrio alginolyticus	August 27, 2012	Cheung Sha Wan	Red drum	AFCD
Vibrio alginolyticus	October 5, 2012	Sok Kwu Wan	Black croaker	AFCD
Vibrio alginolyticus	May 11, 2015	Tai Tau Chau	Hybrid grouper	AFCD
Vibrio alginolyticus	June 5, 2015	O Pui Tong	Green grouper	AFCD
Vibrio alginolyticus	September 14, 2015	Sok Kwu Wan	Crescent sweetlips	AFCD
Vibrio alginolyticus	September 30, 2015	Sok Kwu Wan	Pompano	AFCD
Vibrio alginolyticus	September 30, 2015	Sok Kwu Wan	Red pargo	AFCD
Vibrio alginolyticus	September 30, 2015	Sok Kwu Wan	Red snapper	AFCD
Vibrio alginolyticus	November 20, 2015	Cheung Sha Wan	Cobia	AFCD
Vibrio alginolyticus	January 15, 2016	Lo Tik Wan	Pompano	AFCD
Vibrio alginolyticus	February 18, 2016	Tai Tau Chau	Hybrid grouper	AFCD
Vibrio alginolyticus	March 8, 2016	Kau Sai	Hybrid grouper	AFCD
Vibrio alginolyticus	January 17, 2017	Sha Tau Kok	Hybrid grouper	AFCD
Vibrio alginolyticus	June 29, 2017	Sok Kwu Wan	Pompano	AFCD
Vibrio alginolyticus	October 13, 2017	Sok Kwu Wan	Pompano	AFCD
Vibrio alginolyticus	November 29, 2017	Kau Sai	Hybrid grouper	AFCD
Vibrio harveyi	March 15, 2015	Yim Tin Tsai (East)	Hybrid grouper	AFCD
Vibrio parahaemolyticus	April 5, 2016	Cheung Sha Wan	Star snapper	AFCD
Vibrio parahaemolyticus	June 29, 2017	Sok Kwu Wan	Pompano	AFCD
Vibrio parahaemolyticus	August 7, 2017	Sok Kwu Wan	Pompano	AFCD
Vibrio parahaemolyticus	October 30, 2017	Sok Kwu Wan	Emperor snapper	AFCD
Vibrio vulnificus	October 31, 2013	Sok Kwu Wan	Black croaker	AFCD
Vibrio vulnificus	October 31, 2013	Sok Kwu Wan	Cobia	AFCD

Vibrio vulnificus	October 31, 2013	Sok Kwu Wan	Red drum	AFCD
Vibrio vulnificus	November 18, 2015	Lo Tik Wan	Hybrid grouper	AFCD
Vibrio vulnificus	September 7, 2016	Yim Tin Tsai	Hybrid grouper	AFCD
Vibrio vulnificus	November 15, 2016	Kau Sai	Pompano	AFCD
Vibrio vulnificus	January 5, 2017	Cheung Sha Wan	Pompano	AFCD
Vibrio sp.	October 10, 2017	Sok Kwu Wan	Yellow croaker	AFCD
Vibrio sp.	October 5, 2018	Tai Po	Pompano	FHAS
Unknown bacteria (suspected)	October 15, 2018	Sai Kung	Rabbit fish	FHAS
Unknown bacteria (suspected secondary infection)	October 30, 2018	Sai Kung	Pompano	FHAS
Unknown bacteria (suspected secondary infection)	October 31, 2018	Sai Kung	Pompano	FHAS
Unknown bacteria (suspected secondary infection)	November 12, 2018	Sai Kung	Pompano	FHAS
Unknown bacteria	December 17, 2018	Sai Kung	Pompano	FHAS
Unknown bacteria	April 12, 2019	Yim Tin Tsai	Hybrid grouper	FHAS
Unknown bacteria	April 17, 2019	Yim Tin Tsai	Hybrid grouper	FHAS
Unknown bacteria	April 24, 2019	Yim Tin Tsai	Hybrid grouper	FHAS
Unknown bacteria	April 26, 2019	Yim Tin Tsai	Hybrid grouper	FHAS
Unknown bacteria	April 30, 2019	Yim Tin Tsai	Hybrid grouper	FHAS
Unknown bacteria	May 2, 2019	Yim Tin Tsai	Hybrid grouper	FHAS
Unknown bacteria	May 6, 2019	Yim Tin Tsai	Hybrid grouper	FHAS
Unknown bacteria	May 7, 2019	Sai Kung	Pompano	FHAS
Unknown bacteria	May 17, 2019	Sai Kung	Pompano	FHAS
Unknown bacteria	May 22, 2019	Cheung Sha Wan	Croaker	FHAS
Unknown bacteria	June 21, 2019	Tai Tau Chau	Grouper	FHAS
Unknown bacteria	July 2, 2019	Tai Tau Chau	Grouper	FHAS
Unknown bacteria (secondary bacterial infection)	August 27, 2019	Tai Po	Batfish	FHAS
Unknown bacteria	September 3, 2019	Tai Po	Batfish	FHAS
Suspected bacterial outbreak	November 20, 2018	Lamma Island	Hybrid grouper	FHAS
Suspected bacterial outbreak	November 30, 2018	Lamma Island	Hybrid grouper	FHAS
Parasites				
Copepoda				
Sea lice (<i>Caligus</i>)	November 9, 2012	Yim Tin Tsai (East)	Green grouper	AFCD
Sea lice (<i>Caligus</i>)	March 15, 2015	Yim Tin Tsai (East)	Hybrid grouper	AFCD
Sea lice (<i>Caligus</i>)	June 8, 2015	Yung Shue Au	Hybrid grouper	AFCD
Sea lice (<i>Caligus</i>)	July 15, 2015	Yung Shue Au	Hybrid grouper	AFCD
Sea lice (<i>Caligus</i>)	December 14, 2015	Yung Shue Au	Hybrid grouper	AFCD
Sea lice	January 17, 2017	Sha Tau Kok	Hybrid grouper	AFCD
Sea lice	November 29, 2018	Yim Tin Tsai	Hybrid grouper	FHAS
Sea lice	February 28, 2019	Sam Mun Tsai	Grouper	FHAS
Sea lice	April 12, 2019	Yim Tin Tsai	Hybrid grouper	FHAS
Sea lice	April 17, 2019	Yim Tin Tsai	Hybrid grouper	FHAS
Sea lice	April 24, 2019	Yim Tin Tsai	Hybrid grouper	FHAS

Sea lice	April 26, 2019	Yim Tin Tsai	Hybrid grouper	FHAS
Sea lice	April 30, 2019	Yim Tin Tsai	Hybrid grouper	FHAS
Sea lice	May 2, 2019	Yim Tin Tsai	Hybrid grouper	FHAS
Sea lice	May 6, 2019	Yim Tin Tsai	Hybrid grouper	FHAS
Sea lice	March 17, 2020	Cheung Sha Wan	Hybrid grouper	FHAS
Sea lice	April 1, 2020	Cheung Sha Wan	Hybrid grouper	FHAS
Sea lice	April 2, 2020	Tai Tau Chau	Hybrid grouper	FHAS
Sea lice	April 8, 2020	Cheung Sha Wan	Hybrid grouper	FHAS
Sea lice	April 14, 2020	Tai Tau Chau	Hybrid grouper	FHAS
Sea lice	April 15, 2020	Cheung Sha Wan	Hybrid grouper	FHAS
Sea lice	April 21, 2020	Tai Tau Chau	Hybrid grouper	FHAS
Isopoda				
Unknown	February 8, 2011	Yim Tin Tsai	Giant grouper	AFCD
Unknown	November 6, 2012	Yim Tin Tsai	Green grouper	AFCD
Microsporea				
Glugea	February 18, 2016	Tai Tau Chau	Hybrid grouper	AFCD
Glugea	March 8, 2016	Kau Sai	Hybrid grouper	AFCD
Glugea	March 30, 2016	O Pui Tong	Green grouper	AFCD
Glugea	May 27, 2016	Tai Tau Chau	Hybrid grouper	AFCD
Glugea	January 17, 2017	Sha Tau Kok	Hybrid grouper	AFCD
Unknown	February 8, 2011	Yim Tin Tsai	Giant grouper	AFCD
Monogenea				
Benedenia sp.	February 18, 2016	Tai Tau Chau	Hybrid grouper	AFCD
Benedenia sp.	March 8, 2016	Kau Sai	Hybrid grouper	AFCD
Benedenia sp.	January 5, 2017	Cheung Sha Wan	Pompano	AFCD
Benedenia sp.	January 17, 2017	Sha Tau Kok	Hybrid grouper	AFCD
Dactylogyrus sp.	June 26, 2012	Yung Shue Au	Green grouper	AFCD
Dactylogyrus sp.	November 6, 2012	Yim Tin Tsai	Green grouper	AFCD
Dactylogyrus sp.	November 9, 2012	Yim Tin Tsai (East)	Green grouper	AFCD
Dactylogyrus sp.	August 16, 2013	Kau Sai	Hybrid grouper	AFCD
Dactylogyrus sp.	October 31, 2013	Sok Kwu Wan	Black croaker	AFCD
Dactylogyrus sp.	October 31, 2013	Sok Kwu Wan	Cobia	AFCD
Dactylogyrus sp.	October 31, 2013	Sok Kwu Wan	Red drum	AFCD
Dactylogyrus sp.	June 5, 2015	O Pui Tong	Green grouper	AFCD
Dactylogyrus sp.	September 14, 2015	Sok Kwu Wan	Crescent sweetlips	AFCD
Dactylogyrus sp.	July 31, 2020	Lamma Island	Hybrid grouper	FHAS
Unknown	December 17, 2018	Sai Kung	Pompano	FHAS
Unknown	April 12, 2019	Yim Tin Tsai	Hybrid grouper	FHAS
Unknown	April 17, 2019	Yim Tin Tsai	Hybrid grouper	FHAS
Unknown	April 24, 2019	Yim Tin Tsai	Hybrid grouper	FHAS
Unknown	April 26, 2019	Yim Tin Tsai	Hybrid grouper	FHAS
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Unknown	April 30, 2019	Yim Tin Tsai	Hybrid grouper	FHAS
Unknown	May 2, 2019	Yim Tin Tsai	Hybrid grouper	FHAS
Unknown	May 6, 2019	Yim Tin Tsai	Hybrid grouper	FHAS
Protozoa				
Amyloodinium	December 17, 2018	Sai Kung	Pompano	FHAS
Brooklynella hostilis	May 22, 2012	Sok Kwu Wan	Sweetlip	AFCD
Brooklynella	July 12, 2011	Sok Kwu Wan	Duskytail grouper	AFCD
Brooklynella	June 11, 2012	Sok Kwu Wan	Duskytail grouper	AFCD
Brooklynella	November 22, 2012	Sok Kwu Wan	Pompano	AFCD
Brooklynella	October 30, 2017	Sok Kwu Wan	Crescent sweetlips	AFCD
Cryptocaryon irritans	November 8, 2011	Leung Shuen Wan	Green grouper	AFCD
Cryptocaryon irritans	November 8, 2011	Leung Shuen Wan	Mangrove snapper	AFCD
Cryptocaryon irritans	November 8, 2011	Leung Shuen Wan	Pompano	AFCD
Cryptocaryon irritans	October 5, 2012	Sok Kwu Wan	Black croaker	AFCD
Cryptocaryon irritans	November 6, 2012	Yim Tin Tsai	Green grouper	AFCD
Cryptocaryon irritans	November 9, 2012	Yim Tin Tsai (East)	Green grouper	AFCD
Cryptocaryon irritans	June 6, 2013	Tiu Cham Wan	Pompano	AFCD
Cryptocaryon irritans	October 31, 2013	Sok Kwu Wan	Black croaker	AFCD
Cryptocaryon irritans	October 31, 2013	Sok Kwu Wan	Cobia	AFCD
Cryptocaryon irritans	October 31, 2013	Sok Kwu Wan	Red drum	AFCD
Cryptocaryon irritans	September 14, 2015	Sok Kwu Wan	Crescent sweetlips	AFCD
Cryptocaryon irritans	September 14, 2015	Sok Kwu Wan	Greater amberjack	AFCD
Cryptocaryon irritans	September 30, 2015	Sok Kwu Wan	Pompano	AFCD
Cryptocaryon irritans	November 20, 2015	Cheung Sha Wan	Cobia	AFCD
Cryptocaryon irritans	December 14, 2015	Yung Shue Au	Hybrid grouper	AFCD
Cryptocaryon irritans	November 15, 2016	Kau Sai	Pompano	AFCD
Cryptocaryon irritans	June 22, 2017	Cheung Sha Wan	Blackspotted croaker	AFCD
Cryptocaryon irritans	June 22, 2017	Cheung Sha Wan	Pompano	AFCD
Cryptocaryon irritans	August 7, 2017	Sok Kwu Wan	Pompano	AFCD
Cryptocaryon irritans	September 18, 2017	Sok Kwu Wan	Pompano	AFCD
Cryptocaryon irritans	October 2, 2018	Sai Kung	Pompano	FHAS
Cryptocaryon irritans	October 22, 2018	Sai Kung	Whitemouth croaker	FHAS
Cryptocaryon irritans	October 22, 2018	Sai Kung	Pompano	FHAS
Cryptocaryon irritans	October 30, 2018	Sai Kung	Pompano	FHAS
Cryptocaryon irritans	October 31, 2018	Sai Kung	Pompano	FHAS
Cryptocaryon irritans	November 12, 2018	Sai Kung	Pompano	FHAS
Cryptocaryon irritans	April 13, 2019	Sai Kung	Pompano	FHAS
Cryptocaryon irritans	April 29, 2019	Sai Kung	Pompano	FHAS
Cryptocaryon irritans	May 7, 2019	Sai Kung	Pompano	FHAS
Cryptocaryon irritans	May 17, 2019	Sai Kung	Pompano	FHAS

Cryptocaryon irritans	May 20, 2019	Sai Kung	Pompano	FHAS
Cryptocaryon irritans	July 30, 2020	Tai Po	Batfish	FHAS
Scuticocilia	October 30, 2018	Sai Kung	Pompano	FHAS
Scuticocilia	October 31, 2018	Sai Kung	Pompano	FHAS
Scuticocilia	November 12, 2018	Sai Kung	Pompano	FHAS
Trichodina	February 8, 2011	Yim Tin Tsai	Giant grouper	AFCD
Trichodina	July 12, 2011	Sok Kwu Wan	Duskytail grouper	AFCD
Trichodina	November 8, 2011	Leung Shuen Wan	Green grouper	AFCD
Trichodina	July 5, 2012	Yim Tin Tsai	Potato cod	AFCD
Trichodina	August 16, 2013	Kau Sai	Hybrid grouper	AFCD
Trichodina	September 14, 2015	Sok Kwu Wan	Crescent sweetlips	AFCD
Trichodina	January 13, 2016	Yim Tin Tsai (East)	Hybrid grouper	AFCD
Trichodina	January 15, 2016	Lo Tik Wan	Pompano	AFCD
Trichodina	February 18, 2016	Tai Tau Chau	Hybrid grouper	AFCD
Trichodina	September 7, 2016	Yim Tin Tsai	Hybrid grouper	AFCD
Trichodina	December 28, 2016	Yim Tin Tsai	Pompano	AFCD
Trichodina	January 5, 2017	Cheung Sha Wan	Pompano	AFCD
Trichodina	June 29, 2017	Sok Kwu Wan	Pompano	AFCD
Trichodina	September 18, 2017	Sok Kwu Wan	Pompano	AFCD
Trichodina	October 13, 2017	Sok Kwu Wan	Pompano	AFCD
Trichodina	October 16, 2018	Sai Kung	Japanese eel	FHAS
Trichodina	December 17, 2018	Sai Kung	Pompano	FHAS
Uronema	March 8, 2016	Kau Sai	Hybrid grouper	AFCD
Uronema	March 30, 2016	O Pui Tong	Green grouper	AFCD
Uronema	April 5, 2016	Cheung Sha Wan	Star snapper	AFCD
Uronema	August 7, 2017	Sok Kwu Wan	Pompano	AFCD
Uronema	October 13, 2017	Sok Kwu Wan	Pompano	AFCD
Suspected scuticocilia	October 22, 2018	Sai Kung	Pompano	FHAS
Trematoda				
Sanguinicola	September 18, 2017	Sok Kwu Wan	Pompano	AFCD
Sanguinicolids	January 15, 2016	Lo Tik Wan	Pompano	AFCD
Unclassified				
Turbellarians	November 15, 2016	Kau Sai	Pompano	AFCD
Unknown	June 24, 2019	Sai Kung	Pompano	FHAS
Unknown (suspected parasitic problem)	September 5, 2019	Lamma Island	Cobia	FHAS
Gill parasite	March 17, 2020	Cheung Sha Wan	Star snapper	FHAS
Unknown issues				
Unknown	October 9, 2018	Sai Kung	Grouper	FHAS

¹ Some zones in this table are not found in the map of fish culture zones in Hong Kong (Figure x) (i.e. Tai Po, Sai Kung, Sam Mun Tsai, Lamma Island) and are the general location of the farm from which the pathogen/disease was reported (this is how the location was recorded in the original database). However, to
be able to compare the zones, according to their locations, farmed species, disease issues, etc., and for clarification, the information below is provided:

- 7 Tai Po includes two fish culture zones, Lo Fu Wat and Yung Shue Au
- Sai Kung includes following fish culture zones: Tap Mun, Kau Lau Wan, Sham Wan, Leung Shuen Wan, Tiu Cham Wan, Kai Lung Wan, Kau Sai, Ma
 Nam Wat, and Po Toi O
- 10 Sam Mun Tsai includes two fish culture zones, Yim Tin Tsai and Yim Tin Tsai (East)
- 11 Lamma Island includes two fish culture zones, Sok Kwu Wan and Lo Tik Wan

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Figure 1. Marine fish production in Hong Kong during the years 1969-2018. The figure has been produced for this study using the data related to the
 production of major marine fish species farmed in Hong Kong (groups under the following names: areolate grouper, barramundi, goldlined seabream, greasy
 grouper, Hong Kong grouper, mangrove red snapper, orange-spotted grouper, Russell's snapper, silver seabream, snappers nei, snubnose pompano) from
 FishstatJ (FAO 2020).



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20 Figure 2. A summary of pathogens commonly reported from marine fish net-pen farms in Hong Kong according to the Hong Kong disease databases used in

21 this study (AFCD and City University of Hong Kong). Yellow, blue, and brown colours represent viral, bacterial, and parasitic species, respectively.