



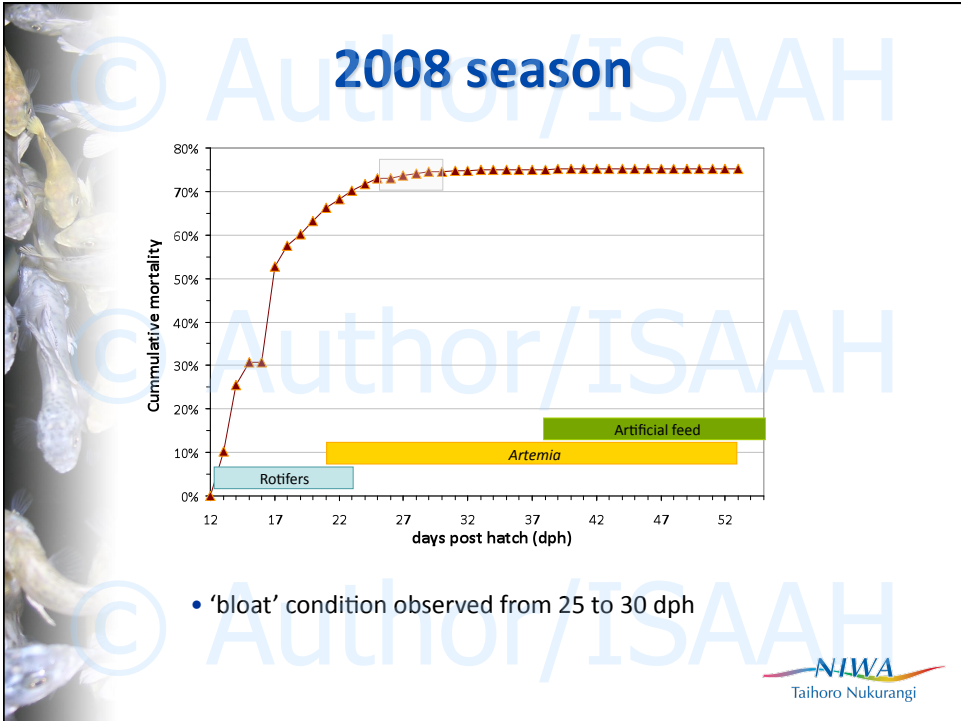
The importance of live feed management: lessons learned from New Zealand groper (*Polyprion oxygeneios*) juveniles

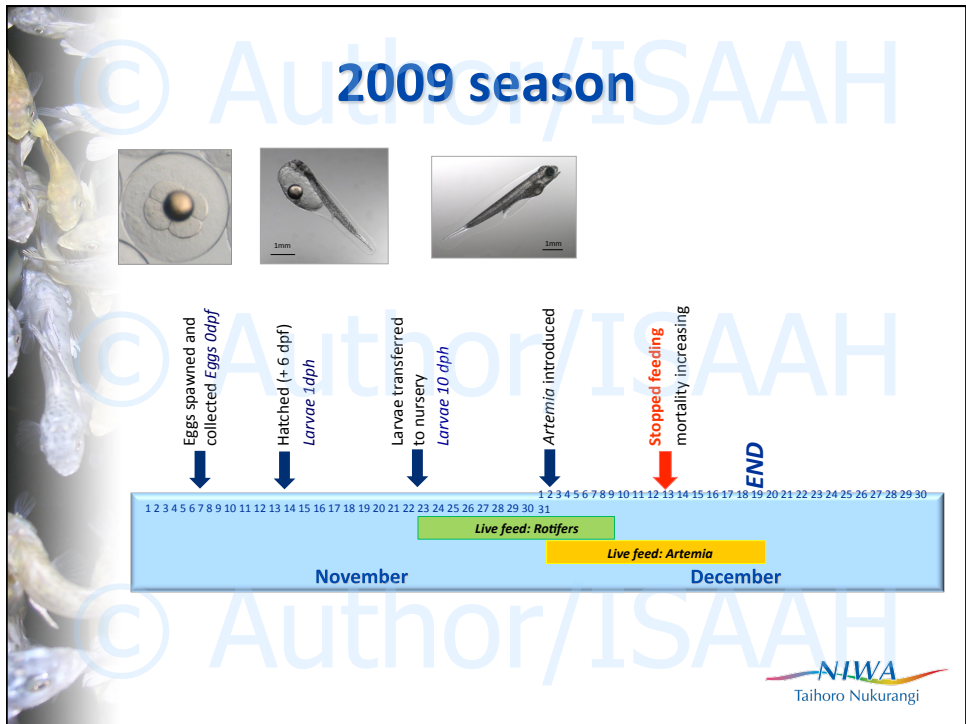
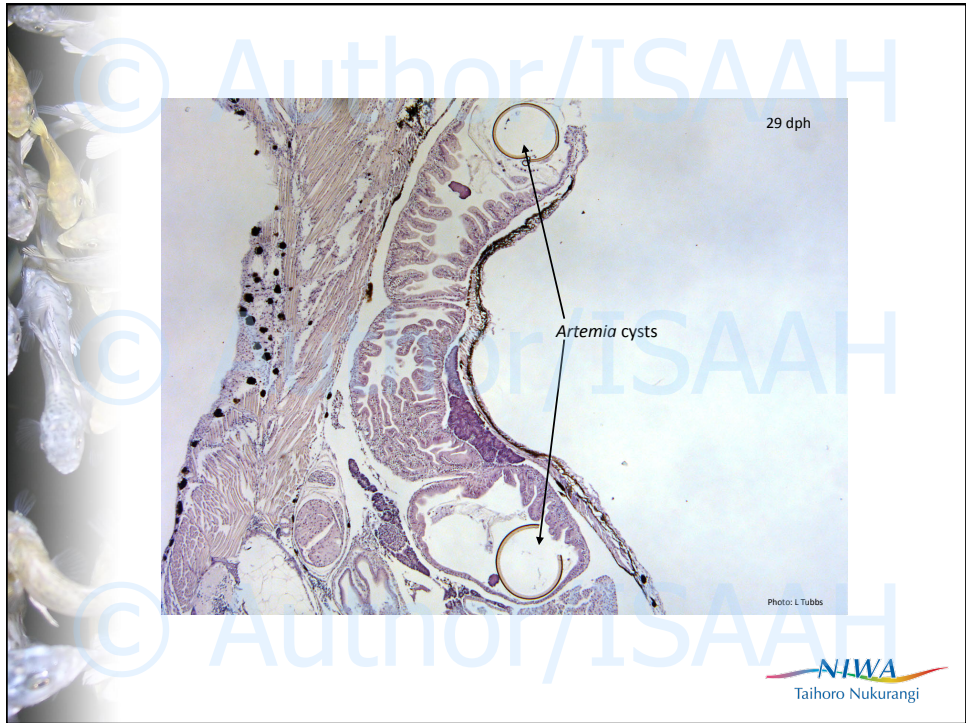
Sally Anderson*, I. Salinas, L. Tubbs, J.S. Lumsden, B. Diggles, V. Webb, Y. Gublin, S. Pether, G. Irvine and S. Walker

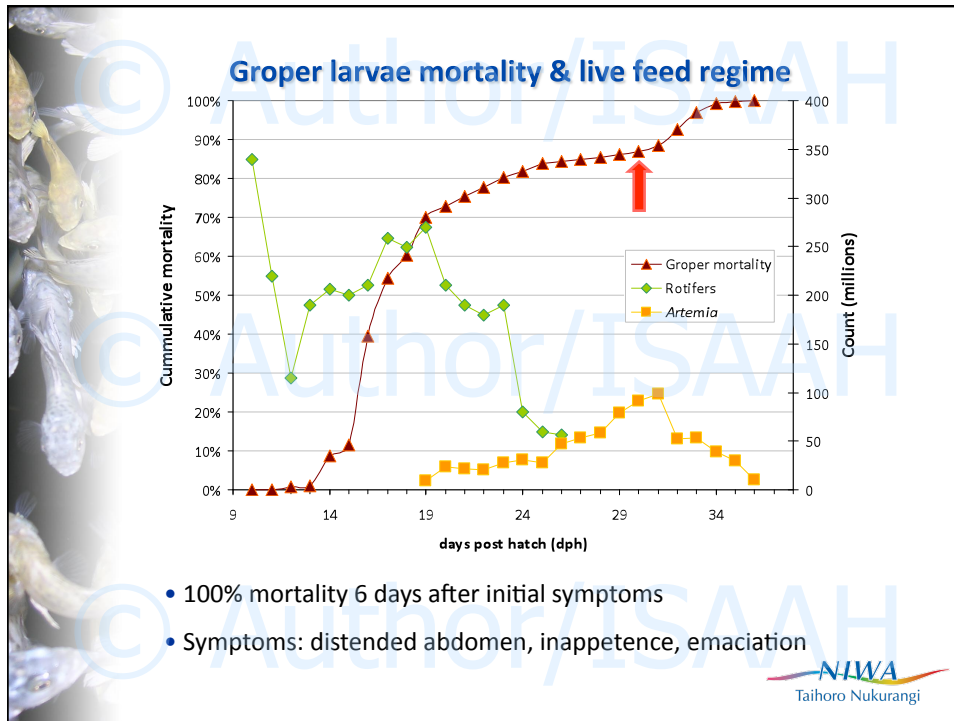


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Date	Sample Type	Analysis
15 Dec 2009	Whole larvae, 31 dph Alive and dead specimens	Histology, 4% PFA
15 Dec 2009	Excised gut tissue from larvae, 31 dph	Microbiology
15 Dec 2009	Artemia collected directly from tank	Microbiology
16 Dec 2009	Artemia culture - prepared as live feed	Microbiology
18 Dec 2009	Excised gill tissue from larvae, 34 dph	Microbiology

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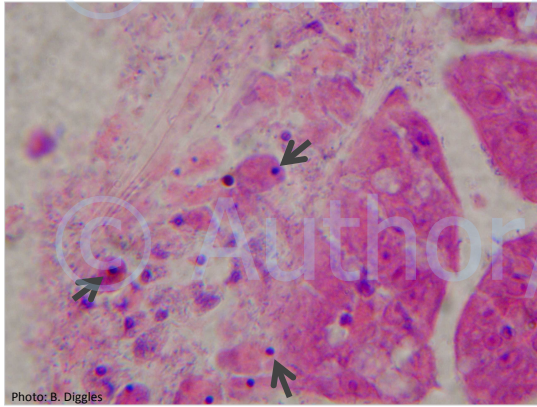


Photo: B. Diggle

- Bacterial enteritis
- Large numbers of bacteria surround pyknotic intestinal epithelium (arrows)
- Cellular necrosis
- Sloughing of epithelial cells into intestinal lumen

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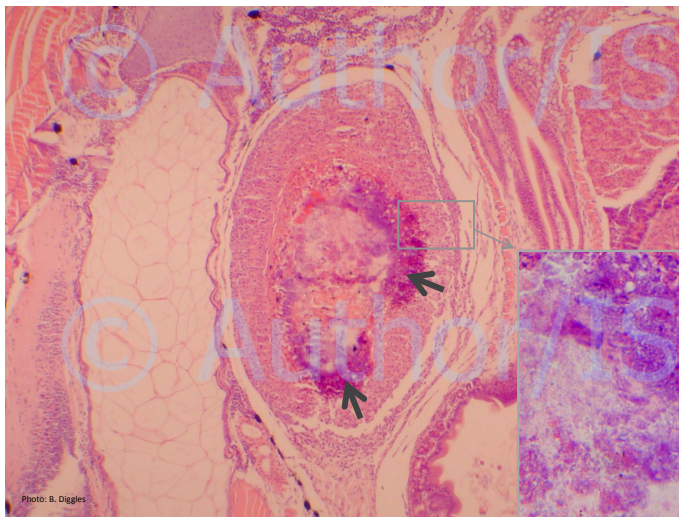
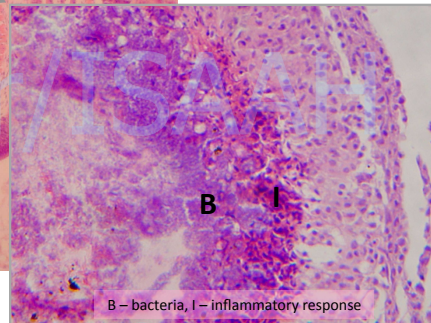


Photo: B. Diggle

- swimbladder filled with bacteria
- inflammatory host response
- peritonitis
- significant necrosis & systemic bacteremia



B – bacteria, I – inflammatory response

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Microbiology

- Bacterial isolation – marine agar, TCBS
- Isolate characterisation - Gram, catalase, oxidase, haemolysis
- Isolate identification - 16S rDNA sequencing, RFLP - *Hae*III digest

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NCBI Blast:Gropser: gut (Art3)

>|_gb|DQ003270.1 Vibrio ichthyenteri isolate HQ010223-1 16S ribosomal RNA gene, partial sequence
Length=1429

Score = 2562 bits (1387), Expect = 0.0
Identities = 1403/1410 (99%), Gaps = 4/1410 (0%)
Strand=Plus/Plus

Query 3 AGTCGAGCGTAAACAGAGAAAAGCTTGCTTT
Sbjct 15 AGTCGAGCGTAAACAGATAGAAAAGCTTGCTTT

Query 63 TAATGCCTGGGAATATGCCTTGATGTGGGGAT
Sbjct 75 TAATGCCTGGGAATATGCCTTGATGTGGGGAT

Query 123 CATAATGCTACGGGCCAAGAGGGGATCTTC
Sbjct 135 CATAATGCTACGGGCCAAGAGGGGATCTTC

Query 183 GTGGGATTAGCTAGTTGGTGGGTAATGGCTCA
Sbjct 195 GTGGGATTAGCTAGTTGGTGGGTAATGGCTCA

Query 243 AGAGGATGATCAGCCACTGGAACCTGAGACG
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Query 303 TGGGGAATATTGCACAATGGGCGCAAGCCTGAT
Sbjct 315 TGGGGAATATTGCACAATGGGCGCAAGCCTGAT

Query 363 CCTCGGGTTGTAAGCACTTTCAGTCGTGAGG
Sbjct 375 CCTCGGGTTGTAAGCACTTTCAGTCGTGAGG

Vibrio ichthyenteri isolate HQ010223-1 16S ribosomal RNA gene, partial sequence

GenBank: DQ003270.1

FASTA Graphics

Features Sequence

LOCUS DQ003270 1429 bp DNA linear BCT 02-MAY-2005

DEFINITION Vibrio ichthyenteri isolate HQ010223-1 16S ribosomal RNA gene, partial sequence.

ACCESSION DQ003270

VERSION DQ003270.1 GI:42912506

KEYWORDS .

SOURCE Vibrio ichthyenteri

ORGANISM Vibrio ichthyenteri
Bacteria; Proteobacteria; Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio.

REFERENCE 1 (bases 1 to 1429)
Chen, C., Fang, H. and Zhang, X.
TITLE Identification and phylogenetic analysis of pathogenic bacteria isolated from flounder (Paralichthys olivaceus)

JOURNAL Unpublished

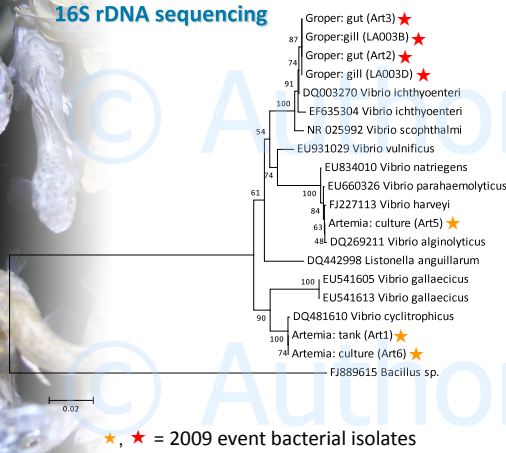
REFERENCE 2 (bases 1 to 1429)
Chen, C., Fang, H. and Zhang, X.
TITLE Direct Submission
JOURNAL Submitted (09-APR-2005) Department of Animal Science, Hebei Normal University of Science and Technology, Chengli, Qinhuangdao, Hebei 046600, P.R.China

FEATURES
location/Qualifiers
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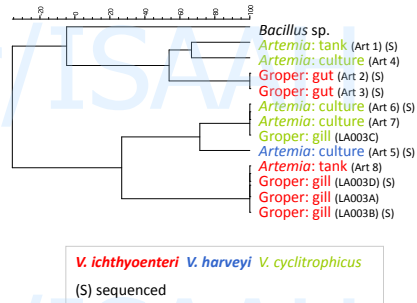
Microbiology

- Bacterial isolation – marine agar, TCBS
- Isolate characterisation - Gram, catalase, oxidase, haemolysis
- Isolate identification - 16S rDNA sequencing, RFLP - *Hae*III digest

16S rDNA sequencing

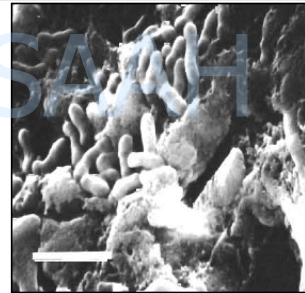


RFLP - *Hae*III digest



Vibrio ichthyenteri


- Bacterial pathogen, intestinal necrosis
 - Japanese Flounder Larvae (*Paralichthys olivaceus*)
 - *Vibrio* spp. INFL (intestinal necrosis of flounder larvae) (Muroga *et al.* 1990)
- Clinical symptom: Opaque intestine = intestinal necrosis
- Histology: necrosis and sloughing of the intestinal mucosa
- Disease reproduced by oral challenge via live feed (Muroga *et al.* 1990, Kim *et al.* 2004)




V. ichthyenteri on the mucosal surface of a flounder larva intestine (from Muroga *et al.* 1990)

Muroga *et al.* 1990. *Dis. aquat. Org.* 9:121-125; Kim *et al.* 2004. *J. Fish Dis.* 27: 497-505







2008	2009
<ul style="list-style-type: none"> • inappetance • distended abdomen • extracellular bacteria in GI tract • hatched <i>Artemia</i> cysts in gut • bacterial enteritis • elevated mortality (5 days) • survivors 	<ul style="list-style-type: none"> • inappetance, emaciation • distended abdomen • bacterial enteritis • necrosis of the intestinal epithelium • systemic bacteriemia • isolated <i>V. ichthyenteri</i> from larval gut and gill tissue • <i>V. ichthyenteri</i> associated with <i>Artemia</i> • 100% mortality (~6 days) • no survivors



Summary

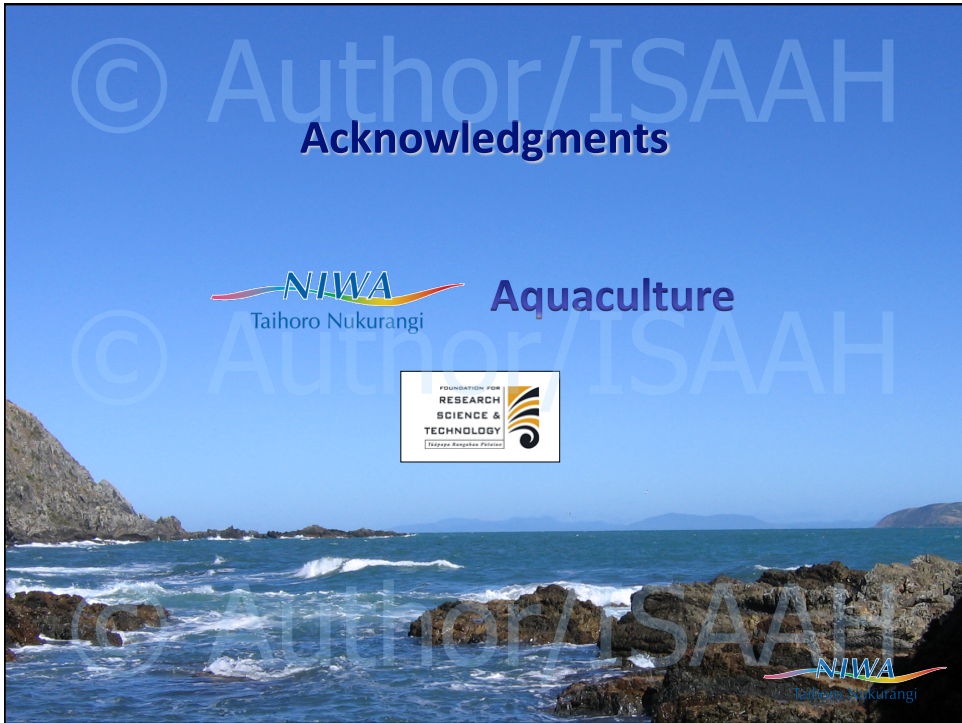
- New Zealand groper larvae:
 - susceptible at ~ 25 to 30 dph
 - '08 and '09 – bacterial pathogen, *Vibrio* sp.
 - *Artemia* food source
- Improvements in live feed production:
 - *Hygiene improvements* – equipment cleaning
 - *Minimise bacterial contamination* associated with live feed
 - disinfection
 - *Alternative therapies*
 - probiotics

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Acknowledgments



Aquaculture



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