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**Pathogens and probionts of ornate spiny lobster**  
**(*Panulirus ornatus*) phyllosoma**

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A thesis submitted for the degree of Doctor of Philosophy of the  
University of New England

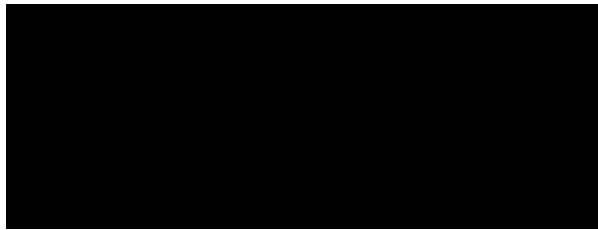
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## Declaration

I certify that the substance of this thesis has not already been submitted for any other degree and is not currently being submitted for any other degree or qualification.

I certify that the help received in preparing this thesis and all sources used have been acknowledged in this thesis.



Evan Goulden

3<sup>rd</sup> December, 2011

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## Abstract

Wild capture production of economically important spiny lobster (Palinuridae) fisheries has stagnated or declined in recent decades, and with an array of global climatic, economic and demographic challenges limiting the opportunities to enhance production, alternative supplies need to be sourced through aquaculture. Among the prospective aquacultures species is the ornate spiny lobster (*Panulirus ornatus*), however sustainable farming will only be met through refining larviculture technologies and the development of disease management strategies to counter epizootics. This study investigated bacterial pathogens causing epizootics in *P. ornatus* larviculture and examined a potential biocontrol strategy using probiotics.

The type strain of *V. owensii* (DY05) was validated by Koch's postulates as the aetiological agent of disease causing rapid and reproducible mortalities of early stage *P. ornatus* phyllosoma larvae. The pathogenicity and infection cycle of *V. owensii* DY05 were investigated using *in vivo* experimental infection models and a *V. owensii* DY05 transconjugant expressing a fluorescent protein (FP), which showed *Artemia*-vectored challenge to be a crucial determinant in the infection process. Soon after ingestion by phyllosomas (6 h), fluorescently labelled cells were monodispersed in the proventriculum (foregut) and hepatopancreas (midgut gland), which preceded mass proliferation in the hepatopancreas and evacuation of planktonic cells into the ambient environment. Continued bacterial proliferation lead to systemic infection and the sustained presence of cells in phyllosoma tissues *post mortem*. Clinical pathologies included hepatopancreatic epithelial cell necrosis, and *in vitro* enzyme assays identified proteases, phospholipases, and haemolysins as potential virulence factors. The observations indicated *V. owensii* DY05 is a specialist enteropathogen of *P. ornatus* phyllosomas, using vector-mediated transmission and release from host-associations to a planktonic existence to perpetuate transfer.

Over 500 bacterial isolates from natural environments associated with wild *P. ornatus* phyllosomas and culture collections were screened *in vitro* for antagonistic activity towards *V. owensii* DY05. Taxonomic grouping and further *in vitro* screening using miniaturised coculture and biofilm assays enabled rapid processing and selection of a two-strain probiotic

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combination (*Vibrio* sp. PP05 and *Pseudoalteromonas* sp. PP107) which additively and reproducibly protected *P. ornatus* phyllosomas from *V. owensii* DY05 infection. The probiont-pathogen-host interactions were investigated *in situ* using FP-expressing transconjugant strains which revealed that the *V. owensii* DY05 infection cycle was intercepted during vectored transmission. It was hypothesised that probiont niche specialisation contributed to the additive protective benefit on phyllosomas. These results indicate that the ingestion of the pathogen by phyllosomas could be averted through administration of *Artemia* preemptively treated with the probiotic strains in scaled-up hatchery experiments. This study provides the first step towards combating epizootic pathogens in *P. ornatus* larviculture using a biocontrol strategy consisting of antagonistic bacteria.

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## Research Publications

### *Chapters 1 and 6 (excerpts)*

**Goulden, E.F.**, Høj, L., and Hall, M.R. (submitted). Microbial management for bacterial pathogen control in invertebrate hatcheries. In G. Allan and G. Burnell (Eds.), *Advances in aquaculture hatchery technology*. Woodhead Publishing, Cambridge, UK.

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**Goulden, E.F.**, Hall, M.R., Bourne, D.G., Pereg, L.L., and Høj, L. (2012). Pathogenicity and infection cycle of *Vibrio owensii* in larviculture of ornate spiny lobster (*Panulirus ornatus*). *Appl. Environ. Microb.* **78**: 2841–2849.

### *Chapter 4*

Goulden, E.F., Hall, M.R., Pereg, L.L., and Høj, L. (accepted). Identification of an antagonistic probiotic combination protecting ornate spiny lobster (*Panulirus ornatus*) larvae against *Vibrio owensii* infection. *PLoS One*.

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### *Other relevant publications*

Cano-Gómez, A., **Goulden E.F.**, Owens, L., and Høj, L. (2010). *Vibrio owensii* sp. nov., isolated from cultured crustaceans in Australia. *FEMS. Microbiol. Lett.* **302**: 175-181.

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**Goulden, E.F.**, Hall, M.R., Bourne, D.G., Payne, M.S., Wietz M, Pereg-Gerk, L., and Høj L. (2009). Potential use of probiotics in ornate spiny lobster (*Panulirus ornatus*) larviculture. Oral presentation, 4th FRDC Aquatic Animal Health Subprogram Scientific Conference, 22-24 July 2009, Cairns, Australia.

Høj, L., **Goulden, E.F.**, Cano Gomez, A., Dorigny, A., Owens, L., Bourne, D.G., Pereg-Gerk, L, and Hall MR (2009). *Vibrio* sp. DY05, a new pathogen of larvae of the ornate spiny lobster (*Panulirus ornatus*). Poster presentation, Vibrio 2009, 4-6 September, 2009, Rio de Janeiro, Brazil.

Hall, M., Smith, G., **Goulden, E.**, and Høj, L. (2009). Diet and microbial interactions in Palinurid lobster larvae. Oral presentation, 5<sup>th</sup> Fish & Shellfish Larviculture Symposium, 7-10 September, 2009, Ghent, Belgium.

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Hall, M., Smith, G., **Goulden, E.** and Høj, L (2010). Microbial and diet interactions in Palinurid lobster larvae. Oral presentation, Australasian Aquaculture Conference 2010, 23-26 May 2010, Hobart, Australia.

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Høj, L., Cano Gomez, A., **Goulden, E.F.**, Owens, L., Andreakis, N., Pereg, L., and Hall, M.R. (2011). *Vibrio owensii* - a pathogen for prawn and lobster larvae. Invited presentation, Marine Pathogens Symposium, Annual scientific meeting 2011, Australian Society for Microbiology, 4-8 July 2011, Hobart, Australia.

**Goulden, E.F.**, Hall, M.R., Pereg, L.L., and Høj, L. (2011). Probiotic protection against *Vibrio owensii* and *in situ* visualisation of bacterial interactions associated with the phyllosoma of *Panulirus ornatus*. Oral presentation, 1st FRDC Australasian Scientific Conference on Aquatic Animal Health, 5-8 July, 2011, Cairns, Australia.

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