

***Kudoa neurophila* in striped trumpeter: identification,
diagnostic development and histopathology**

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Declaration of originality

This thesis contains no material which has been accepted for a degree or diploma by this university or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis.

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The following people and institutions contributed to the publication of the work undertaken as part of this thesis:

G.W. Gossel, **I. Dyková¹**, **J. Handler²** and **B.L. Munday³**. *Pentacapsula neurophila* sp.n. (Multivalvulida) from the central nervous system of striped trumpeter, *Latris lineata* (Forster). Journal of Fish Diseases 2003, 26, 315-320.

C.M. Whipps⁴, G.W. Gossel, **R.D. Adlard⁵**, **H. Yokoyama⁶**, **M.S. Bryant⁷**, B.L. Munday and **M.L. Kent⁸**. The phylogeny of the Multivalvulidae (Myxozoa: Myxosporea) based upon comparative rDNA sequence analysis. Journal of Parasitology 2004, 90, 618-622.

G.W. Gossel, J. Handler, **S. Battaglione⁹** and B.L. Munday. Diagnostic polymerase chain reaction assay to detect *Kudoa neurophila* (Myxozoa: Multivalvulida) in a marine finfish hatchery. Diseases in Aquatic Organisms 2005, 64, 141-149.

J.M. Cobcroft¹⁰, S.C. Battaglione, **M.P. Bransden¹¹**, and G.W. Gossel. Management of larval health in cultured striped trumpeter *latris lineata*. C.I. Hendry, G. Van Stappen, M. Wille and P. Sorgeloos (Eds) European Aquaculture Society, Special Publication No. 36, Oostende, Belgium, 2005.

G.W. Gossel, **J.T. Ellis¹²**, J. Handler and B.L. Munday. Small subunit rDNA phylogeny places *Pentacapsula neurophila* (Myxosporea: Multivalvulida) within Kudoidae. Submitted to Systematic Parasitology and under review.

Contribution:

Author 1. I. Dyková. Institute of Parasitology, Czechoslovak Academy of Sciences, Branisovka, Czech Republic. Described the morphological characteristics of the parasite.

Authors 2 & 3. J. Handler and B.L. Munday. Tasmanian Aquaculture & Fisheries Institute, Fish Health Unit, Launceston, Tasmania, Australia. School of Human Life Sciences, University of Tasmania, Launceston, Tasmania, Australia respectively. Provided guidance and supervision in all aspects of producing publishable quality manuscripts.

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Authors 5, 6, 7 & 8. R.D. Adlard, H. Yokoyama, M.S. Bryant and M.L. Kent. Various appointments. Provided genetic sequence information and phylogenetic analysis and assisted to produce a publishable quality manuscript.

Authors 9, 10 & 11. S. Battaglene, M.P. Bransden and J.M. Cobcroft. Tasmanian Aquaculture & Fisheries Institute, Marine Research Laboratories, Taroona, Tasmania, Australia. S. Battaglene provided guidance and supervision in all aspects of producing publishable quality manuscripts. J.M. Cobcroft and M.P. Bransden were the main contributing authors.

Author 12. J.T. Ellis. Department of Cell and Molecular Biology, University of Technology, Sydney, NSW, Australia. Provided guidance and supervision in all aspects of producing publishable quality manuscripts.

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Journal articles and presentations to learned societies arising from the work described in this thesis.

Articles.

G.W. Grossel, I. Dyková, J. Handler and B.L. Munday. *Pentacapsula neurophila* sp.n. (Multivalvulida) from the central nervous system of striped trumpeter, *Latris lineata* (Forster). Journal of Fish Diseases 2003, 26, 315-320 (Appendix 6.1).

C.M. Whipps, **G.W. Grossel**, R.D. Adlard, H. Yokoyama, M.S. Bryant, B.L. Munday and M.L. Kent. The phylogeny of the Multivalvulidae (Myxozoa: Myxosporea) based upon comparative rDNA sequence analysis. Journal of Parasitology 2004, 90, 618-622 (Appendix 6.2).

G.W. Grossel, J. Handler, S. Battaglene and B.L. Munday. Diagnostic polymerase chain reaction assay to detect *Kudoa neurophila* (Myxozoa: Multivalvulida) in a marine finfish hatchery. Diseases in Aquatic Organisms 2005, 64, 141-149. (Appendix 6.3).

J.M. Cobcroft, S.C. Battaglene, M.P. Bransden, and **G.W. Grossel**. Management of larval health in cultured striped trumpeter *Latris lineata*. C.I. Hendry, G. Van Stappen, M. Wille and P. Sorgeloos (Eds) European Aquaculture Society, Special Publication No. 36, Oostende, Belgium, 2005.

Articles under review.

G.W. Grossel, J.T. Ellis, J. Handler and B.L. Munday. Small subunit rDNA phylogeny places *Pentacapsula neurophila* (Myxosporea: Multivalvulida) within Kudoidae. Submitted to Systematic Parasitology and under review (Appendix 6.4).

Presentations to learned societies.

G.W. Grossel, J.T. Ellis, J. Handler, I. Dyková and B.L. Munday.

Meningoencephalomyelitis causing mortality in striped trumpeter *Latris lineata* due to infection with a *Pentacapsula* sp. (Myxosporea). Impacts of Myxozoan Parasites in Wild and Farmed Fish, 31st July-2nd August, 2002, Abstract p. 20, Nanaimo. Malaspina University-College Printing and Duplication, Nanaimo.

G.W. Grossel, J. Handler and B.L. Munday. Polymerase chain reaction diagnostic assay to detect *Pentacapsula neurophila* (Multivalvulida), the cause of brain disease in cultured striped trumpeter *Latris lineata* (Forster 1801). Australian Society for Parasitology Conference, Darwin, N.T., 6th – 10th July 2003.

G.W. Grossel, J. Handler and S. Battaglione. Pathology, epizootiology and control of *Kudoa neurophila* in cultured striped trumpeter. Australian Society for Parasitology Conference, Fremantle, W.A., 26th – 30th September 2004.

Abstract

Striped trumpeter, *Latris lineata* (Forster), are being experimentally cultured by the Tasmanian Aquaculture and Fisheries Institute (TAFI) at Taroona, Hobart, Tasmania, Australia. Fish that survive beyond 30 days develop nervous aberrations associated with a severe granulomatous meningoencephalomyelitis. The myxozoan parasite *Pentacapsula neurophila* was described as the parasite causing the disease in the striped trumpeter juveniles. Molecular Bayesian phylogenetic analysis using small subunit ribosomal DNA (ssu rDNA) gene sequence and the covariotide evolutionary model, has shown *P. neurophila* to reside firmly within the clade comprised of *Kudoa* species, histozoic parasites of fish from the order Multivalvulida with 4 or more shell valves containing polar capsules. This has provided molecular evidence resulting in the proposed new combination of the Kudoidae to include this *Pentacapsula* species.

A polymerase chain reaction (PCR) diagnostic assay was developed from the ssu rDNA gene sequence to detect *Kudoa neurophila* (formerly known as *Pentacapsula neurophila*). The assay is sufficiently species specific and sensitive enough to detect a small fragment of the parasite ssu rDNA gene (0.1 spore or 60 fg DNA or 4 spores g⁻¹ / 25 µl PCR reaction). Specifically, the test is capable of detecting early stages of the life cycle within the fish host and consequently diagnosing an infection not normally detected using histology. The PCR test can also be used to screen water supplies and prey cultures throughout the hatchery system to determine bio-security efficacy, assist in epizootiology studies, identify infected alternative or other primary hosts indicating the location of the disease reservoir, and enable a targeted approach to disease prevention in an aquaculture situation.

Histology and *in situ* hybridisation techniques were incorporated into the study of the histopathology of the disease caused by this parasite to elucidate its entry point on the fish host and migratory pathway to terminal stage sporulation. *Kudoa neurophila* enters the fish via epithelial cells as early as 25 days post hatch (dph). Parasite cells then appear within plasmodia in skeletal muscle tissue between 50 – 80 dph. This appears to be the first key proliferation stage which is followed by another plasmodial stage in the peripheral nerve pathways near the spinal cord (70 – 115 dph). It is during this stage that clinical signs of the disease, such as whirling, become apparent. The presporogonic cells then enter the spinal cord where terminal stage sporulation occurs throughout the brain and spinal cord (105 – 130 dph) causing acute pathology, eventually resulting in the death of the animal.

The information gained from the research conducted in this thesis, along with incorporated epizootiology studies, form the foundation of understanding that has assisted the hatchery management team to make informed health management decisions with an outlook to produce healthy juvenile striped trumpeter for on-going research into the development of this species for marine sea cage aquaculture.