ALTERNATIVE SOURCES OF OMEGA-3 OILS FOR BARRAMUNDI, *Lates calcarifer*, AQUACULTURE

By

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B.Sc., Grad. Dip. Animal Husbandry

A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

National Centre for Marine Conservation and Resource Sustainability

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The research associated with this thesis abides by the international and Australian codes on animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University. The University of Tasmania Animal Ethics Committee approved the experimental plan for works included in this thesis through permits number A0010171 and A0011588.

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STATEMENT OF CO-AUTHORSHIP

The following people contributed to the publication of the work undertaken and included in the thesis body, which is:

1- Alhazzaa, R., Bridle, A.R., Nichols, P.D. and Carter, C.G. (2011): Replacing dietary fish oil with Echium oil enriched barramundi with C_{18} PUFA rather than long-chain PUFA. *Aquaculture* 312, 162-171.

2- Alhazzaa, R., Bridle, A.R., Nichols P.D. and Carter, C.G. (2011): Up-regulated desaturase and elongase gene expression promoted accumulation of polyunsaturated fatty acid (PUFA) but not long-chain PUFA in Lates calcarifer, a tropical euryhaline fish fed a stearidonic- and γ-linoleic acid enriched diet. *Journal of Agricultural and Food Chemistry* 59, 8423–8434.

3- Alhazzaa, R., Bridle, A.R., Nichols, P.D. and Carter, C.G.: Tropical ectotherm coping with sub-optimal temperature: modifications in fatty acid influenced by dietary lipid. *In preparation*.

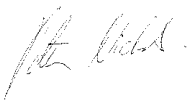
4- Alhazzaa, R., Bridle, A.R., Mori, T, A. and Barden A, E., Nichols, P.D. and Carter, C.G.: Dietary lipid modulation of fatty acid composition and immunity in barramundi, *Lates calcarifer*, following disease challenge. *In preparation.*

5- Alhazzaa, R., Bridle, A.R., Nichols, P.D. and Carter, C.G. (2012): Sesamin modulation of lipid class and fatty acid profile in early juvenile teleost, *Lates calcarifer*, fed different dietary oils. *Food Chemistry* 134, 2057–2065.

Bridle, A. R., Nichols, P. D. and Carter, C. G. assisted with the general supervision of all aspects of producing this thesis. These included experimental design, interpretation of data and proofreading of manuscripts (10% of Chapters)

Mori, T, A. and Barden A, E. assisted with laboratory analysis of eicosanoid, interpretation of data and proofreading of Chapter 5 (10% of the Chapter)

We the undersigned agree with the above stated "proportion of work undertaken" for each of the above published or in preparation manuscripts contributing to this thesis.

Supervisor	Prof. C. G. Carter	 .....
Supervisor	Dr. P. D. Nichols	 .....
Supervisor	Dr. A. R. Bridle	 .....
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ABSTRACT

Fish oil (FO) is the major source of dietary lipid in carnivorous fish feeds including barramundi, *Lates calcarifer*, which is widely farmed in Asia and Australia. However, recent increases in FO prices, increased demand and the foreseen inability of wild fisheries to meet future requirements have created a need for cheaper and more sustainable alternatives. Vegetable oils (VO) can be produced in sufficient quantities to meet the growing aquaculture demand, although they lack the long-chain ($\geq C_{20}$) polyunsaturated fatty acids (LC-PUFA) beneficial to human consumers. Some VO like rapeseed oil (RO), echium oil from *Echium plantagineum* (EO) and linseed oil (LO) have high levels of n-3 and n-6 short-chain ($\leq C_{18}$) PUFA that can accumulate or be converted into LC-PUFA by some fish species, although generally at low efficiency, and not to docosahexaenoic acid. In a series of comparative and factorial experiments, I investigated the growth and lipid changes of barramundi fed different dietary oils: FO, RO, LO and EO over conditions covering: a range of salinities and temperatures, subject to immunity stress or supplemented with plant-derived bioactive ingredients. In general, growth performance parameters were comparable for FO and VO treatments, and resulted in accumulation of VO-derived n-3 and n-6 PUFA. Salinity has no direct effect on growth or lipid metabolism regardless of the dietary lipid source. Endogenous conversion by barramundi of dietary PUFA into LC-PUFA is limited by more than one rate-limiting step and there is a preference for incorporation of LC-PUFA into the polar lipid fraction rather than neutral lipid. The growth of barramundi slowed at sub-optimal (20°C) temperature compared to optimal (30°C) temperature. PUFA from dietary VO deposits in muscle and are maintained under rapid temperature decreases. In contrast, excess LC-PUFA from FO depleted faster than occurs in VO fed fish. The production of pro-inflammatory eicosanoids in fish fed FO was lower than for fish fed VO following bacterial infection. EO significantly suppressed the production of the pro-inflammatory mediators compared to RO. Sesamin, a lignan in sesame seed, enhanced the conversion of dietary PUFA into LC-PUFA for the n-3 series rather than n-6 in early juvenile barramundi. However, sesamin had negative impact on fish growth at this early life-stage. Barramundi fed on VO are a rich source of LC-PUFA precursors, α-linolenic and stearidonic acid, and grow well under the different environmental conditions that are typical of outdoor barramundi farms. The use of terrestrial VO containing the LC-PUFA precursors and plant-derived bioactive compounds show promise for use in barramundi aquafeed in terms of fish growth and health as either partial or complete alternatives for FO. However, using currently available VO, high content of the n -3 LC-PUFA is not achieved.

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