5 GENERAL DISCUSSION/CONCLUSION

This thesis reported on the use of *in vivo* and *in vitro* systems to elucidate the LCPUFA synthesis capabilities of freshwater, anadromous and marine fish species. Chapter 2 reported on the effect of GSE as a natural antioxidant substitute for the synthetic antioxidant ethoxyquin in the feed of YTK. This was assessed by studying the lipid oxidation and fatty acid compositional changes associated with fillet storage. Although Chapter 2 did not specifically investigate the *in vivo* enzymatic capabilities of the LCPUFA synthesis pathway in YTK, it did generate a number of interesting findings about the end product of the pathway, DHA. The human consumer should be aware that there is large variability in the proportion of fillet DHA within YTK. We also found that the proportion of DHA in the fillet was decreased after storage at 4°C for 4 days, regardless of ethoxyquin or GSE antioxidant protection. The apparent genetic variation in the capability of YTK to bioaccumulate DHA may be enzymatically controlled, at least in part, by the LCPUFA synthesis pathway. Selecting YTK with superior LCPUFA synthesis capabilities for breeding may increase the human nutritional value of this species further in the future.

Chapter 3 reported on the use of an *in vitro S. cerevisisae* over-expression system to characterise the SBT Elovl5 and Δ6desaturase enzymes. This *in vitro* system allowed us to examine the accumulation of PUFA and the synthesis of LCPUFA following supplementation with individual or multiple PUFA substrates. This system was particularly useful in determining the Elovl5 substrate preference for C_{18} and C_{20} PUFA rather than C_{22} PUFA substrates, together with a preference for n-3 PUFA substrates rather than n-6 PUFA substrates. Likewise, when multiple substrates of various chain lengths were supplemented, we could determine that the Elovl5 preferred C_{18} PUFA substrates rather than C_{20} PUFA substrates. The SBT Δ6desaturase was expressed at very low levels in the *S. cerevisiae* expression system and functionality was not observed. It was expected that an obligate carnivore like SBT may have reduced LCPUFA synthesis enzyme capabilities due to the structure of the marine food chain. This thesis used the *S. cerevisisae* over-expression system to show that the SBT Elovl5 has high enzymatic activity but it can only be speculated

that a Δ6desaturase enzyme deficiency may be the limiting factor in the production of DHA from ALA in SBT.

Chapter 4 used *in vitro* cell systems to examine the LCPUFA synthesis capabilities of freshwater and anadromous fish species. The FHM and CHSE-214 cell lines were supplemented with PUFA to examine the accumulation of the PUFA substrate and the subsequent LCPUFA products in the cell phospholipids. Conversion of immediate substrates to either desaturated or elongated products revealed that the CHSE-214 cells had functional Δ6desaturase, Elovl5 and Elovl2 enzymes. In contrast, the FHM cell line displayed the ability to elongate PUFA substrates but did not efficiently desaturate PUFA substrates. This finding was unexpected as freshwater and anadromous fish species are considered to be able to convert C_{18} PUFA to C_{22} and C_{24} LCPUFA using the LCPUFA synthesis pathway. The fish cell lines were used in an attempt to model the accumulation of PUFA and LCPUFA in the cell phospholipids in fish *in vivo*. The absence of Δ5desaturase and Δ6desaturase activity in the FHM cell line highlighted the need for caution when using cell lines for predicting LCPUFA synthesis in whole fish.

Insight into possible future research has been gained from each of the chapters in this thesis. YTK are an example of an aquaculture species which may benefit from genetic selection for fish with a higher proportion of fillet DHA. YTK with a higher capability to accumulate DHA could be used in breeding programs to enhance the fillet fatty acid profile of YTK. The marketability of YTK fillets with a higher DHA content will increase, as the loss of DHA during storage, which was shown in Chapter 2, may be offset. Likewise, when SBT are routinely being bred in captivity in the future they could be genetically screened for selection of fish with superior LCPUFA synthesis enzymatic capabilities. The findings in Chapter 3 suggest that the SBT Elovl5 enzyme has high activity while more work needs to be performed with the *S. cerevisiae* expression system to determine the Δ6desaturase enzymatic capabilities. If future research confirms my speculations regarding the apparent low level of Δ6desaturase enzyme activity, introducing a foreign Δ6desaturase into SBT may be a method of increasing the LCPUFA synthesis capability of SBT. More research into the LCPUFA synthesis capabilities of freshwater and anadromous fish species needs to be conducted to uncover suitable host genes for transgenic

modification of marine species. *In vitro* cell systems will continue to be used in the future as an affordable and convenient way to examine the LCPUFA synthesis capabilities of freshwater and anadromous fish species, as was done in Chapter 4. Future research must be conducted with characterized fish cell lines which have a relatively low passage number to ensure the *in vitro* model is a good representative of the *in vivo* system.

This thesis has used *in vivo* and *in vitro* systems to elucidate the LCPUFA synthesis capabilities of a variety of fish species. We have shown that the capability of YTK to bioaccumulate DHA into the fillet is variable and may be controlled in part by the enzymes in the LCPUFA synthesis pathway. The *S. cerevisisae* over-expression system enabled the functional characterisation of a SBT fatty acyl elongase, Elovl5, from the LCPUFA synthesis pathway which preferred C_{18} and C_{20} n-3 PUFA substrates. Finally, the CHSE-214 cell line was shown to have a functional LCPUFA synthesis pathway which made it a good model for the *in vivo* pathway in Chinook salmon. However, the FHM cell line was found to have a different LCPUFA synthesis capability to the *in vivo* pathway in fathead minnow which may be the result of a loss of enzymatic activity in culture.