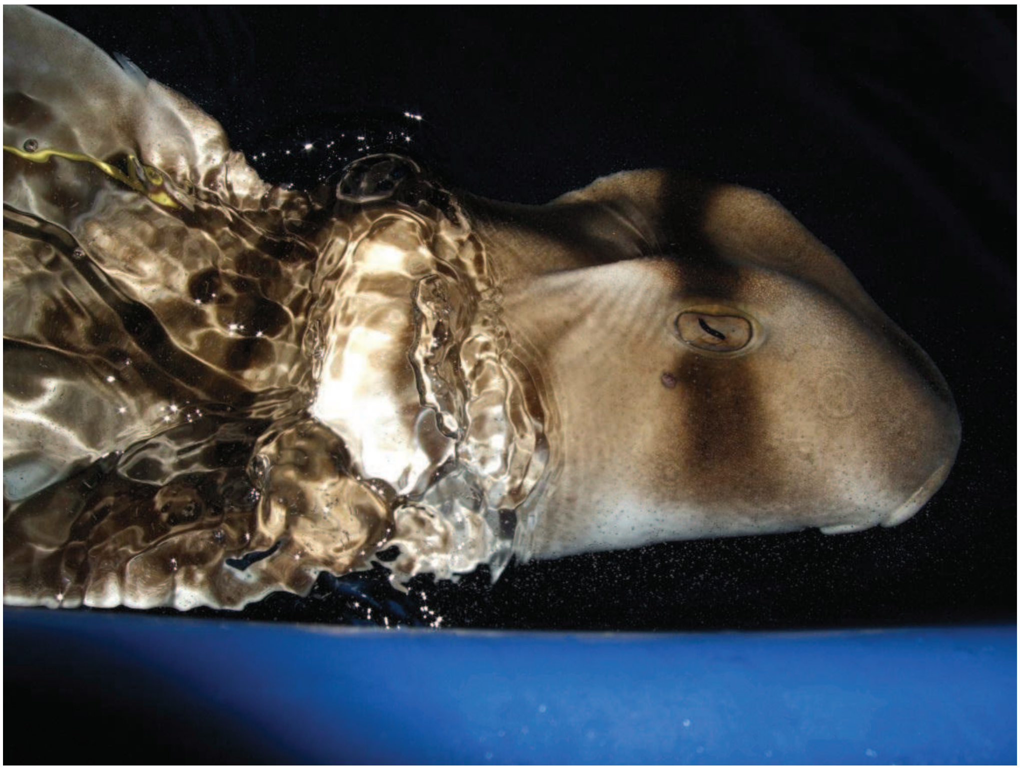
Fatty acid profiles of a benthic chondrichthyan*:* captive feeding trials and ecological applications



Crystal L. Beckmann

BSc Marine Biology (Hons)

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School of Biological Sciences

Flinders University

Adelaide, Australia

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Summary

Fatty acid analysis is a tool for dietary investigation that complements traditional stomach content analyses. There is little known about how sharks alter dietary fatty acids following incorporation into tissues, the stability and turn-over rates of fatty acid in tissues, and whether specific fatty acids are selectively retained within tissues. Four controlled feeding experiments were used to determine the extent to which the fatty acid composition of diet is reflected in the tissues of the Port Jackson shark *Heterodontus portusjacksoni* and to improve our understanding of which fatty acid biomarkers can be used to reliably distinguish prey types. The first experiment tested the fatty acid profile of muscle and liver tissues and used two groups of sharks fed exclusive diets of prawns or squid, and an unfed control group. The liver and muscle fatty acid profiles showed significant differences between the groups fed exclusive diets and unfed control sharks, suggesting that the extent of dietary change was strong enough that both tissues could be used as indicators of diet. The different diet fed to sharks could, however, only be distinguished based on the liver fatty acid profiles, with prawn-fed sharks comparatively higher in 18:1n-7, 22:5n-3, 20:0 and 18:1n-9, while squid-fed sharks were higher in 16:0 and 22:6n-3. The lack of differences in the muscle fatty acid profiles suggesting that diet was not different enough to cause a change in the muscle fatty acid in the duration of the experiment. The second experiment further investigated liver and muscle fatty acid profile dynamics during a dietary change and assessed the fatty acid profiles of sharks fed squid for six weeks followed by prawns for an additional six weeks. This experiment showed significant differences in the liver and muscle fatty acid profiles within three weeks of a dietary switch driven by 22:6n-3 in the liver and muscle and 16:1n-7 in the liver. Higher levels of dissimilarity were observed before and after the dietary change in the muscle tissue which may indicate that dietary fatty acids are preferentially used in the muscle following a dietary change. Changes in fatty acid profiles over time and different incorporation rates between tissues were also evident when sharks were fed exclusive diets of artificial fish or poultry oil pellets for a period of 18 weeks. The fatty acid profiles from the liver and blood serum of fish oil and poultry oil fed sharks were significantly different within 12 weeks while the muscle fatty acid profiles of fed sharks did not differ until week 18. The drivers of dissimilarity which aligned with dietary input were 14:0, 18:2n-6, 20:5n-3, 18:1n-9 and 22:6n-3 in the liver and blood serum. The fourth experiment used yolks sampled from viable egg cases and recently hatched neonates fed a known diet to determine whether the fatty acids present in high levels in yolks or in diets are reflected in the liver and muscle tissues of hatchlings. The fatty acid profiles of hatchling tissues were more similar to yolk than to diet, demonstrating the conservative transfer of fatty acid from egg yolks to hatchlings as well as the preferential retention of some fatty acids in the muscle and liver. Specifically, arachidonic acid (ARA, 20:4n-6) was preferentially retained likely as a result of eicosanoid production during growth; dietary docosapentaenoic acid (DPA, 22:5n-3) was not reflected in shark tissues and is likely catabolised for energy; docosahexaenoic acid (DHA, 22:6n-3) was reflected in tissues and was a good dietary indicator; and high proportions of saturated fatty acids were retained in the muscle at the expense of dietary polyunsaturated fatty acids.

The application of fatty acid profile analysis to investigate dietary information of wild specimens was assessed through the comparisons of fatty acid profiles and stomach contents from three locations. Fatty acid profile analysis indicated significant differences between the three locations sampled, however, stomach content analysis did not show significant differences between the two closest locations (Gulf St Vincent and Spencer Gulf). The discrepancy in the results from the two methods highlighted the ability of fatty acid profiles to complement information obtained from stomach content analysis. For example, soft-bodied prey such as molluscs which have their shells crushed and are rapidly digested, were underrepresented in the stomach content analysis, but could be readily detected in 16:1n-7 levels. Fatty acid analysis is increasingly powerful as a tool in studies of trophic ecology in marine ecosystems. This biochemical technique has become useful in deciphering spatial and temporal variability in diets, identifying predation on key species, and providing dietary information which is not always obtainable using more traditional methods.

Declaration

Statement of Originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis. 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. This thesis may be available for loan and limited copying in accordance to the *Copyright Act 1968*.

Crystal L. Beckmann

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Statement of Co-authorship

Chapter 1

The introduction was my own work. My supervisors, Charlie Huveneers and Jim Mitchell proofread earlier versions of this chapter.

Chapters 2–6 of this thesis have been prepared as scientific manuscripts and I am the primary author for each paper. Chapters which have been published are identified on the title page for the chapter. In all cases experimental design, field and laboratory work, data analysis and interpretation, and manuscript preparation were the primary responsibility of the candidate. However, they were carried out in collaboration with supervisors. Contributions of co-authors are outlined below.

Chapter 2 and 3

Charlie Huveneers provided advice on the experimental design. Co-authors provided information and important citations, and assisted to the drafting of the chapters by critically revising them.

Chapter 4, 5 and 6

Charlie Huveneers and David Stone provided advice on the experimental design. Co-authors provided information and important citations, and assisted to the drafting of the chapters by critically revising them.

Chapter 7

The conclusion was my own work. My supervisor, Charlie Huveneers proofread earlier versions of this chapter.

List of publications

Peer-Reviewed publications relevant to this thesis

Beckmann CL, Mitchell J, Seuront L, Stone DAJ and Huveneers C (2014) From egg to hatchling: preferential retention of fatty acid biomarkers in young-of-the-year sharks. Journal of Fish Biology. DOI: 10.1111/jfb.12451

Beckmann C, Mitchell JG, Stone DAJ and Huveneers C (2014) Inter-tissue differences in fatty acid incorporation as a result of dietary oil manipulation in Port Jackson sharks (*Heterodontus portusjacksoni)*. Lipids 49(6):577–590.

Beckmann CL, Mitchell JG, Stone DAJ, Huveneers C (2013) A controlled feeding experiment investigating the effects of a dietary switch on muscle and liver fatty acid profiles in Port Jackson sharks *Heterodontus portusjacksoni*. Journal of Experimental Marine Biology and Ecology 448:10–18.

Beckmann CL, Mitchell JG, Seuront L, Stone DAJ and Huveneers C (2013) Experimental Evaluation of Fatty Acid Profiles as a Technique to Determine Dietary Composition in Benthic Elasmobranchs. Physiological and Biochemical Zoology 86:266–278.

Beckmann CL., Mitchell JG, Stone DAJ and Huveneers C (in review), Stomach content and fatty acid biomarkers as indicators of spatial variations in the diet of Port Jackson shark (*Heterodontus portusjacksoni*) in southern and eastern Australia, Marine Ecology Progress Series.

Conference Presentations relevant to this thesis

Oceania Chondrichthyan Society and Australian Society for Fish Biology joint conference, Adelaide 2012, "Indicator fatty acids in multiple shark tissues and blood serum resulting from different artificial pellet diets"

Flinders University Postgraduate Conference, June 2012, "Validation of the use of Fatty Acid profiles to assess diet in sharks"

Oceania Chondrichthyan Society, Gold Coast 2011, "Validation of the use of Fatty Acid profiles to assess diet in sharks"

Flinders University Postgraduate Conference, June 2011, "Validation of the use of Fatty Acid profiles to assess diet in sharks"

Sharks International, Cairns 2010, "Using fatty acid signatures to infer diet in elasmobranchs"

Other Publications or contributions

Huveneers, C, Rogers PJ, **Beckmann C,** Semmens JM, Bruce BD and Seuront L (2013) The effects of cage-diving activities on the fine-scale swimming behaviour and space use of white sharks, Journal of Marine Biology 160(11):2863–2875

Huveneers C, Rogers PJ, Semmens J, **Beckmann C**, Kock AA, Page B and Goldsworthy S (2013) Behavioural effects of an electric field on white sharks: in situ testing of an electric deterrent. Plos One. 8(5): e62730.

Huveneers C, Rogers PJ, Semmens J, **Beckmann C**, Kock AA, Page B and Goldsworthy S.D (2012) Effects of the Shark Shield ™ electric deterrent on the behaviour of white sharks (*Carcharodon carcharias*). Final report to SafeWork South Australia. Version 2. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2012/000123-1. SARDI Research Report Series No. 632. 61pp.

Huveneers C, Rogers PJ, **Beckmann C**, Semmens J, Bruce B, and Seuront L (2012) Effects of a cage-diving operation on the fine-scale movement of white sharks (*Carcharodon carcharias*). Final report to the Department of Environment, Water and Natural Resources Wildlife Conservation Fund and the Nature Foundation of South Australia. South Australian Research and

Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2012/000417-1. SARDI Research Report Series No. 657. 48pp.

Beckmann, C & Huveneers C (2011) *Notoraja sticta*. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.1. www.iucnredlist.org.

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