Australian and NZ Fish oils - an update on current and future sources of long-chain omega-3 oils and their quality

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NZ Fats and Oils Symposium: November 8-10, 2016
**LC Omega-3 Oils: Coverage today**

- **Health** – Nutritional need for & inadequate intake of **LC Omega-3 (≥C20)**
- **Seafood** – Update on **LC omega-3 profiles (farmed fish)**
- **Supply** – Resource (sustainability) aspect; alternate sources needed; Australian perspective
- **Plant Technology** – Results so far; further R&D occurring to increase **LC Omega-3 yields in oilseed crops**
- **Approval & Acceptance** – CSIRO consumer research on the plant technology

- **Summary**
Essential Fatty Acid Families

ω6 family

18:2ω6
Corn Oil
Safflower Oil
Sunflower Oil

20:4ω6
Meat, Eggs, Brains

Linoleic (LA)

ω3 family

18:3ω3
α-Linolenic (ALA)
Flaxseed Oil
Canola Oil
Soybean Oil

20:5ω3
Eicosapentaenoic
EPA

22:6ω3
Docosahexaenoic
DHA

Microalgae
Seafood

Anti-thrombotic
Anti-inflammatory

LC Omega-3 Oils: ≥C20, two or more double bonds
Why long-chain omega-3?

**Increasing demand from:**
- Ageing populations
- High-growth economies
- Dietary supplement markets (especially preventative health)
- Pharmaceutical pipeline

**Supporting evidence:**
- Over 30,000 papers
  \(\rightarrow\) \(~80\%\) positive outcomes

**Plants: short-chain ALA, some SDA \(\text{C}_{18}\):**
- Limited health benefits
- Low conversion, especially to DHA
Global Fisheries – are there enough fish anyway - ?

We estimate that large predatory fish biomass today is only about 10% of pre-industrial levels.

Loved to death: our fish stocks in crisis

Aquaculture – Has the Good Oil gone missing?

July 2002, INFORM, AOCS: “warned some species of farm-raised fish may have little or no omega-3 fatty acids...”

(Stoll, Harvard Med. School)
Australian Farmed Fish – Good Oil (2002-15)

Global fish catches static or declining
• Fish oil used in aquaculture – replaced by other oils

LC Omega-3 oils have decreased cf 2002

• Content decreased by 10-50+% in 2010-15

• $\omega_3/\omega_6$ ratio <1 in 2012-15

Nichols et al. Nutrients 2014 - (2002-2013 results)
Farmed Atlantic salmon - %LA, %EPA, %DHA

Changing diets:
- % LA increasing
- % EPA+DHA decreasing
- ω3/ω6 ratio decreasing with increased Chicken Fat in feed
- ω3/ω6 ratio <1 since mid 2013

Nichols et al. Nutrients 2014 - (2002-2013 results)
Other Sources of LC Omega-3. I.

Microalgae oil: several Australian University-Industry consortia

- **Phototrophs** (open ponds)
- **Heterotrophs** (fermenters)
- Recent move in algal biofuels R&D towards **HTP** forming **biodiesel (FAME)**
- **LC Omega-3** directed activities (e.g. new CRC underway)

Krill Oil: new collaboration of **Aker-IMAS/Utas** underway (ARC-Linkage), resource monitoring & catch limits overseen by CCAMLR (Hobart HQ); MSC certified fishery (Aker)
Other Sources Needed. II.
CSIRO Agriculture: **Oilseed LC Omega-3 Oils**

- LC Omega-3 oils essential for human & marine fish health
- Global fish catches static or declining
- Microalgae biosynthesize the LC omega-3 oils that fish consume & store. Fish do not make EPA+DHA

**CSIRO-wide project** (1997 idea; commenced 2003)

*Goal: Isolate omega-3 genes from microalgae & transfer them to crop plants to sustainably produce LC omega-3 oils*
Partnership commenced - 2010
The LC Omega-3 Oilseed Journey

- Genes
- Combinations
- Crop
- DNA Constructs
- Plant Transformation
- Field trials
- Detailed Oil Analyses
- Application trials
LC Omega-3 in Marine Microalgae

![Bar chart showing percent composition of AA, EPA, and DHA across different algal classes.]

- **Rhodophyceae**
  - Yellow-green: EPA
  - Red: AA
  - Green: DHA

- **Eustigmatophyceae**
  - Red: AA
  - Yellow-green: EPA
  - Green: DHA

- **Chlorophyceae**
  - Yellow-green: EPA
  - Red: AA
  - Green: DHA

- **Prasinophyceae**
  - Yellow-green: EPA
  - Red: AA
  - Green: DHA

- **Bacillariophyceae**
  - Yellow-green: EPA
  - Red: AA
  - Green: DHA

- **Prymnesiophyceae**
  - Yellow-green: EPA
  - Red: AA
  - Green: DHA

- **Cryptophyceae**
  - Yellow-green: EPA
  - Red: AA
  - Green: DHA

- **Dinophyceae**
  - Yellow-green: EPA
  - Red: AA
  - Green: DHA
LC Omega-3 Oils - Engineering in land plants

16:0 → 18:0 → 18:1 → 18:2 → α-18:3

Land Plants

Δ6-des
18:4 SDA

Δ6-elo
20:4

Δ5-des
20:5 EPA

Δ5-elo
22:5

Δ4-des
22:6 DHA

Marine Algae
Land Plant Achievements

First land plant with **EPA** + **DHA** in its seed oil

**Arabidopsis thaliana**

Robert et al. FPB - 2005
DHA Biosynthesis - *Isolation of an efficient synthesis pathway*

18:3[^9,12,15]  
α-Linolenic acid, **ALA**

↓

*Micromonas pusilla* Δ6-des

18:4[^6,9,12,15]  
Stearidonic acid, **SDA**

↓

*Pyramimonas cordata* Δ6-elo

20:4[^8,11,14,17]  
Eicosatetraenoic acid, **ETA**

↓

*Pavlova salina* Δ5-des

20:5[^5,8,11,14,17]  
Eicosapentaenoic acid, **EPA**

↓

*Pyramimonas cordata* Δ5-elo

22:5[^7,10,13,16,19]  
Docosapentaenoic acid, **DPA**

↓

*Pavlova salina* Δ4-des

22:6[^4,7,10,13,16,19]  
Docosahexaenoic acid, **DHA**

**Microalgal**

Petrie et al. 2010; *Metab Eng.* 12:233-240

**Microalgal**


**Microalgal**

Zhou et al. 2007; *Phytochem.* 6:785-796

**Microalgal**


**Microalgal**

Zhou et al. 2007; *Phytochem.* 6:785-796

**Rapid assessment of seed constructs in leaf**
Fish oil-like levels of DHA in plant seed (& high ω3/ω6 ratio)

**Tobacco Benth leaf TAG**

- 16:0
- 18:2ω6
- 18:3ω6
- 18:4ω3
- 18:3ω3
- 18:1ω9
- 18:0
- 18:4ω3
- EPA 20:5ω3
- 20:4ω3
- DPA 22:5ω3
- DHA 22:6ω3 15.9%

**Arabidopsis seed**

- 16:0
- 17:0(IS)
- 18:0
- 18:1ω9
- 18:2ω6
- 18:4ω3
- 18:3ω3
- 20:1
- 20:2ω6
- 20:3ω3
- EPA 20:5ω3
- 20:4ω3
- DPA 22:5ω3
- DHA 22:6ω3 15.1%

Petrie et al. PLOS ONE 2012
Timeline of DHA biosynthesis in oilseeds

Petrie et al., PLOS One 2013; Inform 2013

Nuseed/GRDC/CSIRO 2013, Canola
Nuseed/GRDC/CSIRO 2013, Camelina
Ruiz-Lopez et al. 2013, Arabidopsis
Nuseed/GRDC/CSIRO 2012, Arabidopsis
CSIRO 2010, Arabidopsis
CSIRO 2005, Arabidopsis
Wu et al. 2005, Brassica juncea
Kinney et al. 2005, Soybean

Engineered oilseed crops with fish oil DHA levels

James B. Petrie, Peter B. Nickels, Michelle O'Brien, and Nicholas R. Stump

- Developing an efficient source of omega-3 long-chain PUFA (n-3 LC-PUFA) in oilseeds is critical for humans as fish oil is being a major food item in diets. It is more than essential for individuals suffering from cardiovascular disease.

- The presented data in this article is based on the molecular and metabolic studies conducted in the laboratory of Dr. James B. Petrie. The findings provide insights into the potential of oilseeds as a viable source of n-3 LC-PUFA.

- The article presents a proposal for the development of oilseeds rich in n-3 LC-PUFA and discusses its potential implications for human health.

Marine microorganisms are the primary origin of omega-3 LC-PUFA

Fish consume marine microorganisms and accumulate n-3 LC-PUFA in their body tissue.

Fish are sources of omega-3 LC-PUFA in our diet

ω-3 LC-PUFA can be transferred to plants

ω-3 LC-PUFA can be transferred to plants
A sustainable, land-based platform

Canola:

- High quality and healthy oil
- Commercially available in multiple regions
- Canada/US/AUS >10M Ha
- Decades of breeding and commercial optimization
- Efficient plant, high oil yield
The new **canola-DHA** oil profile: plus a bit more

<table>
<thead>
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<th>16:0</th>
<th>18:1</th>
<th>LA</th>
<th>ALA</th>
<th>SDA</th>
<th>EPA</th>
<th>DPA</th>
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<tr>
<td>Parent 1</td>
<td>5.3</td>
<td>44.0</td>
<td>18.6</td>
<td>22.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>Parent 2</td>
<td>4.3</td>
<td>72.2</td>
<td>14.0</td>
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<td>-</td>
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<tr>
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<td>6.0</td>
<td>28.1</td>
<td>7.0</td>
<td>27.5</td>
<td>4.6</td>
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<td>12.3%</td>
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<tr>
<td>High copy</td>
<td>5.3</td>
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<td>6.8</td>
<td>25.1</td>
<td>5.9</td>
<td>0.8</td>
<td>1.2</td>
<td>19.3%</td>
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</table>

Total Omega-3: 48.1%  
Total LC Omega-3: 16.0%  
Total Omega-6: 7.4%  
Total LC Omega-6: 0.1%  
Omega-3:6 ratio: 6.5 : 1  
LC Omega-3 to Omega-6 ratio: 135:1
CSIRO Consumer Research

Trials – Australia, USA, Europe, Asia

Take home message:

A large proportion of the population are accepting of GM land plant LC omega-3 oil that:

- Provides a health benefit,
- Was supported by health claims from a trusted source,
- Was indirectly consumed (e.g. food for farming fish)

(Cox et al. 2007, 2008, 2010)
Analytical / Quality - NZ Fish oil capsule study / Oil Quality

NZ paper *Nature Scientific Reports* (Albert et al., Jan 2015)

Two issues reported:

(i) **EPA+DHA did not meet label claim**, markedly so for many (69%) products

(ii) **Oils highly oxidized** (high PV, pAV) – health implications raised, including in media.
Products DO generally meet label claims

mg of EPA + DHA per Capsule

Nichols et al. Nutrients 2014 - (FO & KO results, SO unpublished data)
II. TGA Follow up Analyses – ANZ Fish Oils

• Responding to the NZ NSR paper & media, the Therapeutic Goods Administration (TGA, Aust Govt Dept of Health) surveyed fish oil products on Australian market.

• **15 products** tested. The type of ‘fish oil’
  - **8 products** captured under TGA Compositional Guideline for ‘fish oil - Natural’
  - **7 products** captured under the BP (British Pharmacopoeia) monograph on ‘Concentrated Omega-3 triglycerides - fish’

• Products analysed using BP methods for testing fish oils.

• **Testing for oxidation gave satisfactory results for all products** in relation to PV. **4 products** gave high results for pAV which can be attributed to the presence of excipient fragrances or flavourings (aldehydes) which interfere with the test.

• **All 15 products gave acceptable results for content of omega-3 fatty acids**, that is they were all above the legislated (or official) lower limit of 90% of label claim.
Summary – Oil Quality. I.

• NZ NSR paper – 2015. 29/32 products did NOT meet EPA+DHA
  30/36 products exceeded PV
  9/36 products exceeded pAV

• Previous Australian analyses – Industry, CSIRO, re-testing, other analyses
  → products all met specs/claims

• TGA – 2015. All 15 products tested met PV specs and EPA+DHA claims.
  No action with industry undertaken.

• GOED (+ Coun. Respon. Nutr.) 2016. 2171/2187 products met PV spec
  (Lipid Tech – Review paper) 2092/2117 products met pAV spec

• O3C - Updates/responses placed at O3C website, and sent to - AAOCS &
  AOCS, Omega List, NYT/Frontline, CMG, CMA, ABC Four Corners, others
III. New O3C Analyses – 2016

• O3C – (New analyses completed June 2016). All 10 products (5 x 18/12 oils, 5 x concentrates) were purchased in Melbourne in May 2016 and tested by standard/accepted (BP) methods by a validated laboratory for – PV, pAV, EPA+DHA

• All 10 products met n-3 (EPA+DHA) content claims and PV specs. 8/10 met pAV spec. 2 contained additives, well known to interfere with pAV. GOED cautions regards use of pAV analyses for oils with additives.

• Aust & NZ fish oil supplements –

  DO meet LC Omega-3 Claims & are NOT oxidized

Nichols et al. Nutrients 2016
Summary – Oil Quality. III.

New O3C Analyses – 2016

n-3 PUFA Content
10/10 (100%) meet label claim

EPA+DHA Content
10/10 (100%) meet label claim

In agreement with 2015 TGA analyses

NZ NSR paper:
29/32 products (90%) did NOT meet EPA+DHA label claim

Nichols et al. Nutrients 2016
New O3C Analyses – 2016

Peroxide Value

10/10 (100%) meet PV spec

NZ NSR paper:
30/36 products (83%) exceeded PV

- Aust & NZ fish oil supplements –
  DO meet LC Omega-3 Claims & are NOT heavily oxidized

Nichols et al. Nutrients 2016
NZ Oils Study #2 – Am J Physiol - July 2016

- Pregnant rats fed **heavily Oxidized** Fish Oils
- High infant mortality
- Increases maternal insulin resistance

**Study relevance?**  
*A&NZ Fish Oils are NOT oxidized*

- Dose was equivalent to 40 g/adult; exceptionally high
  
  -- *animal ethics approval?*

**Ethics** issue raised at 2016 Sydney O3C Symposium

- Senior Univ Auckland (UoA) scientist has raised methods issues for analyses by their UoA-Liggins colleagues
FA analyses – Care is needed by analysts

- Issues likely due to analytical methods used for LC Omega-3 profiling
- Cross checking of FA, PV, pAV data is needed where ‘anomalies’ occur
- Consideration of use of standard methods, reference materials, etc

Areas not covered today

- Institutional review process, Journal review process, Journal editorial process, Media review process

Australian & NZ fish oil supplements

- Generally DO meet Omega-3 Claims & are NOT oxidized
Positive Australian & NZ News re LC Omega-3

General:

Joint Omega-3 Symposium O3C-AAOCS, Newcastle, November 2013: Published in Nutrients Special Issue (2014). Book also published in late 2014.

“Recent Advances in Omega-3: Health Benefits, Sources, Products and Bioavailability”. See:

http://www.mdpi.com/journal/nutrients/special_issues/omega-3_conference

➢ 12 papers in the Special Issue. Australian / NZ emphasis
Summary

• **LC Omega-3** health benefits - ongoing recognition; marine resource and thereby supply issues

• *Farmed seafood* in Aust & NZ generally higher **LC Omega-3** content than wild harvest seafood; Aust & NZ wild harvest fishing is sustainable

• **LC Omega-3** content in *farmed fish* has decreased, as has the previously high omega-3 / omega-6 ratio; need to revisit

• Alternate sources of **LC Omega-3** are required for future aquaculture
  - Excellent progress with new land plants (Canola-DHA, CSIRO-Nuseed- GRDC); field trials completed – 2014-16
    
    ![Image](image.png)
    
    1 Ha of Canola-DHA at 12% DHA = DHA from 10,000 fish

• Aust & NZ fish oil supplements –
  - **DO meet LC Omega-3 Claims & are NOT oxidized**
Thank you

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