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

* OCEANS & ATMOSPHERE, ^ AGRICULTURE
www.csiro.au

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**
- **Analytical/Oils Quality** – ***NZ - Nature Sci Reports paper***
- **Summary**

Essential Fatty Acid Families

ω6 family

18:2ω6 — Linoleic (LA)

Corn Oil
Safflower Oil
Sunflower Oil

20:4 ω6 — Arachidonic AA

Meat, Eggs, Brains

Thrombotic
Inflammatory

ω3 family

18:3ω3 — α-Linolenic (ALA)

Flaxseed Oil
Canola Oil
Soybean Oil

20:5ω3 — Eicosapentaenoic EPA

22:6ω3 — Docosahexaenoic DHA

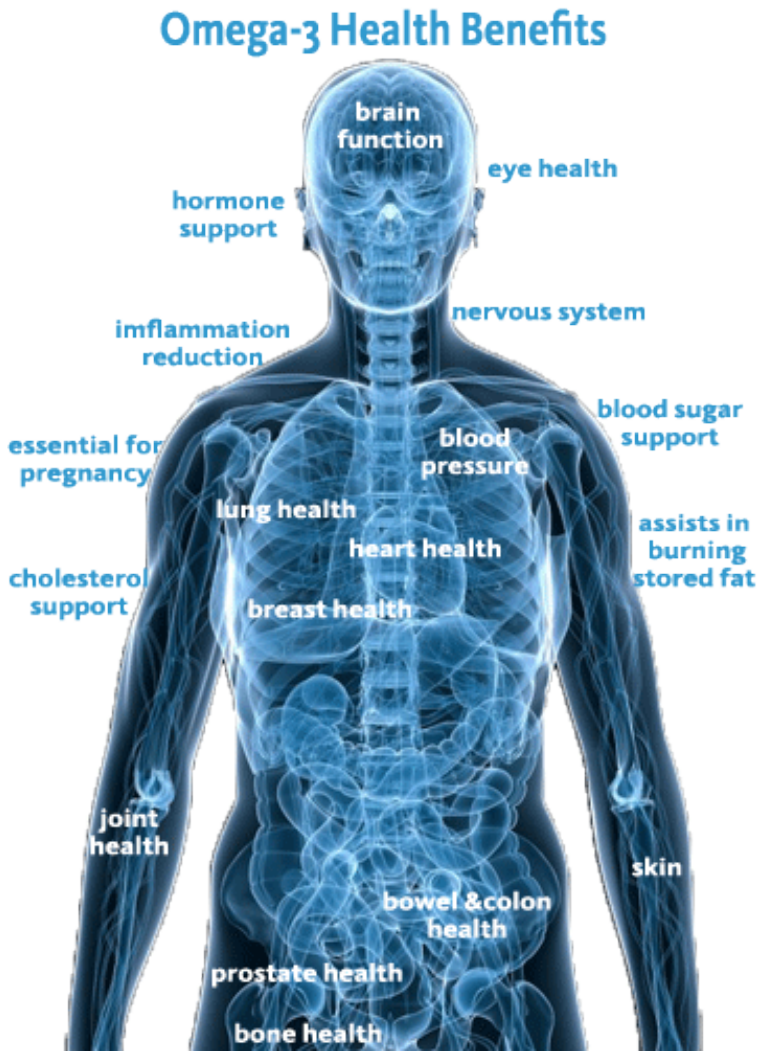
Microalgae
↓
Seafood

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:

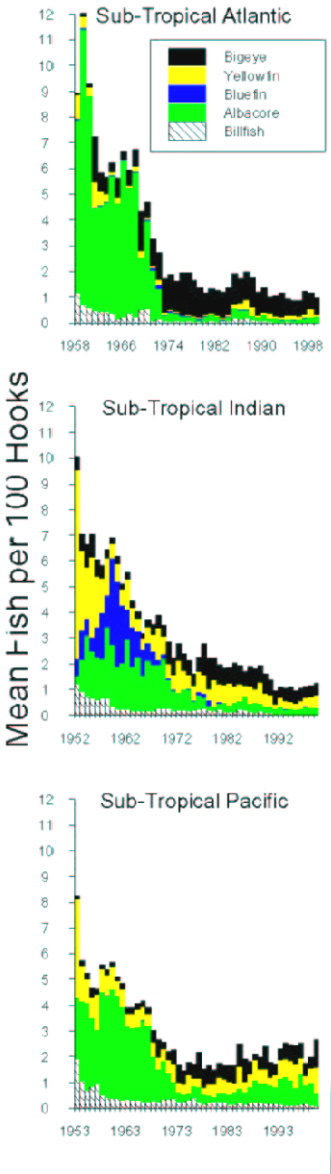
- Over 30,000 papers

  → ***~80% positive outcomes***

Plants: short-chain ALA, some SDA (C_{18})

- Limited health benefits
- Low conversion, especially to **DHA**

Global Fisheries – are there enough fish anyway - ?



letters to nature

Rapid worldwide depletion of predatory fish communities

Ransom A. Myers & Boris Worm

Biology Department, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1

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

July 2002, INFORM, AOCS:

"warned some species of farm-raised fish may have little or no omega-3 fatty acids……"

(Stoll, Harvard Med. School)

Loved to death: our fish stocks in crisis

Aquaculture –
Has the Good Oil gone missing?

Australian Farmed Fish – Good Oil (2002-15)

Global fish catches static or declining

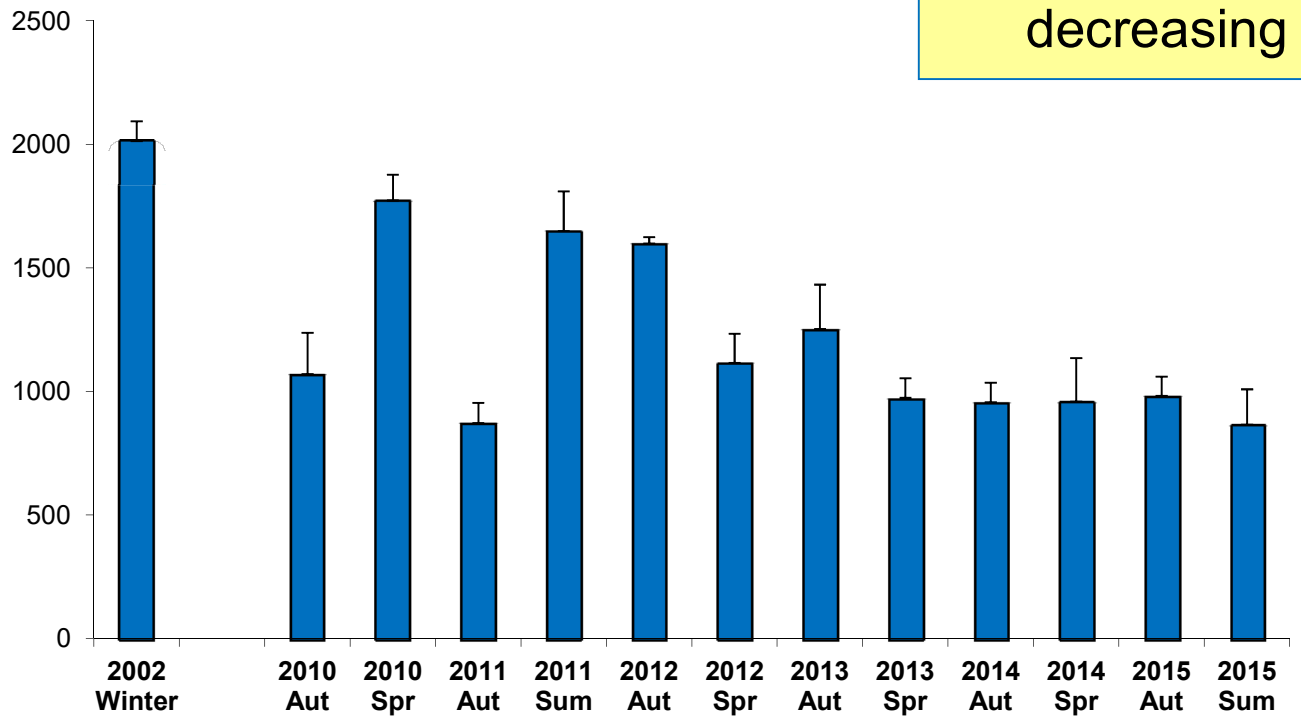
- ***Fish oil used in aquaculture – replaced by other oils***

LC Omega-3 oils have decreased cf 2002

- ***Content decreased by 10-50+% in 2010-15***
- ***ω3/ω6 ratio <1 in 2012-15***

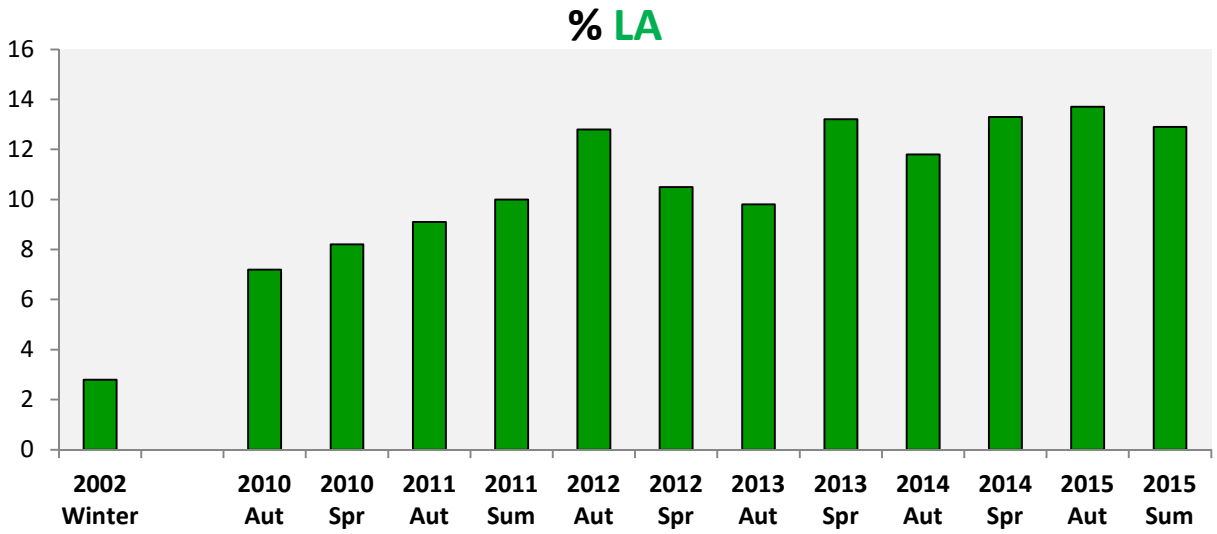
Changing diets:

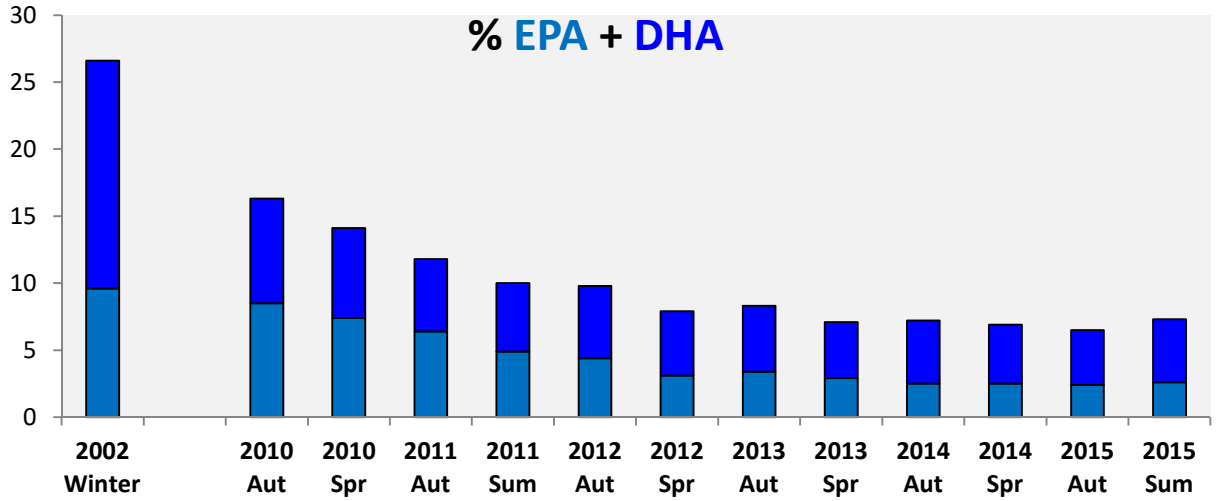
- % **LA** increasing
- % **EPA+DHA** decreasing



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

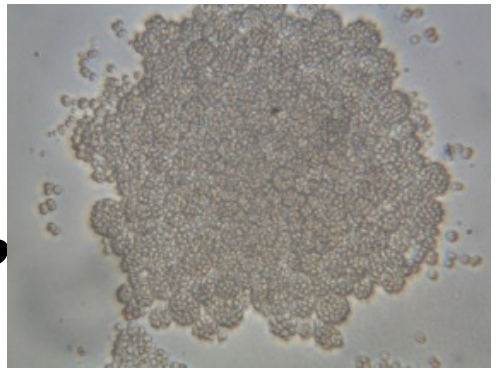
➡ ***Environmental Signature***

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***

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Partnership commenced - 2010

The LC Omega-3 Oilseed Journey

Genes

Combinations

Crop

DNA Constructs

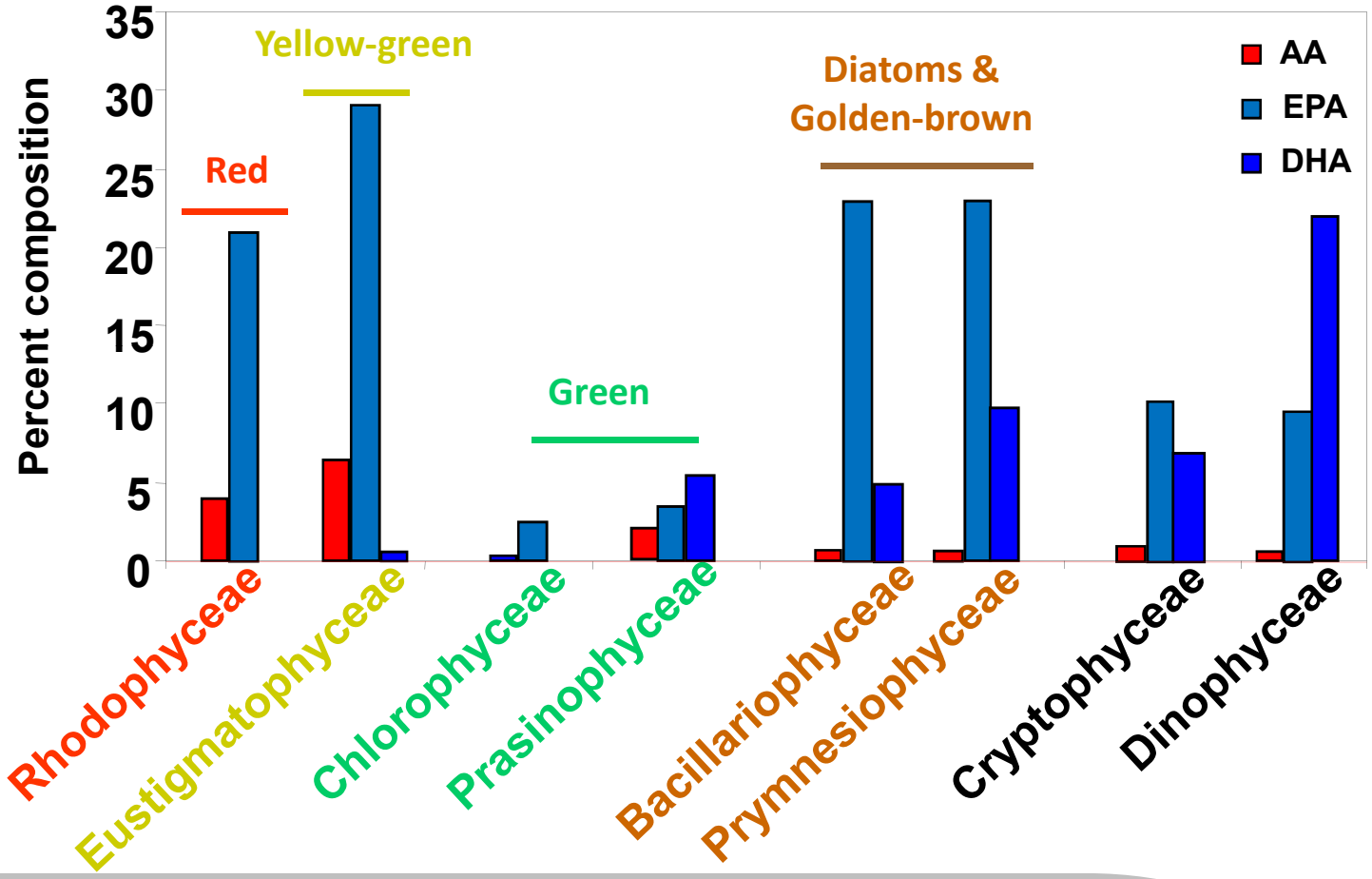
Plant Transformation

Field trials

Detailed Oil Analyses

Application trials

LC Omega-3 in Marine Microalgae



LC Omega-3 Oils - Engineering in land plants

16:0 ➡ 18:0 ➡ 18:1 ➡ 18:2 ➡ α–18:3

Land Plants

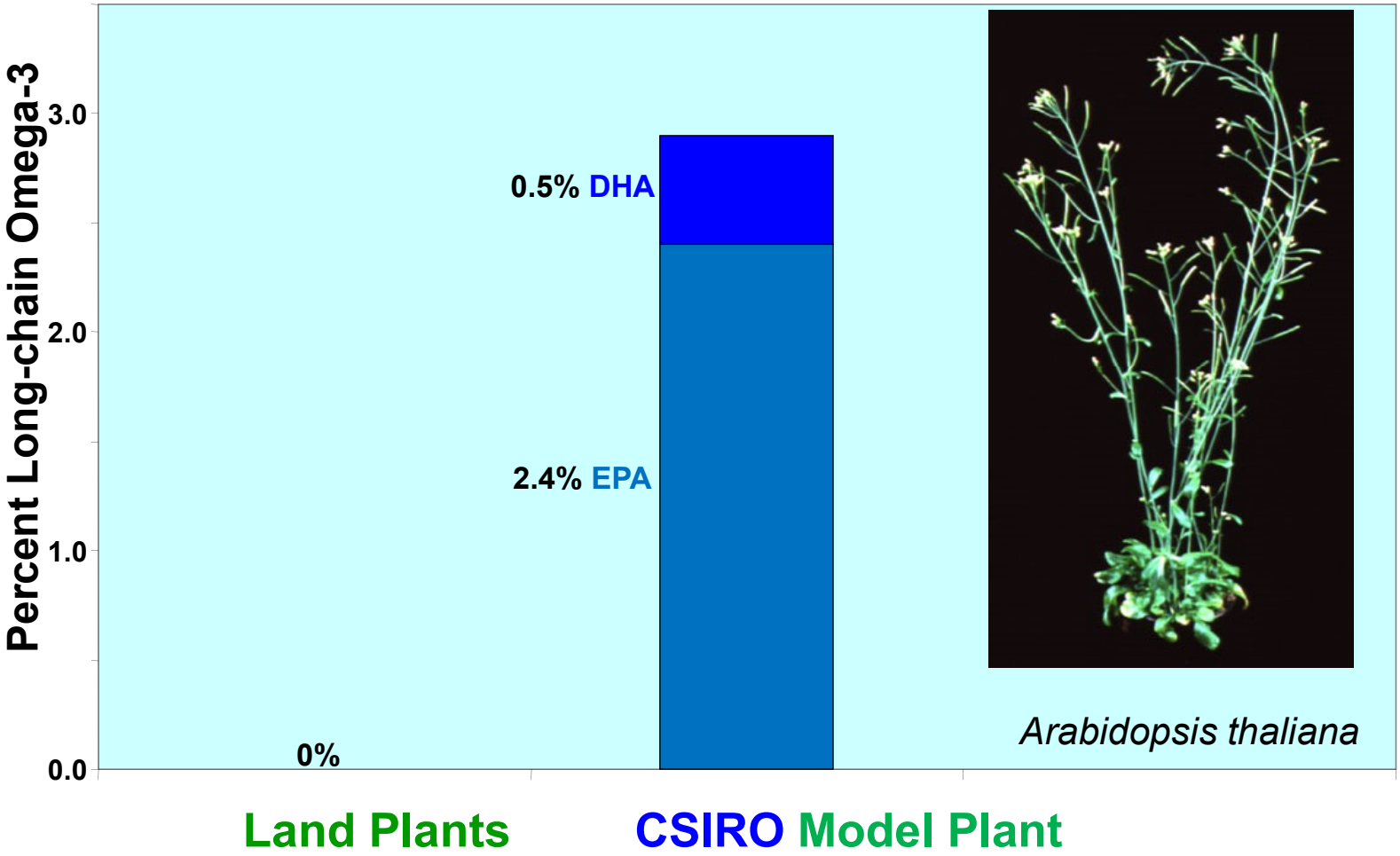


Δ6-des
18:4 SDA
Δ6-elo
20:4
Δ5-des
20:5 EPA
Δ5-elo
22:5
Δ4-des
22:6 DHA

Marine Algae

Land Plant Achievements

First land plant with EPA + DHA in its seed oil



Robert et al. FPB - 2005

DHA Biosynthesis - *Isolation of an efficient synthesis pathway*

$18:3^{\Delta 9,12,15}$
α-Linolenic acid, **ALA**

Micromonas pusilla Δ6-des ↓ — **Microalgal** Petrie et al. 2010; *Metab Eng.* 12:233-240

$18:4^{\Delta 6,9,12,15}$
Stearidonic acid, **SDA**

Pyramimonas cordata Δ6-elo ↓ — **Microalgal** Petrie et al. 2010; *Marine Biotechnol.* 4:430-438

$20:4^{\Delta 8,11,14,17}$
Eicosatetraenoic acid, **ETA**

Pavlova salina Δ5-des ↓ — **Microalgal** Zhou et al. 2007; *Phytochem.* 6:785-796

$20:5^{\Delta 5,8,11,14,17}$
Eicosapentaenoic acid, **EPA**

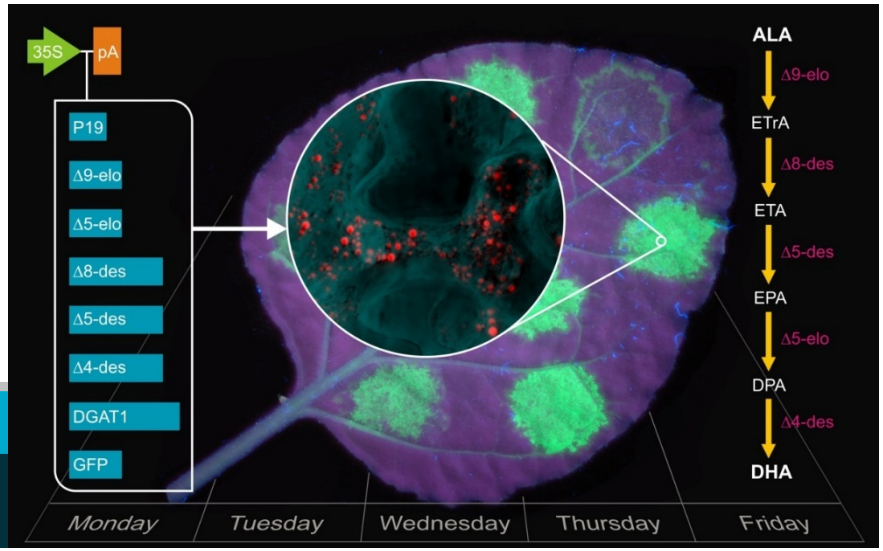
Pyramimonas cordata Δ5-elo ↓ — **Microalgal** Petrie et al. 2010; *Marine Biotechnol.* 4:430-438

$22:5^{\Delta 7,10,13,16,19}$
Docosapentaenoic acid, **DPA**

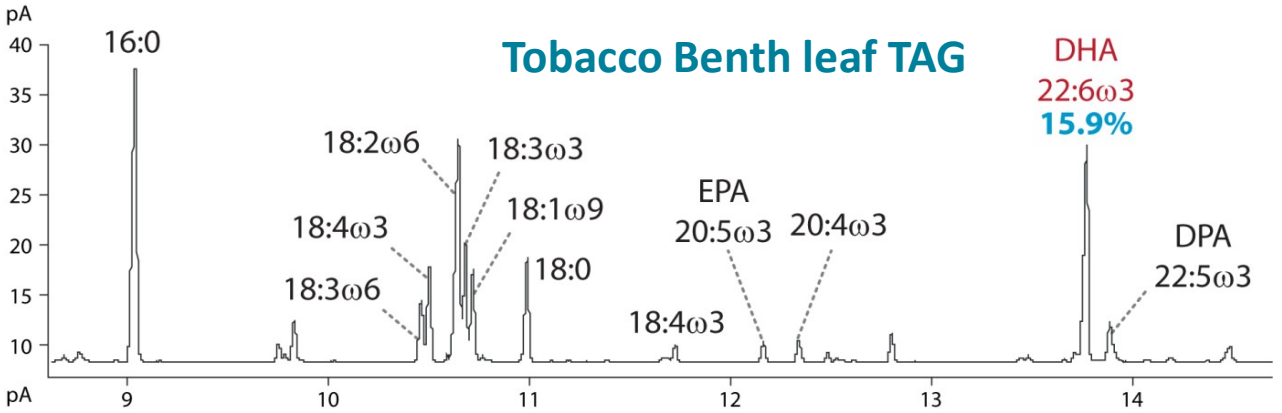
Pavlova salina Δ4-des ↓ — **Microalgal** Zhou et al. 2007; *Phytochem.* 6:785-796

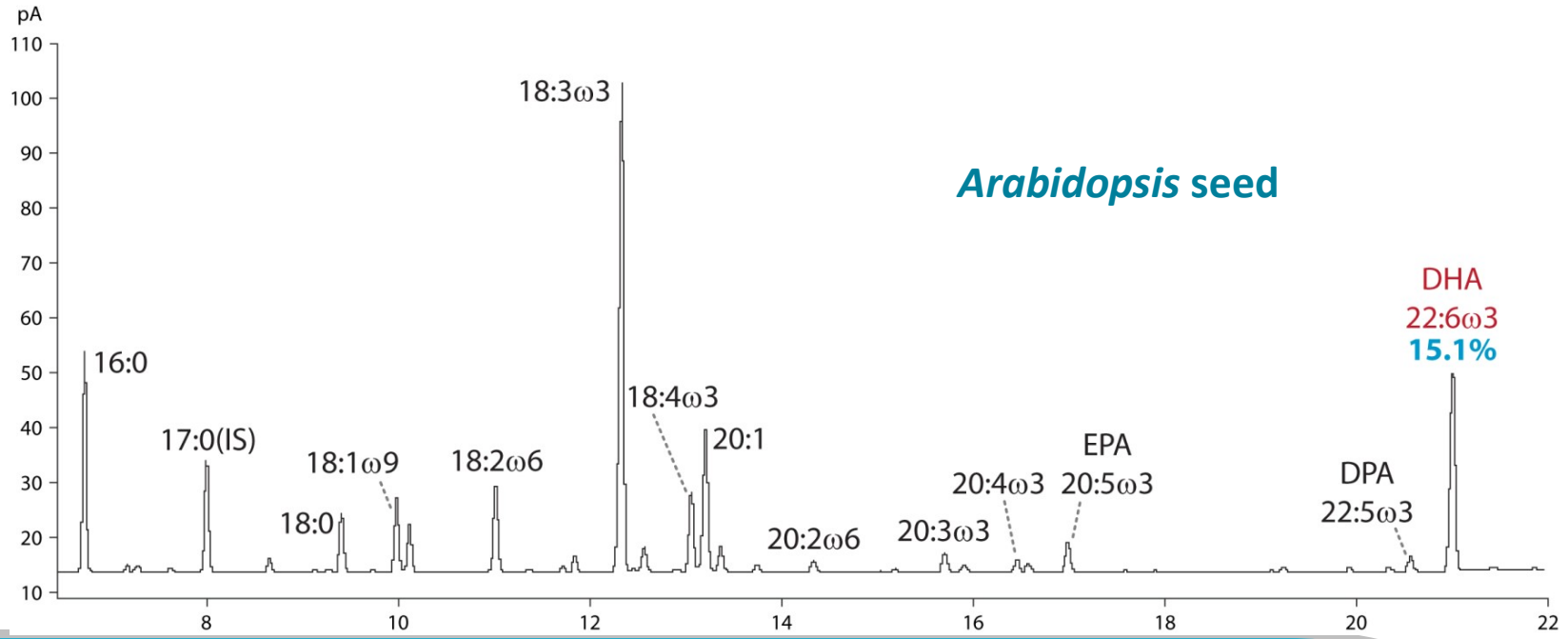
$22:6^{\Delta 4,7,10,13,16,19}$
Docosahexaenoic acid, DHA

Rapid assessment of seed constructs in leaf



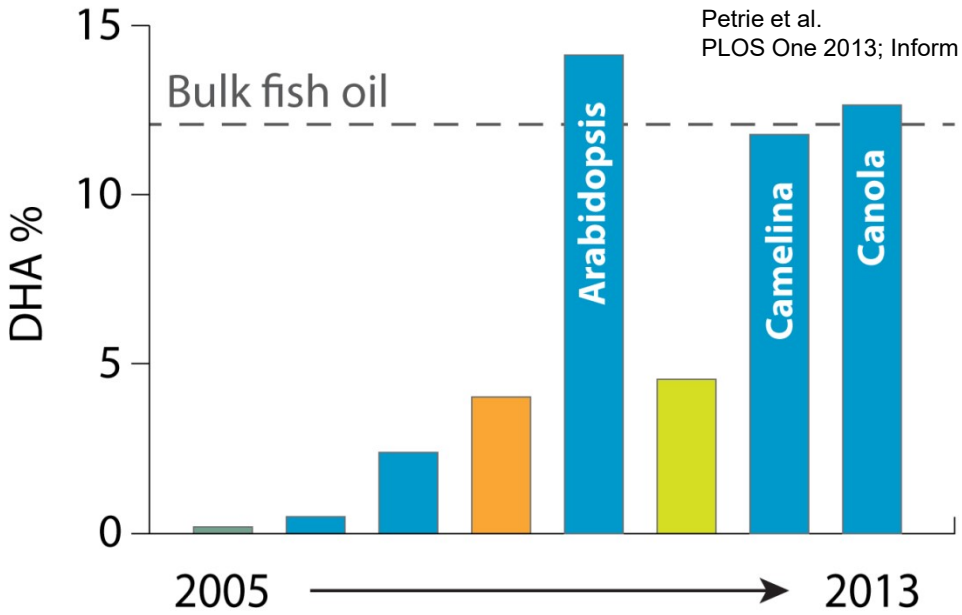
Fish oil-like levels of DHA in plant seed (& high ω3/ω6 ratio)





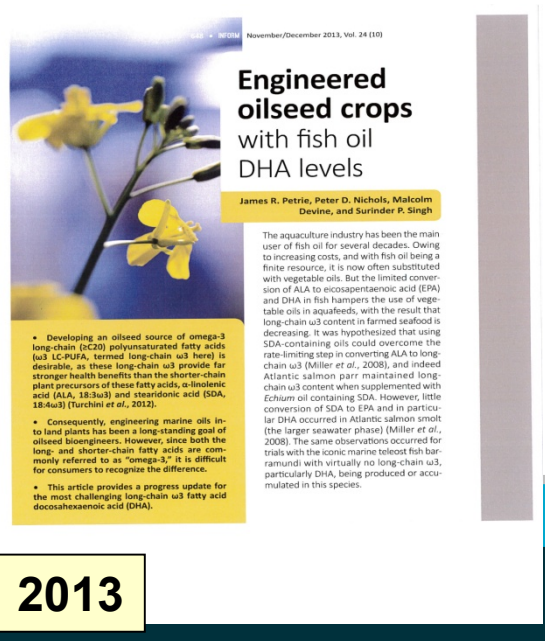
Petrie et al . PLOS ONE 2012

Timeline of DHA biosynthesis in oilseeds



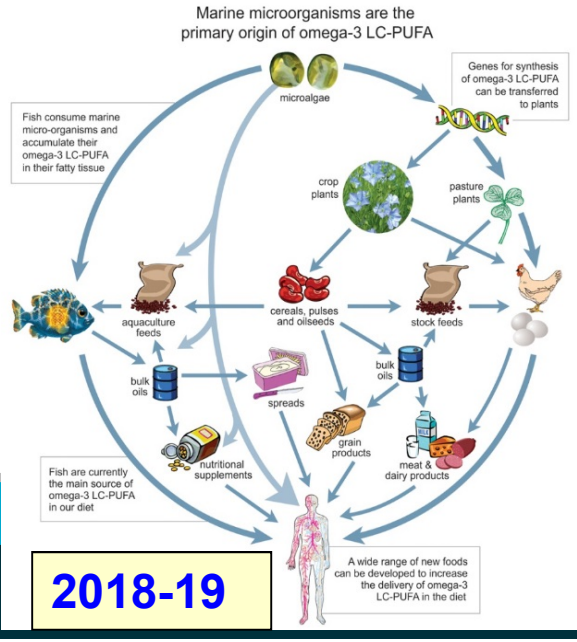
Petrie et al.
PLOS One 2013; Inform 2013

- **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



2013

2015



2018-19

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

	16:0	18:1	LA	ALA	SDA	EPA	DPA	DHA
Parent 1	5.3	44.0	18.6	22.2	-	-	-	-
Parent 2	4.3	72.2	14.0	2.5	-	-	-	-
Low copy	**6.0**	**28.1**	**7.0**	**27.5**	**4.6**	**0.5**	**0.8**	**12.3%**
High copy	5.3	24.4	6.8	25.1	5.9	0.8	1.2	**19.3%**

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)

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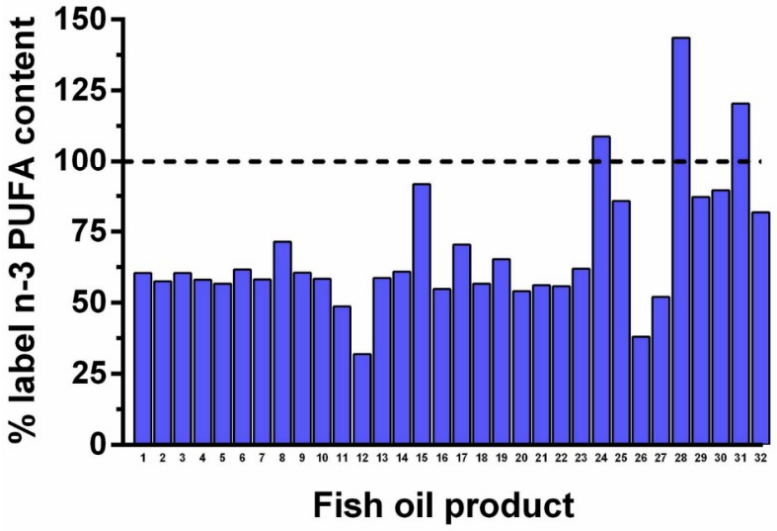
Analytical / Quality - NZ Fish oil capsule study / Oil Quality

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

NSR – Product results



(ii) **Oils highly oxidized** (high PV, pAV) – health implications raised, including in media.

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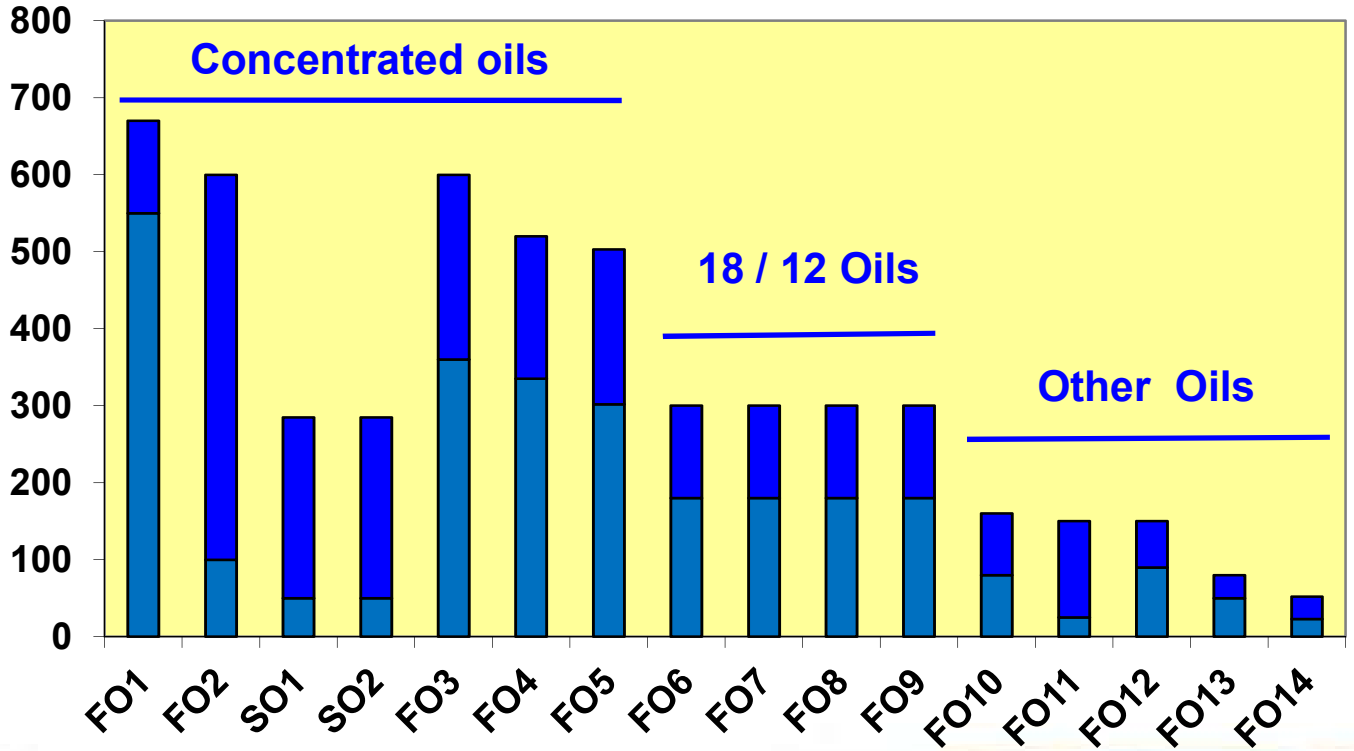
Previous Australian Studies I : Fish, Squid & Krill Oil Capsules

Products DO generally meet label claims

mg of EPA + DHA per Capsule



Nichols et al. Nutrients 2014 - (FO & KO results, SO unpublished data)

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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.

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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

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Summary – Oil Quality. II.

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

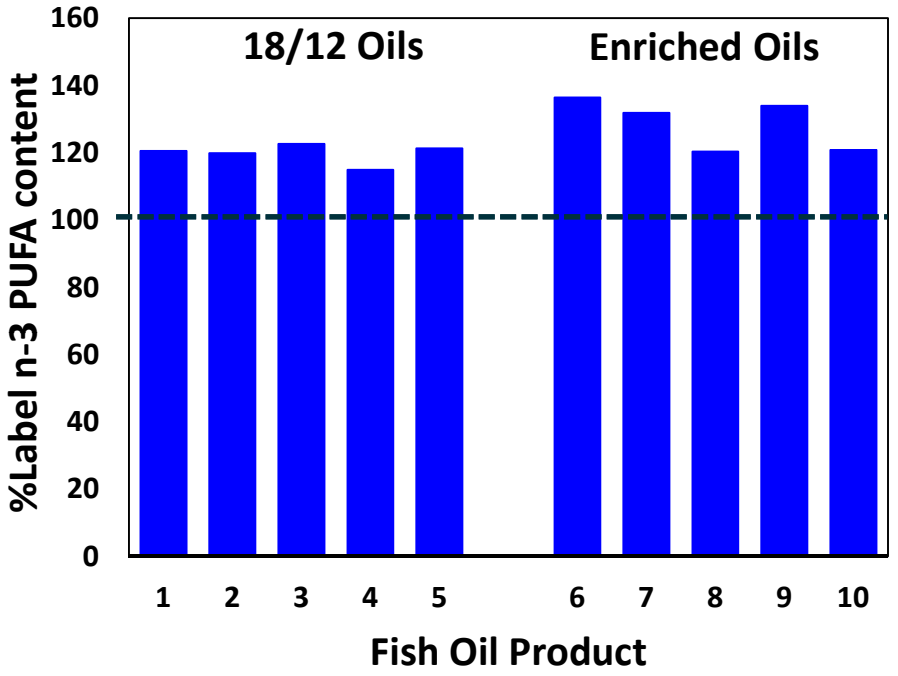
n-3 PUFA Content

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

Summary – Oil Quality. III.

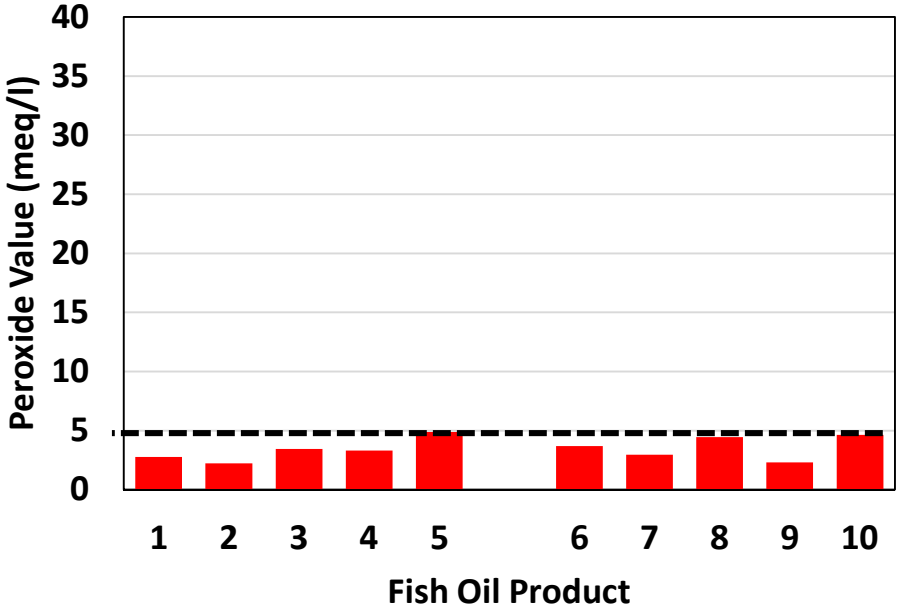
New O3C Analyses – 2016

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

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Summary – Oil Quality. IV.

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

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Summary – Oil Quality. V.

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***

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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**

  ➡ **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

Surinder Singh
Srinivas Belide
Allan Green
Dawar Hussain
Yoko Kennedy
Geraldine Lester
Qing Liu
Lina Ma
Anne Mackenzie
Peter Mansour
Peter Nichols
Nathalie Niesner
James Petrie
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Thank you

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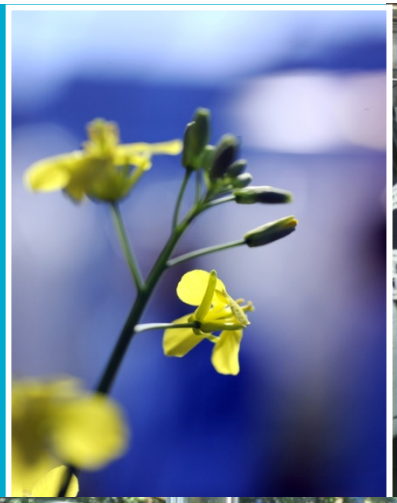
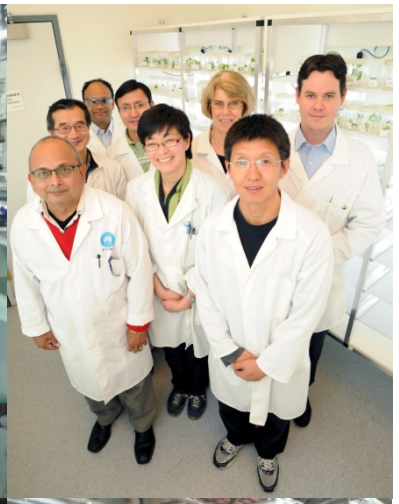
The CSIRO-Nuseed
LC Omega-3 Team
The Omega-3 Centre Team

Andy Sinclair, Lalen Dogan





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