Bioactive compounds from Aquatic Resources

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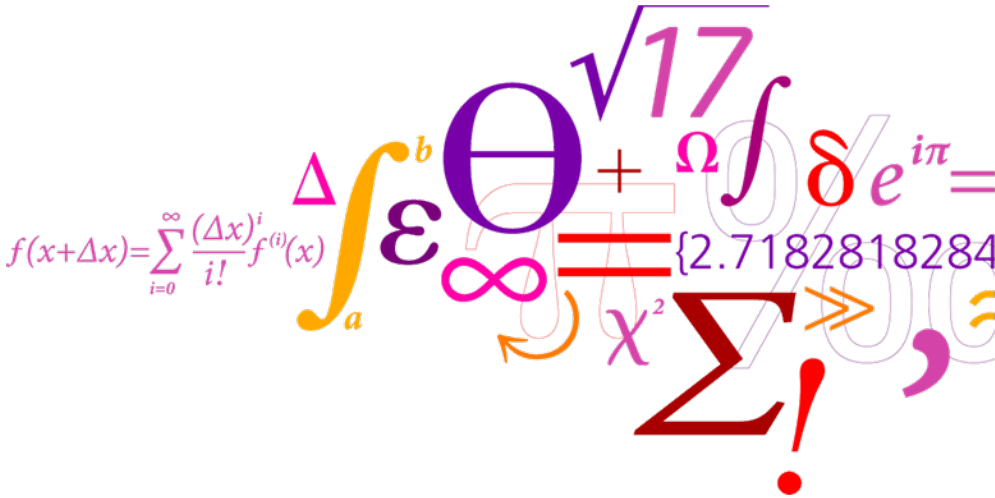

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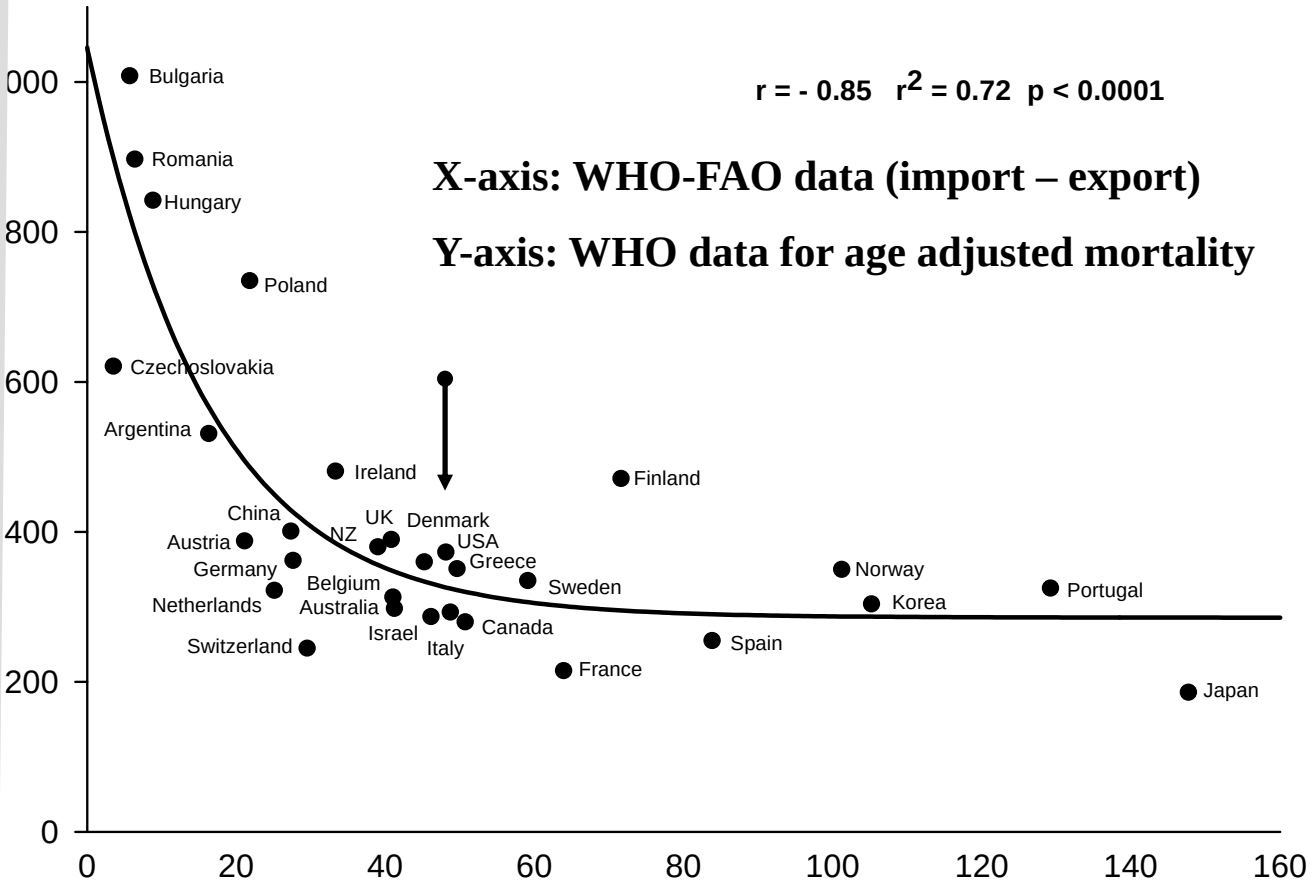
Outline

- Introduction

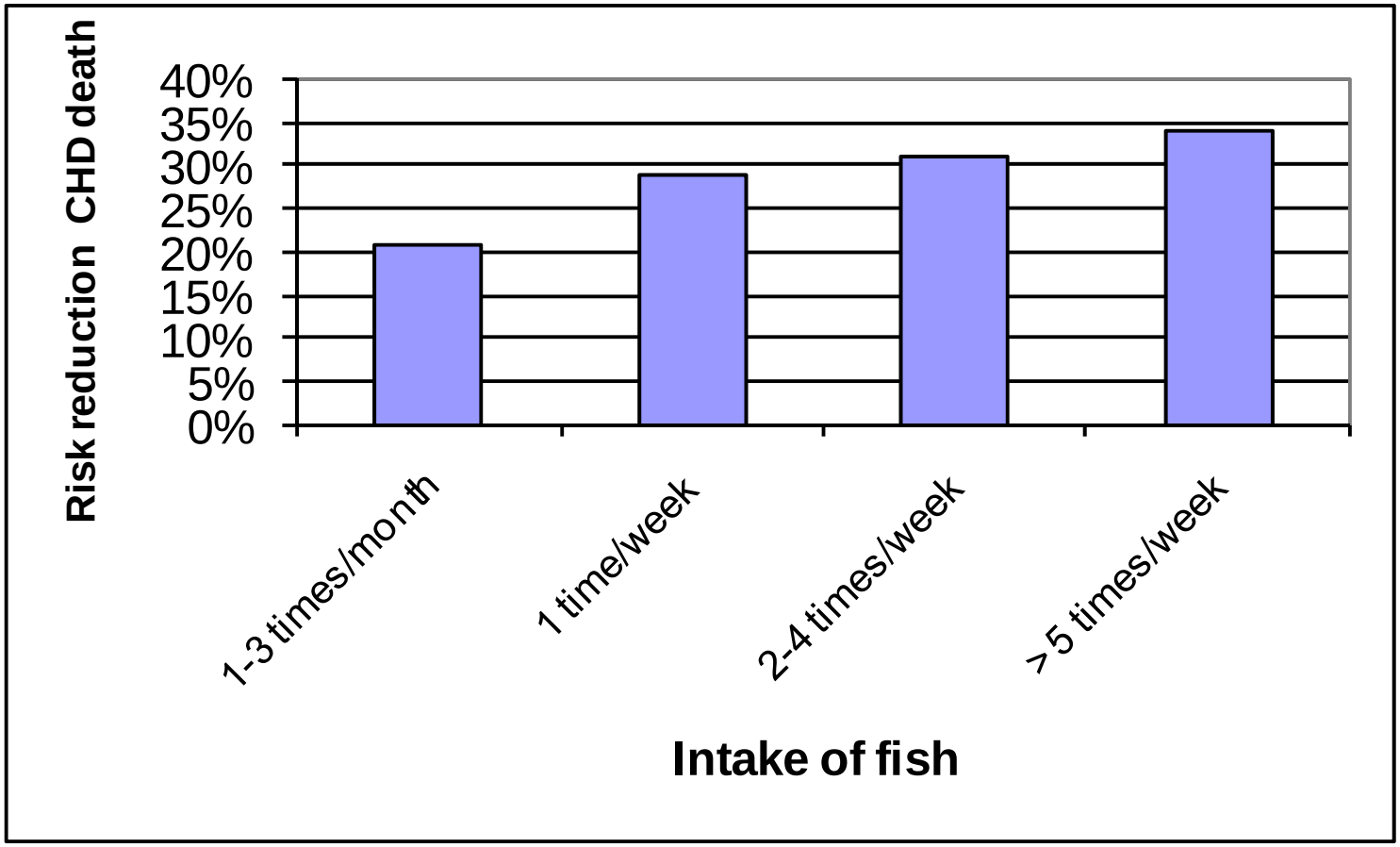
- Bioactive compounds in:
  - Fish
  - Algae
  - Krill
  - Jelly fish and sea cucumber

Prevalence of cardiovascular diseases

CVD vs seafood intake



Effect of fish intake on CHD death among women



Hu et al., JAMA 2002

Getting information on health effects

Human studies, clinical trials

Highest level of evidence

-Firm control of diet, study length, type of subjects, etc.

-Expensive

-Impossible to conduct on some diseases

Observational (Epidemiological) studies

Segment of population observed

-Fish intake associated with diseases

-Indication of correlation

-Many confounding factors

Animal Studies

Extrapolation to humans is limited

-Can excert tight control over experimental conditions

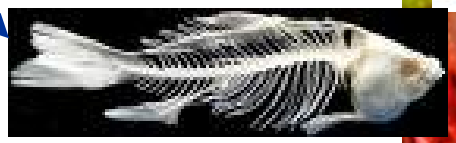
-Insights into designing human studies

In vitro (test tube) studies

Hardest to extrapolate to humans

-Important in early phases

-Can give directions



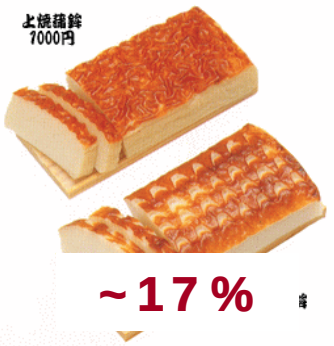
Muscle

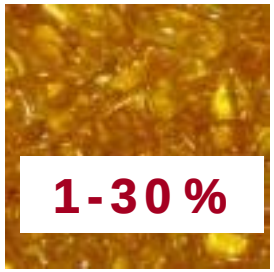
Bones / shells / cartilage

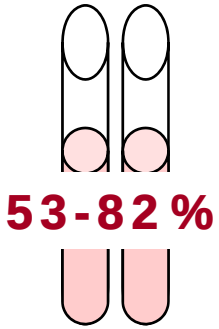
Proteins

Lipids

Water solubles







-Chitosan
-Glucosamine
-Chondroitin sulfate

-Peptides

-LC n-3 fatty acids

-Phospholipids
-Vitamin D

-Free amino acids (taurine)
-Minerals (Selenium)

Omega-3 fatty acids

Well documented effects:

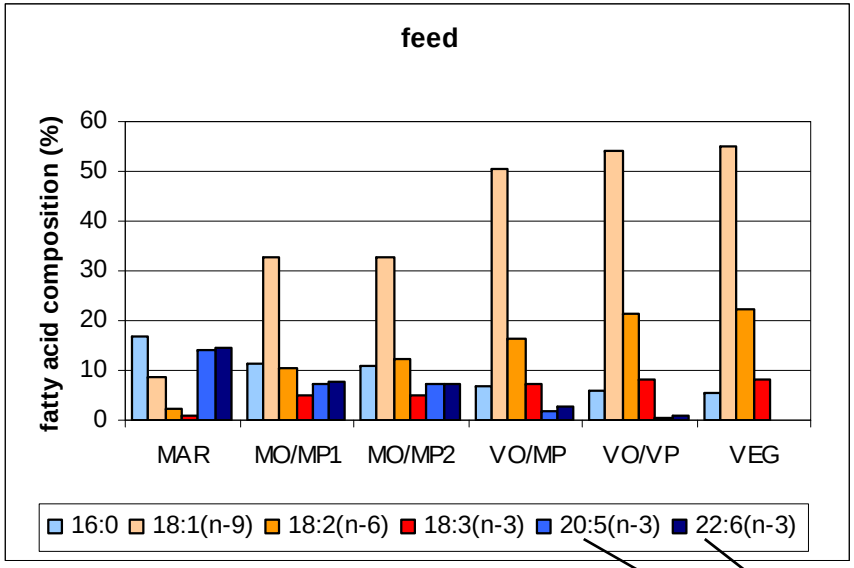
- Preventive effect on development of CVD
- Lowers blood pressure (1-2 mm Hg)
- Reduces lipid levels in blood(triglycerides)
- Alleviates symptoms from rheomatoid arthritis

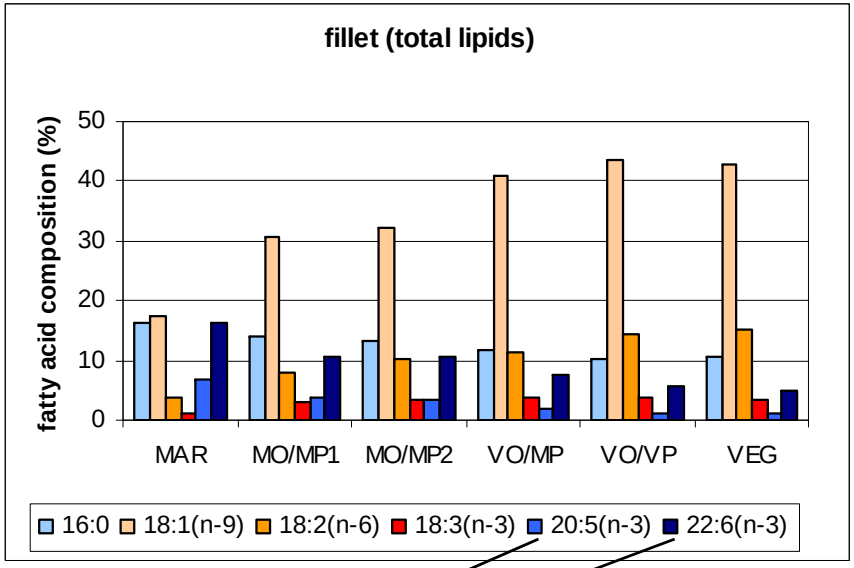
Omega-3 fatty acids

Less well documented:

- Reduce pre-term birth
- Important for mental health
- Reduces weight loss and improves health in cancer patients
- Normalising immune response

Fatty acid composition in feed and filet



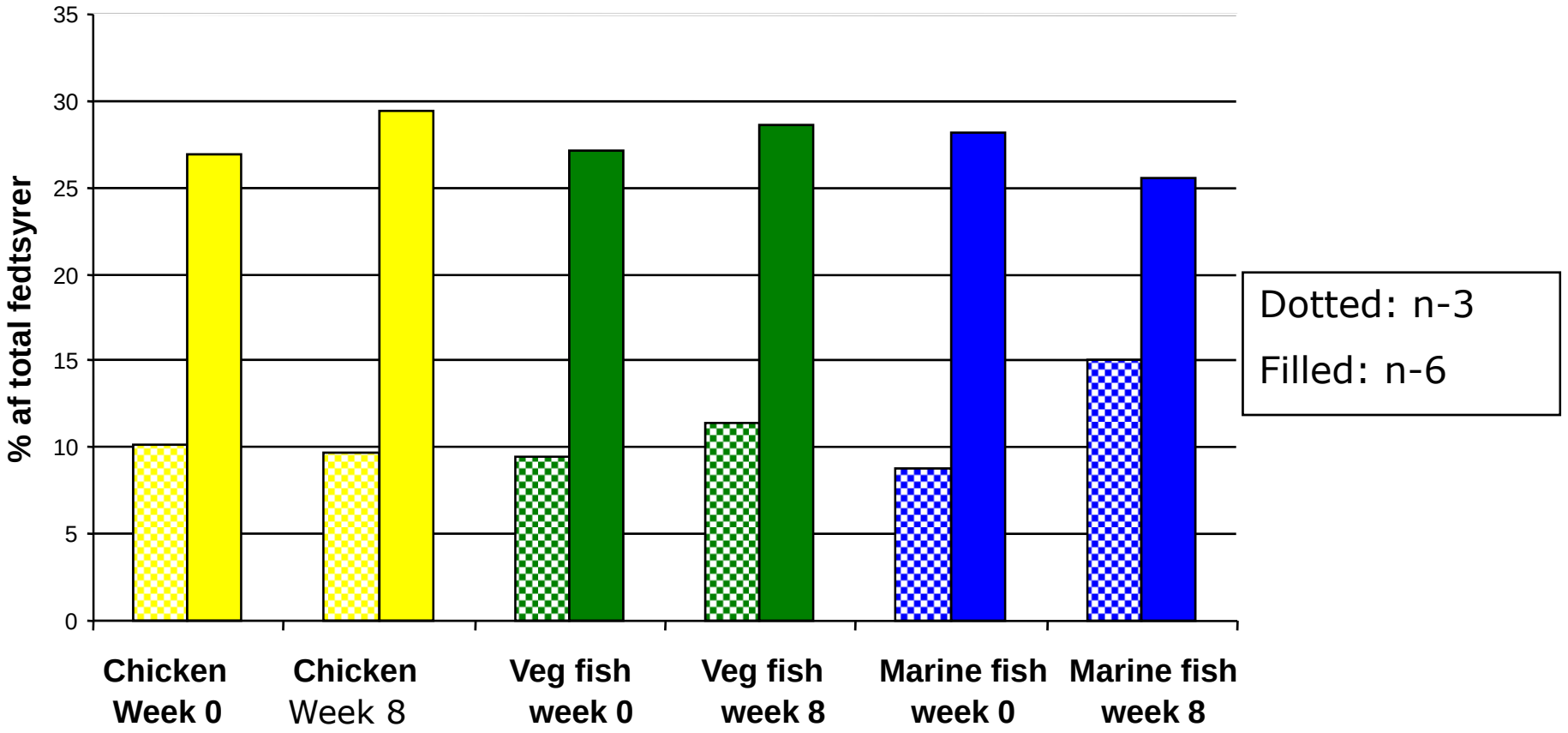


EPA/DHA

Total fat content: feed 24-27%; fillet 8-11%

Fatty acid composition in blood of test persons, who have eaten ”vegetarian” or ”marine” fish

Intake: 150 g fish/chicken per day during 8 weeks



Phospholipids

- The marine PL are also associated to positive health effects, mainly due to their high content of LC n-3 PUFA.
- Some studies have also shown that the bioavailability of the LC n-3 PUFA is higher from PL than from TAG.
- Sphingolipids are suggested to have a role in suppression of colon cancer.
    - However, most studies so far are performed on vegetable and animal PL (not fish) and the mechanisms are not well understood.

Fish protein

- It is generally accepted that a high protein diet generally promotes weight loss. Fish, particularly lean fish is a very good protein source.

- Animal experiments suggest that fish protein may have a reducing effect on blood pressure and improves insulin sensitivity

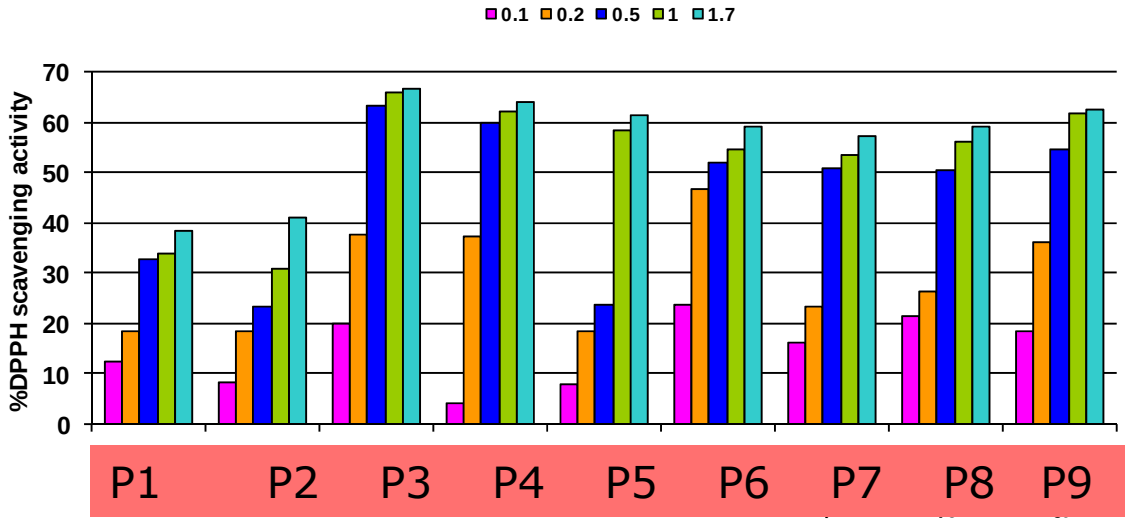
- One human study has shown that fish protein perhaps can improve insulin sensitivity and thereby reduce type 2 diabetes risk

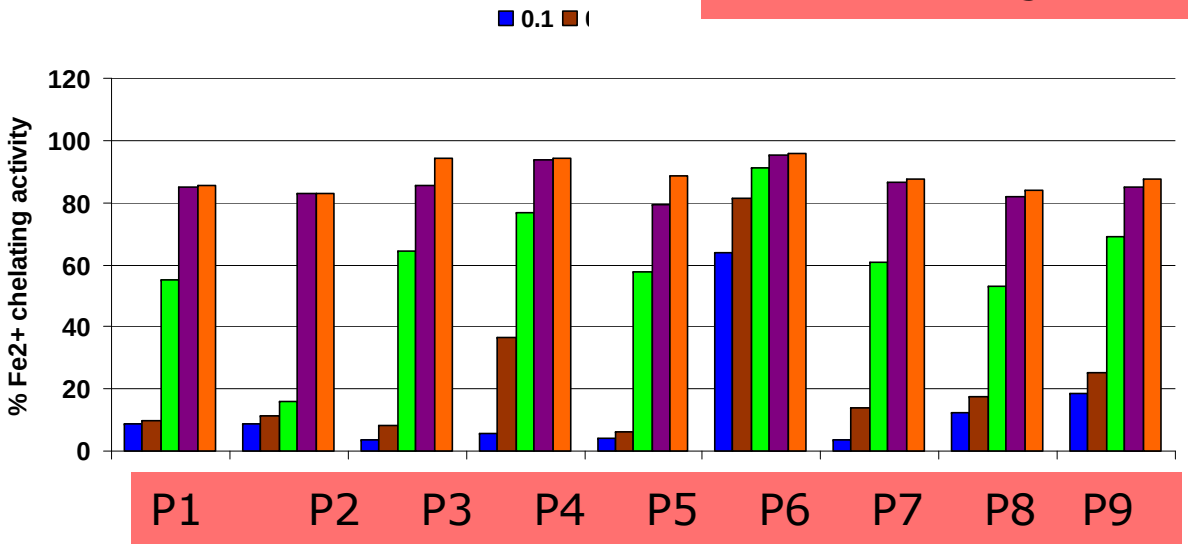
Fish peptides

- Peptides are formed naturally in our digestive system during protein digestion
- In vitro and animal experiments show that fish peptides may:
  - Reduce blood pressure
  - Stimulate immune defense
  - Protect against iron deficiency
  - Work as antioxidants
  - Regulate satiety
  - Reduce growth of cancercells

Antioxidant activity fish peptides

Good ability to scavenge free radicals





Good ability to chelate metal ions, which may catalyse oxidation

Taurine - a free amino acid

- Limited synthesis of taurine in humans. Present in fish in large quantity
- Human and animal studies show a risk reducing effect on CVD, particularly together with omega-3 fatty acids
- Important for excretion of cholesterol via bile salts
- May play a role for our mental health (important component in cell membranes)
- Animal and in vitro studies show that taurine may:
  - Act as an antioxidant
  - Reduce blood pressure
  - Reduce cholesterol levels
  - Reduce coronary thrombosis risk

Vitamin D

- Fish – a very important source of vitamin D
- Particular requirements during the loooong and daaark winter time in Nordic countries
- Associations found between vit D/vit D metabolites and :
  -vascular calcification
  -diabetes type II/metabolic syndrome
  -bone health
- The hormonal calcitriol suggested to have anti-cancer activity
- Newer investigations suggest that vitamin D important for mental health

Minerals – Selenium

- Essential micronutrient
- Seafood has a high content
  - Seafood important source in some areas
  - Primary source is cereals, levels in soil important

- Co-factor in some enzyme systems, e.g. glutathione peroxidases
  - Associated with defense against oxidative stress
  - Involved in detoxification of contaminants
- Reduced cancer risk, prostate cancer (Methylselenol)
- Evidence for health effects not conclusive

Fortification with selenium via fish feed

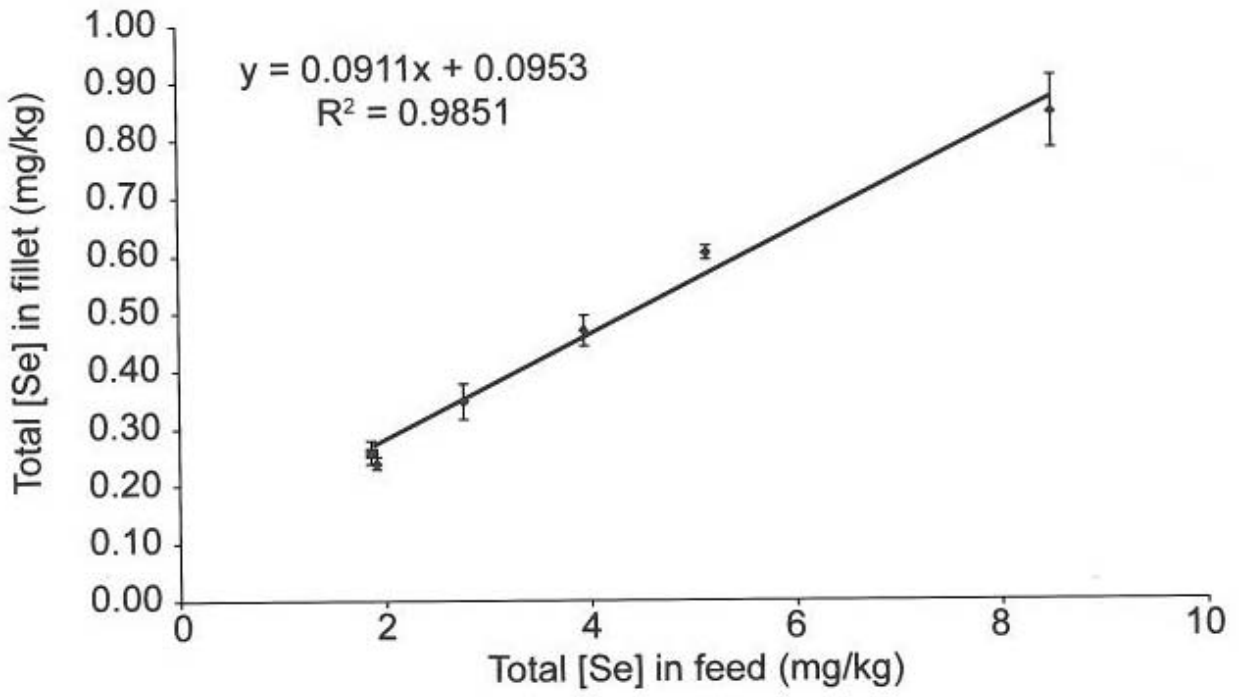


Figure 2. Selenium content in muscle tissue of African catfish farmed with organoselenium from selenium enriched garlic feed (Experiment 3).

From: Luten et al. In Luten et al. Seaf ood research from fish to dish2006

Chitosan and glucosamine

- Shellfish and crustacean shells are rich in chitin, the raw material of chitosan and N-acetyl-glucosamine.
- Low levels of evidence as there is a lack of human studies.
- Several suggested effects:
  - Chitosan shows antibacterial and antioxidative activities
  - In mice, chitosan lowered blood cholesterol and HMW chitosan increased the survival in cancer
  - N-acetyl glucosamine and highly deacetylated chitosan suggested to be used as a substrate for tissue repair.
  - May reduce pain in connection with osteoarthritis.
  - N-acetyl-glucosamine suggested to have some protective effect against Alzheimers disease.

SEAWEEDS

Seaweed composition

Seaweed is known for its high content of polysaccharides, minerals and certain vitamins

Difficult to conclude on the contents of the different components as they vary with geography, environment, within populations and season

Proteins: Generally low content: 5-15% of dry weight, some up to 47 %

Lipids: up to 4%, rich in the omega 3-fatty acids

Polysaccharides: 35-60%

Minerals: Na, K, P, Ca, Mg, Fe, I

Vitamins: Vitamin A, B_1, B_2, B_6, B_{12}, C, D, E

Proteins

Same protein level as in soy beans

Contains:

Phycobili proteins: antioxidative effect (Plaza et al 2008)

Lectin: aggregate blood cells (Murata and Nakazoe 2001)

Lipids

Lipids in seaweed can be divided into:

- Sterols
- Tri-, di- og monoacylglycerols
- **Phopholipids**

Fatty acids:

n-3 Poly Unsaturated Fatty Acids (PUFA)

- α–linolenic acid and
- eicosapentaenoic acid (EPA))
- other n-3 PUFA such as 18:4n-3

Sterols

Rich in sterols, such as fucosterol (especially in Fucus)

-possible reduction of blood cholesterol (Plaza et al 2008)

-anti-inflammatory (inhibits infections; Plaza et al 2008)

Pigments

Fucoxanthin: pigment/cartenoid from *Fucus* species (6%)

- Antioxidant (Le Tutour et al 1998)
- UV-B defense (Heo and Jeon 2008)
- Preventive effect on cerebrovascular diseases
  (change in brain blood flow; Plaza et al 2008)

- Affecting fat metabolism (Plaza et al 2008)
  - Anti-obesity
  - Possible up-regulation of UCP1 in BAT (brown adipose tissue)
  - 2% lipids from *Undaria* reduce White AT (g/kg body weight) of mice and rats (Maeda et al 2008)

Polysaccharides

Table 5. Polysaccharides in seaweed species with characteristics, source and bioactivity

	Characteristics	Source and content	Bioactivity
Polysaccharides	Total	*Saccharina latissima* [a] *Sargassum pallidum* [b,c]	Antitumor action [a, b] Potent anti-coagulant [c] Decrease in LDL-cholesterol in rats [d] Anti-herpetic [e]
Phycolloids	Algins/alginic acid	*Undari pinnatifida* [a]: 24 % [f] *Laminaria digitata*: 32 % [g] *Laminaria* sp. [h] *Sargassum vulgare* [i] *Ascophyllum nodosum*: 28 % [g]	Anticancer [a]
	Carrageenans	*Chondrus crispus* [H] *Eucheuma cottonii* [m]	Antitumor and immunomodulation [j,k] Anti-HIV [l], but no efficacy on humans [i]
	Agar	*Gracilaria* sp., Gigartina sp. etc. [m]	
Fucoidan ranging from typical fucoidans (major components) to low sulphate-containing heteropolysaccharide-like fucans (minor components) [o]	Fucan composed of neutral sugars other than fucose and a high content of uronic acid(s) [o]	*Sargassum horneri* [c] *Sargassum vulgare*: uronic acid, xylose and fucose accounted for >90 % of total sugars [i] *Fucus vesiculosis* [o] *Undaria pinnatifida* [p]	Potential antiviral [c] Slightly anticoagulant activity [o] Anti-herpetic [p]
	Fucoidan=fucan sulphate, containing mainly L-fucose, sulphate, and no uronic acid [o,q]	*Laminaria digitata*: 5.5 % [g] *Laminaria* sp. [h] *Ascophyllum nodosum* [r]: 12 % [g] *Undaria pinnatifida* [p,s,t]: 1.5 % [f] *Fucus vesiculosis* [o] *Eisenia bicyclis* [h]	Potential antiviral (HIV and HSV) [e,s,t,v] Anticoagulant [o,x] Anti-arteriosclerosis [a] Anti-cancer [a,y] Potential antiviral against human cytomegalovirus and avian flue [s] Anti-tumor activity [h] Inhibits growth of *Cryptosporidium parvum* in mice [z]
Mannitol		*Laminaria digitata*: 13 % [g] *Laminaria* sp. [h] *Sargassum mangarevense*: 1-12 % [æ] *Ascophyllum nodosum*: 7.5 % [g]	Effectively protects the photosynthetic apparatus from low-salinity damage [ø,å]
Laminaran	Branched (soluble) and unbranched (unsoluble) polysaccharide: beta 1-3,beta 1-6-glucan [å,β]. 84-94 % sugar and 6-9 % uronic acid [α]	*Laminaria digitata*: 14 % [g] *Laminaria* sp. [h]: 99 % of total sugars [α] *Fucus vesiculosis*: 84 % of total sugars [α] *Ascophyllum nodosum*: 4.5 % [g];90 % of total sugars [α] *Undaria pinnatifida* 3 % [f]	Only found in brown seaweed [a]
Phycarine		*Laminaria digitata* [o]	Immune system, stimulation of macrophage phagocytosis [δ]
Porphyran	Polysaccharide: polymer of acidic saccharide containing sulphate groups, β-1,3-xylan [$]	*Porphyra umbilicalis*: 48 % [g] *Porphyra* sp. [$]	Potential apopototic/programmed cell death activity [$]
Ulvan	Polysaccharide, highly branced polymers of soluble dietary fiber and contain rhamnose, glucuronic acid and xylose [μ,π]. Structurally similar to the mammalian glycosaminoglycans [ω]	*Ulva lactuca* [ω]	Cytotoxicity and cytostaticity, HU colon cell line [ω]

a= (Murata and Nakazoe, 2001), b= (Ye et al., 2008), c= (Athukorala et al., 2007), d=(Amano et al., 2005), e= (Ghosh et al., 2009), f= (Je et al., 2009), g= (MacArtain et al., 2007), h= (Bartsch et al., 2008a), i= (Dietrich et al., 1995), j= (Yan et al., 2004), k= (Zhou et al., 2006a), l= (Skoler-Karpoff et al., 2008), m= (FAO, 2008), n= (Vlieghe et al., 2002), o= (Nishino et al., 1994), p= (Hemmingson et al., 2006), q= (Matsubara et al., 2005), r= (Marais and Joseleau, 2001), s= (Lee et al., 2004), t= (Maruyama et al., 2007), u= (Yamamoto et al., 1984), v= (Schaeffer and Krylov, 2000), x= (Mayer and Hamann, 2004), y= (Han et al., 2008), z= (Smit, 2004), æ= (Zubia et al., 2008), ø= (Gessner, 1971), å= (Lobban and Harrison, 1994), α= (Rioux et al., 2007), β= (Deville et al., 2007), δ= (Mayer et al., 2007), $= (Plaza et al., 2008), ω=(Kaeffer et al., 1999), μ= (Bobin-Dubigeon et al., 1997), π= (Michel and Macfarlane, 1996)

Minerals & vitamins

Seaweeds contain more minerals than any other food. This is mainly due to the the surface cell wall polysaccharides that freely and selectively absorb inorganic nutrients from the sea.

Seaweeds contain all the minerals human needs including trace metals

Table 8. Mineral contents in marine algae[8)]

	Minerals (mg/g)					Vitamins (/100 g)			
	Na	K	Ca	P	Fe	A(IU)	B_1(mg)	B_2(mg)	C(mg)
Enteromorpha compressa (Aonori)	530	3,200	840	740	32.0	12,000	0.56	1.90	40.0
Undaria Pinnatifida (Wakame)	6,100	5,500	960	400	7.0	1,800	0.30	1.15	15.0
Hizikia fusiformis (Hiziki)	1,400	4,400	1,400	100	55.0	310	0.01	0.14	0
Laminaria saccharina (Konbu)	2,800	6,100	710	200	3.9	560	0.48	0.37	25.0
Porphyra complex (Amanori)	120	2,100	390	580	12.0	14,000	1.15	3.40	100.0
Tomato	2	230	9	18	0.3	220	0.05	0.03	20.0
Spinach	21	740	55	60	3.7	1,700	0.13	0.23	65.0
Carrot	26	400	39	36	0.8	4,100	0.07	0.05	6.0
Orange (Valencia)	1	190	20	20	0.1	42	0.01	0.03	40.0

The values refer to the analyzed data of the products which are available in the market.

(Murata and Nakazoe 2001)

Other compounds

Phenols mainly in brown algae (particularly phlorotannins)

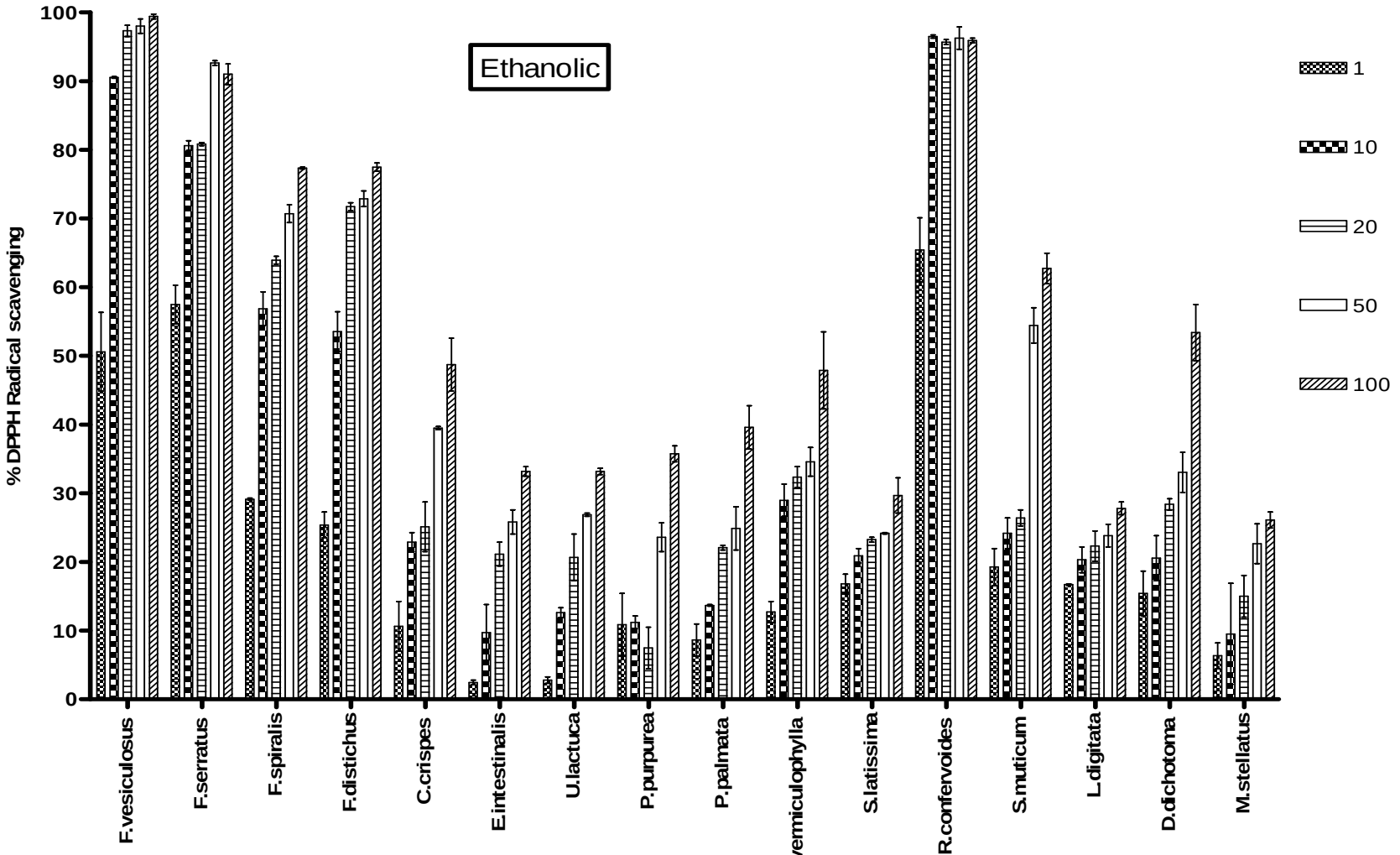
located in the outer membrane in physode-vesicles

Antioxidant and anti-*Staphylococcus* effect (Zubia et al 2008)

Other effects of phlorotannin:

antiherbivour (#1, #111)
may form complexes with alginic acid
they may even be excreted to the surrounding media
antioxidant (in vitro #8, ESR cellul no cytotoxicity on human fetal lung fib
antidiabetes (ref in #8)
radiation protection (ref in #8)
anti-cancer (ref in #8)
anti-HIV (ref in #8)
anti-allergic (ref in #8)
anti-plasmin inhibition (#10)
photochemoprevention (#10)
antiproliferative activities (#18)

DPPH Radical scavenging activity of different seaweed extracts



KRILL

Lipids, minerals & Chitin

- Current interest in krill mainly due to high content of omega-3 PUFA and phospholipids

- Magnesium (high level)
- Phosphorous (high level)
- Flouride (high level)
- Iron (low level)

- Krill is a better source of chitin than crab and shrimp

Proteins

- Amino acid composition reported (excellent composition)
- Protein digestibility and availability determined in rat
- Krill proteins capable of supporting protein synthesis in rats
- No studies on krill protein allergies

Sea cucumber

Jellyfish

Lipids

- SC: EPA 43-57%, DHA 2.0-5.8%.
  Branched chain bacterial FA: *ai*-C15:0 – high levels are thought to be responsible for wound healing

- JF: trans-6-hexadecenoic acid (also found in turtles)
  [Hooper et al., Lipids 8, 1973, 509-516]
- JF: 9.3% tetracosehexaenoic acid (24:6n-3) and 0.8% tetracosapentaenoic acid (24:5n-6) in jellyfish *Aurelia sp.*
  [Nichols et al., Lipids 38, 2003, 1207-1210]
- JF: C26- to C29-sterols. Cholesterol was the major sterol
  [Yasuda, Comparative biochemistry and Physiology B, 1974, 225-230]

Proteins

- JF: Aequorin, a protein, may help protect the brain (prevent brain cell death in neurodegenerative disorders, such as Alzheimer´s, Parkinson´s) – recommended dose 10 mg/day
  [K.A. Gazella: Brain boosters that work; in: Better nutrition, sept 2008]

- SC: glutamic acid was the predominant AA, followed by glycine and aspartic acid. Essential AA (leucine, lysine) were also present at high levels

- JF: The protein Mucin was recently claimed to help in regrowth of cartilage (Physorg.com)

Health effects

- SC: traditional medicine for asthma, hypertension, rheumatism, anemia, sinus congestion. Healing wounds. Tissue repair ability has been associated with high EPA content. Inhibition of lung cancer, improving body immunity, antiaggregation of platelet.

- JF: Immunostimulating effect of collagen

Conclusions

- Fish contains several bioactive compounds
  - Omega-3 PUFA's beneficial effects the most well documented, but more research necessary to fully understand the potential effects
  - More research is also necessary to document the beneficial effects of other compounds such as taurine, fish peptides, selenium and vitamin D

- Exploration of bioactive compounds from the aquatic environment is in its initial stage

- Effects of most of the bioactive compounds needs further documentation

Aurelia sp. – Moon jelly

Acknowledgements

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  (Nordic network for marine functional foods: http://www.marifunc.org/)
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  (EU FP6 programme: http://www.seafoodplus.org/)
  Børresen, T. (ed). *Improving seafood products for the consumer.* Woodhead Publishing Ltd, Cambridge,

- Susan Holdt, Caroline Baron, Nina Skall Nielsen, Maike Timm-Heinrich, Sabeena Farvin, DTU Food