Literature review of available techniques to characterize marine and estuarine food webs

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Literature review of available techniques to characterize marine and estuarine food webs

With emphasis for application in the model OMEGA

H.J. De Lange
N.W. Van den Brink

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ABSTRACT

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Food webs can be characterized by use of markers. These are increasingly used in food web studies since they are the only tool that can be used to infer relations through multiple trophic levels. Such a marker is a characteristic of an organism that can be objectively measured and evaluated as an indicator of normal biologic processes. These include fatty acids, stable isotopes, and molecular markers such as immunological markers and DNA markers.
This report presents the results of a literature survey on these marker techniques, their use in describing marine and estuarine food webs, and an evaluation of their usefulness for the model OMEGA.
Stable isotope analysis is advised as the best method to characterize food webs by means of trophic position and carbon source. Fatty acids can be used to differentiate within trophic groups, especially within the group of primary producers

Keywords: stable isotopes – fatty acids – trophic marker – DNA – immunological marker – food web – marine – estuarine

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Preface

This report presents the results of a literature survey carried out by Alterra on marker techniques to describe food webs, their use in describing marine and estuarine environments, and an evaluation of their usefulness for the model OMEGA.

The model OMEGA has been developed by Rijkswaterstaat-RIZA, to describe possible effects and accumulation of toxicants in freshwater and terrestrial environments. Rijkswaterstaat-RIKZ wants to apply the model OMEGA for marine and estuarine environments, but to do so a complete insight into how marine/estuarine food webs can be modelled is needed.

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Summary

The elucidation of food web structures is a central theme in ecology. Modelling tools can be useful in the description of food webs and the prediction of effects of bottom up or top down stressors, among which contaminants. An example of such a model is OMEGA (Optimal Modelling for Ecotoxicological Assessment), which has been developed for freshwater and terrestrial environments. RIKZ wants to apply the model OMEGA for marine and estuarine environments, but to do so a complete insight into the structure of marine/estuarine food webs is needed. Thus, before the model OMEGA can be used for marine/estuarine food webs, these food webs need to be characterized.

Food webs can be characterized by use of markers. These are increasingly applied in food web studies since they are the only tool that can be used to infer relations through multiple trophic levels. Such a marker is a characteristic of an organism that can be objectively measured and evaluated as an indicator of normal biologic processes. The perfect marker is a compound whose origin can be easily identified, that is inert and non-harmful to organisms, not selectively processed during food uptake and incorporation, and that is metabolically stable and hence transferred from one trophic level to the next in a predictable manner. Unfortunately, such ideal markers are nonexistent, but there is a number of techniques available that meets several of the abovementioned criteria. These include fatty acids, stable isotopes, and molecular markers such as immunological markers and DNA markers.
This report presents the results of a literature survey on these marker techniques, their use in describing marine and estuarine food webs, and an evaluation of their usefulness for the model OMEGA.

The main results of this literature survey are:
- Stable isotopes and fatty acids are analysed in body tissues, and thus give a marker of assimilated food integrated over a longer time period.
- Stable isotope signatures serve as bulk indicators for dietary carbon sources ($\delta^{13}C$) and trophic level identification of consumers ($\delta^{15}N$). $\delta^{34}S$ can be used to distinguish between benthic and pelagic food sources.
- Stable isotope analysis is rather easy, and it generates one value per sample. Expert users advise that stable isotope results should always be combined with ecological and physiological information of the studied species/communities.
- Fatty acids can provide detailed information on the source of a consumer's diet as well as its current nutritional and physiological status.
- Fatty acid analysis is rather difficult, and it generates a large number of fatty acids per sample. The interpretation can be more precise than is possible with stable isotopes.
- Molecular techniques using immunological or DNA markers are used to analyse the gut or faeces content, and thus give a marker of recently ingested food.
- Molecular techniques describe specific trophic relations between (groups) of species.

Our recommendation for use in OMEGA follows a tiered approach:

1. *Stable isotope* analyses of C and N are recommended as the first step in describing food webs, since this gives information on both carbon source and trophic level.
2. *Fatty acids* can be used to differentiate within trophic groups, especially within the group of primary producers.
3. If specific information is required on predator-prey relation, the use of *DNA markers* is suggested. For general purposes it is our estimation that the limitations of molecular methods (time and expenses needed to obtain specific primers or antibodies, and time needed to analyze all possible interactions) outweigh the potential advantages of knowing specific predator-prey interactions.

1 Introduction

1.1 General

The identification of predator-prey relationships and the elucidation of the food web structure is a central theme in ecology. In ecotoxicological studies such information is essential in order to assess the potential of contaminants to accumulate to top-predators. Useful tools in this respect are models that describe food webs and predict effects of bottom up or top down stressors. Modelling tools can also be used to describe accumulation and possible effects of toxicants. An example of such a model is OMEGA, which has been developed by RIZA for freshwater and terrestrial environments.

RIKZ wants to apply the model OMEGA for marine and estuarine environments, but to do so a complete insight into how marine/estuarine food webs can be modelled is needed. Thus, before the model OMEGA can be used for marine/estuarine food webs, these food webs need to be characterized.

There are several methods that can be used to describe food webs and trophic relationships between organisms:

- observational: *e.g.* studying fluctuations in field populations of prey and suspected predator, from such observations food webs can be constructed;
- experimental: *e.g.* laboratory feeding experiments, or field experiments excluding or including predators, used to describe specific predator-prey relations;
- analytical: *e.g.* visual observations of predator attacks on prey, labelling of prey, visual (microscopic) inspection of gut contents and pellets; use of biochemical, immunological or stable isotope patterns.

The information on predator-prey interactions that is obtained is then used to construct a food web. Of the above mentioned approaches and methods, biomarkers are the only tool that can be used to infer relations through multiple trophic levels. These are increasingly used in food web studies.

A biomarker can be defined as a characteristic that can be objectively measured and evaluated as an indicator of normal biologic processes. The perfect biomarker is a compound whose origin can be uniquely and easily identified, that is inert and non-harmful to organisms, not selectively processed during food uptake and incorporation, and that is metabolically stable and hence transferred from one trophic level to the next in both a qualitative and quantitative manner. Unfortunately, such ideal biomarkers are nonexistent, but there are several techniques available that meet one or more of the abovementioned criteria. These include fatty acids, stable isotopes, and molecular markers such as immunological markers and DNA markers.

This report presents the results of a literature survey on these biomarker techniques, their use in describing marine and estuarine food webs, and a comparison of their

usefulness for the model OMEGA. Used methods of the literature search are described in Appendix 1.

1.2 Outline of review

This literature review focuses on description of selected techniques. For each technique, the following questions are discussed:

- How does the technique work?
- What type of marker: qualitative, quantitative, is it inert?
- In what type of research has it been applied as a trophic marker?
- What are the pros and cons?
- What expertise and equipment is needed?
- Is the technique easy to use?

We describe first fatty acids (Chapter 2), then stable isotopes (Chapter 3), and lastly molecular markers (Chapter 4). Finally, in Chapter 5 we compare the described techniques, and give recommendations for use in OMEGA food web modelling.

2 Fatty acid markers

2.1 Background

Fatty acids (FAs) are the main constituents of acyl lipids, such as triacylglycerols (see example in Figure 1), glycolipids, and phospholipids. Algae are the primary producers of FAs in aquatic food webs. There are about 15 to 20 major fatty acids that can be identified in most algal samples (see Appendix 2). When ingested by consumers, the fatty acids are released from the backbone molecule (*e.g.* glycerol in the case of triacylglycerol) during digestion, and enter the circulation of the consumer intact as free fatty acids. In general, these free fatty acids are transferred to the tissues and not transformed or metabolised. Once taken up by tissues in the consumer, fatty acids are either used for energy, or re-esterified to a backbone molecule and stored in adipose (=fatty) tissue (Napolitano, 1999; Iverson *et al.*, 2004).
Some fatty acids are limited to certain taxa, and if these are metabolically stable, they can be used to trace energy transfer through the food web. A relative limited number of fatty acids are elongated or desaturated by animals, thus it is important to distinguish dietary from biosynthesis sources when fatty acids are used as marker (see Appendix 2).

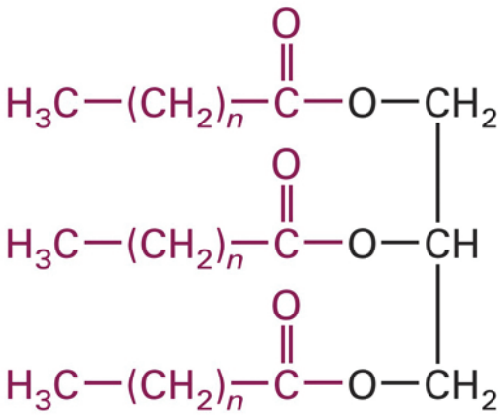

Figure 1: Example of a triacylglycerol, a glycerol backbone (in black) with three fatty acids (in red).

Algae are the base of the food web and the primary producer of fatty acids. Most of the commonly used biomarker fatty acids are marker of an algal taxonomic group, but there are also bacterial fatty acids and terrestrial fatty acids that can be used as biomarker (Table 1). Numerous studies have demonstrated that fatty acids patterns are passed from algal prey to herbivore consumer (*e.g.* Kainz *et al.*, 2004; Persson & Vrede, 2006). There are a few studies available that present results on further transference of fatty acids to higher trophic level animals, such as fish (*e.g.* Kirsch *et al.*, 1998; Kainz *et al.*, 2004), birds (*e.g.* Dahl *et al.*, 2003), and marine mammals (*e.g.* Iverson *et al.*, 2004).

Table 1: Specific fatty acid markers of algal classes, bacterial markers and terrestrial markers (based on Napolitano, 1999 and Dalsgaard et al., 2003); see Appendix 2 for explanation of abbreviations.

Class	Fatty acid trophic marker
Algal markers	
Bacillariophyceae	16:1ω7; 16:4ω1; C16PUFA; EPA
	ratio 16:1ω7/16:0
	ratio EPA/DHA
Chlorophyceae	16:4ω3; C16PUFA; 18:2ω6; 18:3ω3
Cryptophyceae	18:1ω9; 18:2ω6; 18:3ω3
Dinophyceae	18:1ω9; 18:4ω3; 18:5ω3; DHA
	ratio 18:5ω3/18:3ω3
Cyanophyceae	10:0; 16:1ω7; 18:1ω7; 18:2ω6; 18:3ω6; 18:3ω3
Prymnesiophyceae	18:1ω9 + 18:4ω3
Bacterial markers	
gram-positive	iso 15:0; anteiso 15:0; 15:0; anteiso 17:0
gram-negative	cyclopropane, 2- and 3-hydroxy acids
sulfate reducing	10Me16:0; iso 17:0; iso 17:1; cy17:0; 17:0; cy 19:0; 16:1ω7; 18:1ω7
methanotrophic bacteria	16:1ω6; d11-trans-16:1; 18:1ω8
general bacterial markers	odd carbon numbered + branched chain FA
	ratio 18:1ω7/18:1ω9
Other markers	
terrestrial markers	18:2ω6
	18:2ω6 + 18:3ω3 > 2.5 % total FA
	22:0 + 24:0
	Σ 24:0-32:0
trophic class	ratio EPA/DHA decreases toward higher trophic levels
carnivory	18:1ω9
	lower ratio of 18:1ω7/18:1ω9
herbivorous calanoid copepods	20:1ω9; 22:1ω11

An important aspect of fatty acid trophic marker is that the fatty acid composition of the animal represents the time-integrated dietary intake. However, one must realize that lipid metabolism and storage in animals are organ-specific, fatty acid biomarkers should therefore be extracted from specific body parts or tissues. For example, the fatty acids in triacylglycerols in adipose tissues of animals represent dietary fatty acids and are thus particularly useful as trophic markers. In contrast, the liver accounts for most of the fatty acid synthesis by elongation and desaturation, these fatty acids are not useful as trophic marker (Napolitano, 1999).

The fatty acid composition is a qualitative marker. There are however some efforts undertaken to quantify predator diet. This is done by means of the development of a statistical model to provide quantitative estimates of the proportions of prey species in the diets of individual predators using fatty acid signatures (Iverson *et al.*, 2004).

Since fatty acids can be altered by a variety of organisms, the signal may change through the food web, and thus they are not a true inert biomarker. Another aspect is that species which have a large lipid reservoir have the potential to buffer short term dietary changes, as was shown for the krill species *Euphausia superba* (Stübing *et al.*,

2003). Fatty acids are not an inert trophic marker for such species. In addition, temperature and light can influence the fatty acid composition (Stübing *et al.*, 2003). Metabolic condition and reproductive state also affect the fatty acid composition. All these aspects challenge the ecological significance of use of fatty acids as OM biomarkers (*e.g.* Kainz *et al.*, 2006).

2.2 Examples of fatty acids used as trophic marker in marine food webs

Fatty acid profiles have been used in a variety of mostly marine food web studies, see Appendix 3:

- Description or confirmation of predator – prey relation (*e.g.* Kattner *et al.*, 1998; Dahl *et al.*, 2000; Falk-Petersen *et al.*, 2004; Stevens *et al.*, 2004; Ahlgren *et al.*, 2005).
- Transference of FAs through multiple trophic levels (*e.g.* Copeman & Parrish, 2003; Dwyer *et al.*, 2003; Kainz *et al.*, 2004; Mansour *et al.*, 2005).
- Description of feeding behaviour (*e.g.* Borobia *et al.*, 1995; Smith *et al.*, 1996; Auel *et al.*, 2002; Howell *et al.*, 2003; Nyssen *et al.*, 2005).
- Description of food chain: *e.g.* detrital, benthic, plankton, inshore, pelagic (*e.g.* Fukuda & Naganuma, 2001; Lea *et al.*, 2002; Dahl *et al.*, 2003; Herman *et al.*, 2005).
- Combination with contaminants (Mateo *et al.*, 2004; Herman *et al.*, 2005).

2.3 Pros & cons

Pros:

- Presence and combinations of certain FA can be characteristic of particular algal classes. It is possible to differentiate within a trophic level.
- Primary producer-primary consumer interaction (*e.g.* alga – herbivorous zooplankton) can be well described by FAs.
- FAs can be used to resolve ecological niches and complex trophic interactions.

Cons:

- No single FA can be assigned uniquely to any one species, and FA are not necessarily stable. Temporal dynamics can be species-specific and are linked to metabolic condition or reproductive state.
- FAs can be used as qualitative and semi-quantitative food web markers. Use as quantitative marker is not feasible.
- FAs are mostly a biomarker of primary producers; hence the method is most suitable for herbivore species, and less for omnivorous and carnivorous species. In higher trophic levels, FA signatures may originate from a variety of sources, and markers of herbivory become "blurred".
- General lack of validation (often as simple as examining stomach contents)
- Multivariate statistical tools are often necessary to interpret the data, multivariate results are sometimes difficult to interpret or have low power of resolution.

2.4 Ease of technique

Fatty acid analysis is somewhat complicated, and it does require some specialized chemicals, equipment and laboratory skills (see Parrish, 1999, for a detailed description).

Samples
Samples can be taken from various biotic components:

- Plankton needs to be concentrated first. Algae can be filtered or centrifuged. Either 5-10 mg freeze dried material is sufficient, or a sufficient volume (similar as needed for chlorophyll analysis) filtered over a glass fibre filter.
- Zooplankton is usually collected with the use of meshes, and further picked by hand. Storage is done frozen or freeze-dried. A sample size of 5-10 mg for zooplankton is sufficient (Kainz *et al.*, 2004).
- Invertebrates can be picked by hand. These can be analyzed individually, or if the organism is large enough, a subsample can be taken. A sample size of 200-300 mg is sufficient for animal tissues (Meziane & Tsuchiya, 2000).
- Samples from fish are often taken from the dorsal muscle, and then freeze-dried. A sample size of 25-35 mg is sufficient for the analysis (Kainz *et al.*, 2004).
- Samples from large marine mammals are usually taken from the blubber, by biopsy or necropsy techniques. 0.5 to 1.0 g of tissue is sufficient for the analysis (Herman *et al.*, 2005).

As soon as a sample is taken, the lipid content and composition begin to change, catalyzed by enzymes, heat, oxygen and light. Thus, samples should be kept as cold as possible and processed as soon as possible. To avoid contamination, contact with any oils or grease should be avoided. All glassware should be thoroughly rinsed with chloroform, and ashed in a muffle furnace. After processing, the tissue sample or filter should be placed in a clean glass vial containing chloroform. An antioxidant such as BHT (butylated hydroxytoluene) can be added. The airspace above the chloroform should be flushed with nitrogen. Under these conditions, the sample can be stored in a freezer (< -20 °C) for months.

Extraction
Lipids are usually extracted following the extraction procedure (sometimes modified) of Folch *et al.* (1957) involving chloroform and methanol. Care must be taken that the samples are not contaminated with grease, samples should only be handled using chloroform cleansed forceps. Extraction efficiency can vary highly depending on type of sample and followed procedure. This is a complicating factor in fatty acid analysis. Once the lipids are extracted and concentrated in a solvent (usually hexane), the remaining steps are relatively easy.

Fatty acids are released from the acyl lipids and re-esterified to methyl esters. This is usually done in an acidified (3% concentrated H_2SO_4) methanol solution. The fatty acid methyl esters (FAMEs) are extracted with hexane and ready for measurement on a GC.

FAMEs are measured on a GC which should be equipped with a long (>25 m) and very polar silica column, in order to obtain good separation between the different fatty acids. Detection is usually done with a Flame Ionisation Detector (GC-FID). Peaks can be identified using retention times of known standards, and calculating equivalent chain length values. The combination with a mass spectrometer (GC-MS) can be very useful in the identification of unknown peaks. Concentrations are calculated as the peak surface relative to the peak surfaces of quantitative internal standards.

Time needed

The extraction and methyl-esterification steps roughly take up 2 days. Depending on the experience of the person performing the analysis, between 10 and 20 samples can be processed simultaneously. Once the samples are stored in GC vials, they can be stored almost indefinitely in a freezer (< -20 °C).

GC analysis takes roughly 45 minutes (depending on type of column and settings) per sample. Several standards need to be analyzed as well. Having a GC equipped with an auto-sampler is an advantage for processing large number of samples.

Precautions using necropsy samples

Soon after death, triglycerides begin to break-down and form free fatty acids as by-products, hence free fatty acids may serve as a crude measure of decomposition. A rule of thumb may be that when free fatty acids (relative to total lipid) are in excess of 5%, the sample is of suspect integrity.

Unfortunately, the decomposition rate is not precisely known, and this will depend on factors such as temperature, type of organism and cause of death. This should be subject of further research.

3 Stable isotopes

3.1 Background

Isotopes are atoms having the same number of protons, but different number of neutrons, thus only differing in mass and not in chemical properties. Many chemical elements of biological importance (*e.g.* C, H, N, O, S) have two or more stable (non-radioactive) isotopes, of which the lightest is present in far greater abundance in most cases. Each organism has its own isotopic composition, the so-called dynamic stable isotope (SI) fingerprint. This signature is the ratio of the rare, heavy isotope (*e.g.* ^{13}C, ^{15}N, ^{34}S) to the common, lighter isotope (*e.g.* ^{12}C, ^{14}N, ^{32}S), relative to international standards (see Appendix 4 for further explanation).

Analyses of these SI fingerprints across different groups of organisms can be used to characterize food webs, especially sources of carbon and trophic status of consumers. This has proven to be a useful technique, and has been used increasingly in ecological studies since the 1980's. Most ecological research has studied naturally occurring stable isotope ratios of carbon (C) and nitrogen (N), and in a fewer number of studies sulfur (S). Stable isotope analysis gives results on food that is assimilated, and not food that is merely ingested, giving it an advantage over other methods such as gut analysis in elucidating trophic dynamics (Connolly *et al.*, 2004).

Stable isotopes can be used in combination with certain markers, such as lipids, fatty acids and amino acids. This is already applied in microbial ecology studies (see review of Boschker & Middelburg, 2002). Stable isotopes can also be used in labelling studies (*e.g.* Boschker & Middelburg, 2002; Boschker *et al.*, 2005), and in modelling studies (*e.g.* Van Oevelen, 2006).

General procedure in SI analysis is to analyze the isotopic signature of different trophic levels, *e.g.* primary producers, primary/secondary/tertiary consumers. The producer signature is then compared to the consumer signatures. General pattern is that the carbon and sulfur signature is relatively conservative across trophic levels, but the nitrogen signature has a predictable enrichment (fractionation) per trophic level (see paragraph 3.2).

Several criteria must be met to use natural stable isotope signatures in ecology (after Marguillier, 1998):

- The isotope signature of the potential sources must be isotopically distinct from each other.
- The isotope signatures must either not change, or change predictably.
- If isotopes are used as indicators of ecological processes, then the extent of fractionation, discrimination, and segregation must be known for all reactions.

Temporal variation reflected in tissue variation
Tissues with different turnover rates reflect average dietary records over different time windows. Most commonly measured tissues in vertebrates are bone, blood, muscle and feathers (Dalerum & Angerbjörn, 2005). Bone collagen has a slow elemental turnover rate (half life roughly 6 months), and is therefore suitable for long-term trends in dietary patterns. Most other tissues have a relatively quicker turnover rate (half life roughly between 1 and 6 months) and can thus be used to study seasonal patterns. Results from studies of tissues with a fast turnover rate, *e.g.* plasma (half life of 0.5 to several days), reveal the most recent dietary activities (Dalerum & Angerbjörn, 2005). It is also possible to compare measurements from different sections on tissues with progressive growth, such as feathers, hair, claws and teeth. These tissues will retain isotopic values in a chronological order, and can be indicative for the specific period that they are formed (Dalerum & Angerbjörn, 2005).

This is illustrated by a study on the whitefish (*Coreganus lavaretus*). One must realize that ectotherms such as fish have a discontinuous growth pattern over the year. A study of the whitefish revealed that the isotope signature of the muscle reflected food consumed during periods of growth (spring and summer), with a 4-5 months lag time. The isotope signature of the liver, with a continuous protein turnover, had a larger seasonal variation, and reflected the isotope composition of the food with a 1-month lag time (Perga & Gerdeaux, 2005).

3.2 Trophic fractionation

Isotopic fractionation in biochemical reactions (both kinetic and chemical processes) occurs when similar molecules of slightly different mass (i.e. isotopes) react at different rates (Peterson & Fry, 1987). Each metabolic process has its own fractionation value for each element. Fractionation occurring in predator-prey relationships is termed trophic fractionation; this is the change in isotopic signature from prey to predator.

Many previous studies have shown that trophic fractionation is predictable, when comparing large groups of samples (*e.g.* Peterson & Fry, 1987; Vander Zanden & Rasmussen, 2001; Post, 2002). These results show that consumers are similar in isotopic compositions to their diets for carbon and sulfur, but on average 2 to 5 ‰ heavier than dietary nitrogen. Thus, especially the nitrogen isotope value can be used to identify the trophic level of a predator. The carbon and sulfur isotope value can be used to identify the source (primary producer). Biplots with $\delta^{13}C$ (or $\delta^{34}S$) on the x-axis and $\delta^{15}N$ on the y-axis can be used to visualize trophic levels and pathways of biomass (carbon) through the food web (see Figure 2).

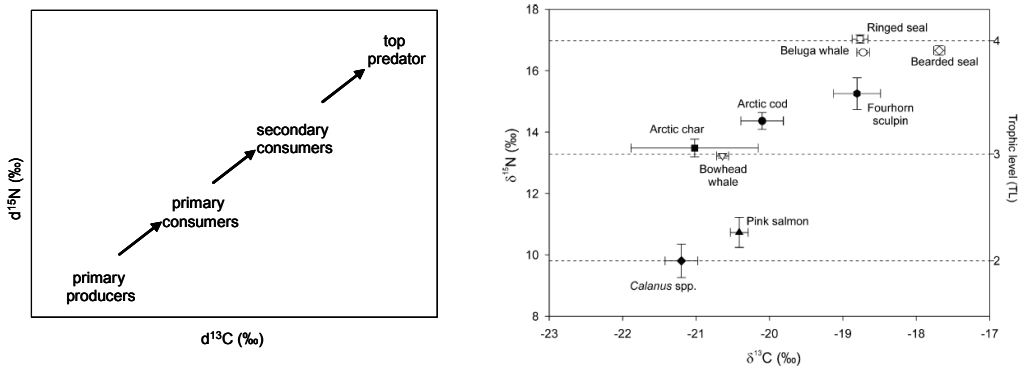

Figure 2: Examples of stable isotope biplots: left panel is a conceptual model plot, right panel is an example taken from Hoekstra et al. (2003).

Carbon

Carbon isotope ratios are relative conservative from primary producer to apex predator, with an enrichment of <1‰ per trophic level (Petersen & Fry, 1987). Since primary producers have different $\delta^{13}C$ values (see Table 2), the $\delta^{13}C$ can be used to estimate the diet of a consumer and follow transfer of organic matter trough a food web. In terrestrial ecosystems, C3 and C4 plants can be distinguished. In freshwater systems, allochthonous versus autochthonous sources of C can be separated. For marine systems, inshore (benthic) versus offshore (pelagic) versus can be distinguished.

Table 2: $\delta^{13}C$ values of CO_2 and different primary producers (from Peterson & Fry, 1987)

origin	$\delta^{13}C$
CO_2 in atmosphere	-7 ‰
total CO_2 in ocean	0 ‰
C3 plants	-28 ‰
C4 plants	-13 ‰
marine algae	-19 to -24 ‰
freshwater algae	-20 to -45 ‰
pelagic source	depleted
benthic source	enriched

Nitrogen

The $\delta^{15}N$ signature of animals is on average 2 to 5 ‰ heavier than their diet $\delta^{15}N$ signature (Peterson & Fry, 1987; Post, 2002). Thus, especially the nitrogen isotope value can be used to identify the trophic level of a predator. The seminal paper of Minagawa & Wada (1984) calculated an average fractionation at a single feeding process of 3.4 ‰, which was independent of habitat or trophic level (Minagawa & Wada, 1984). This average value of trophic fractionation is confirmed by other studies (*e.g.* Vander Zanden & Rasmussen, 2001; Post, 2002), and is widely used in estimating trophic level. Trophic level is then calculated with the following formula:

$$1 + [(\text{average } \delta^{15}N \text{ consumer} - \text{average } \delta^{15}N \text{ primary producer})/3.4]$$

For specific systems or areas, other fractionation factors are used, such as 3.8 for marine arctic food webs (Hobson *et al.*, 2002). Another aspect that receives some debate in calculating trophic position is the amount of variance around this average value of 3.4 (*e.g.* Vander Zanden & Rasmussen, 2001; Post, 2002).

Calculating trophic position in this manner will give a good estimate of trophic position in those (theoretical) food webs where there is a strict vertical food chain. Most food webs will be more diverse, with predators feeding on different prey items. Such omnivorous species will have intermediate trophic positions. Complex food webs with significant omnivory will have a lower trophic enrichment than 3.4 (Marguillier, 1998).

Comparisons between studies reveal that values of $\delta^{15}N$ of the primary producer level can differ considerably. This variation is both spatial and temporal, and is the result of variations in the nitrogen source, nitrogen concentration and species succession of primary producers (especially nitrogen-fixers vs. non-nitrogen fixers) (Cabana & Rasmussen, 1996). Spatial variation of $\delta^{15}N$ of primary producers can be further explained by changes in environmental variables such as salinity, depth and temperature (Jennings & Warr, 2003). Another complicating aspect is that the amount of variation around the average $\delta^{15}N$ value is in general larger for shortlived planktonic organisms than for larger animals at higher trophic levels (*e.g.* Post, 2002).

These two aspects combined result in a baseline value of primary producer $\delta^{15}N$ which has a large amount of variation around the average, and which can differ considerably between systems and between seasons. Thus, there is no solid baseline $\delta^{15}N$ value for all primary producers to which all other trophic positions are compared. A single $\delta^{15}N$ value is not sufficient to infer trophic position; it always has to be compared with $\delta^{15}N$ values of other taxonomic groups within the same study.

This variation at the base of the food web also complicates cross-system comparisons. To be able to make such comparisons, and to reliably estimate trophic position of secondary or higher consumers, it is imperative to have a reliable isotopic baseline. Long lived primary consumers are suitable candidates to obtain such isotopic baselines, such as mussels and snails in freshwater ecosystems (Cabana & Rasmussen, 1996; Post, 2002); and mussels in marine ecosystems (Jennings & Warr, 2003). The formula to calculate trophic position will then be:

$$2 + [(\text{average } \delta^{15}N \underline{\text{consumer}} - \text{average } \delta^{15}N \text{ primary } \underline{\text{consumer}})/3.4]$$

Sulfur

Isotope signature of sulfur reveals information on the source of S. S present in the water column is generally in the form of sulfates and is isotopically enriched (+20‰). Sedimentary sulfides formed by bacterial reduction of sulfates in anaerobic sediments are isotopically lighter (-24‰). Porewater sulfates are more variable and can be more enriched than water column sulfates (+60‰), or more depleted if formed as a result of reoxidisation of sedimentary sulfides. For intertidal plants, rainwater with a signature between 2 and 16 ‰ is also a potential source of S. Producers utilizing different sources of S have therefore different signatures. This allows discrimination between benthic and pelagic producers (Connolly *et al.*, 2004).

Tissue specific trophic fractionation

The principle of trophic fractionation is widely used to characterize food webs and infer predator-prey relations. One should be aware however, that on a species scale,

variations in fractionation occur. The isotopes in different dietary components are not first mixed well (it is not a random process), before allocated to different tissues. Instead, they are routed differently to specific tissues and body compartments. Consequently, tissues often do not reflect the isotopic composition of the bulk diet, but the isotopic composition of the nutrient component from the diet from which the tissue was synthesized. *E.g.* the protein part of the diet is synthesized in the proteins of the consumer. This phenomenon is called isotopic routing, and more laboratory experiments are needed to be able to properly interpret field data (Gannes *et al.*, 1997).

3.3 Examples of marine studies using stable isotopes as trophic marker

The stable isotope method has been used widely to describe both marine and estuarine food webs (Appendix 5).

- Most studies use both C and N signatures, or only N signature.
- A limited number of studies utilize S signature (Kwak & Zedler, 1997; Oakes & Connolly, 2004).
- SI signature can be used to describe a specific predator or trophic group (Hobson *et al.*, 1994; Smith *et al.*, 1996; Kaehler *et al.*, 2000; Jennings *et al.*, 2002a, 2002b; Nyssen *et al.*, 2002; Dahl *et al.*, 2003; Das *et al.*, 2003; Jennings & Warr, 2003; Forero *et al.*, 2004; Boschker *et al.* 2005; Connolly *et al.*, 2005; Iken *et al.*, 2005; Nyssen *et al.*, 2005; Sherwood & Rose, 2005).
- There are many studies where SI signatures are used successfully to describe an entire food web (Minagawa & Wada, 1984; Jarman *et al.*, 1996; Kwak & Zedler, 1997; Yoshii *et al.*, 1999; Hobson *et al.*, 2002; Tittlemier *et al.*, 2002; Fredriksen, 2003; Hoekstra et al, 2003).
- Several studies combined SI signatures with the accumulation of contaminants at different trophic levels (Jarman *et al.*, 1996; Fisk *et al.*, 2002; Tittlemier *et al.*, 2002; Das *et al.*, 2003; Van de Vijver *et al.*, 2003; Elliott, 2005; Herman et al., 2005).

Combination with contaminants

The abovementioned studies which have combined the estimation of trophic position using stable isotopes with measurements of bioaccumulating contaminants are still only fragmentary; no biomagnification estimates can be made yet.

From these studies it may be concluded that concentrations of organic contaminant are usually correlated with $\delta^{15}N$ value, confirming bioaccumulation at higher trophic levels. The pattern of accumulation of metals at different trophic levels is more erratic, accumulation is only observed for cadmium (Das *et al.*, 2003) and mercury (Jarman *et al.*, 1996, but see Das *et al.*, 2003 for contradicting results).

There is one study which combined fatty acids, stable isotopes and organochlorine contaminants to assess the feeding ecology of killer whale (Herman *et al.*, 2005). Both fatty acids and organochlorine levels could distinguish three different feeding types, whereas stable isotopes lacked sufficient resolution. In contrast, fatty acid profiles

could not fully define prey preference, whereas organochlorine and stable isotope analysis were congruent.

3.4 Pros and cons

Pros:
- Trophic position of species can be determined, using δN signature
- Trophic links can be assessed, using δC and/or δS signature
- Whole food web can be analyzed, from particulate organic matter to apex predator

Cons:
- Stable isotopes usually cannot determine the species composition of the diet.
- Primary producer δN signature differs between ecosystems, complicating cross-system comparisons.
- Single isotopic signatures have no relevance, they always need to be compared with isotopic signatures of other food web components/trophic levels.
- Three processes complicate dietary reconstruction from stable isotopes: 1) dietary components can be assimilated with different efficiencies; 2) isotopic fractionation can alter isotope values in tissues relative to the source; 3) metabolic routing can disproportionally distribute the source element among different tissues (Dalerum & Angerbjörn, 2005).

3.5 Ease of technique

Samples and measurement
Stable isotope analysis is relatively easy, once you have the needed specialized equipment. The analysis is done on (freeze-)dried samples, ground into a powder to obtain a homogeneous mixture.
Samples can be taken from various biotic components, very similar to samples for fatty acid analysis:
- Phytoplankton or seston needs to be concentrated first. This can be done by filtration or centrifugation.
- Zooplankton is usually collected with the use of meshes, and further picked by hand.
- Invertebrates can be picked by hand. These can be analyzed individually, or if the organism is large enough, a subsample can be taken.
- Samples from fish are often taken from the dorsal muscle.
- Samples from large marine mammals are usually taken from the blubber, by biopsy and necropsy techniques.

It is advised to extract the lipids from the sample before analysis, because lipids are depleted in ^{13}C compared with whole organisms, and the lipid content of animal tissues is variable (Peterson & Fry, 1987).

The sample is weighed into a small tin boat: approximately 1 mg of animal tissue, 3 mg of plant tissue, or 10-70 mg of sediment sample is sufficient for the analysis.

The sample is then measured on a GC-MS against a standard (see Appendix 4). The measurement process involves complete combustion of organic material to a gas, and separation of pure gases (CO_2, N_2, and SO_2). There are special mass spectrometers designed to measure stable isotope ratios, such as continuous flow isotope ratio mass spectrometers (CF-IRMS).

Time needed
Preparation of the sample is the most laborious part of the analysis. This involves the collection, freeze-drying, grinding and weighing of the sample. Handling time per sample is estimated to be roughly 30 minutes per sample.
Measurement on an IRMS takes approximately 5 minutes per sample. With the use of a sample carrousel tens of samples can be lined up and measured successively.

Precautions using necropsy samples
There are, as far as we know, no studies that have directly addressed decomposition rates and effect on the stability of stable isotopes in the tissues of stranded marine mammals. This is however an interesting and pertinent issue, and should be subject of further research. For example, measuring SI ratio changes in a few large chunks of pinniped/cetacean tissues as function of time and mimic environmental conditions (temperature, sunlight, salinity, etc.) as would be present in true environmental samples.

4 Molecular techniques

Food web structure can be elucidated by identifying all predator-prey relationships. Several techniques have been developed for identifying prey consumed by predators. A widely used approach to identify predator-prey relations is the visual analysis (often microscopic) of gut and/or faecal contents. A limitation of this approach is that only prey items that are (partly) resistant to digestion can be identified. Quantification is only possible when remains are countable (such as fish otoliths, insect head capsules). However, predators may selectively feed on soft parts of the prey, or feed on prey that leave no recognizable remains. Visual inspection of gut/faecal contents may thus lead to a biased interpretation of prey choice (*e.g.* Van Franeker, 2001; Symondson, 2002; Sheppard & Harwood, 2005).

Molecular techniques can be used to identify the prey species, even in (partly) digested or soft tissue items. Still, fully digested or assimilated prey items cannot be identified. Early molecular studies were dominated by the development of immunological markers, *e.g.* polyclonal and monoclonal antibodies. Recently, DNA techniques such as PCR combined with species-specific primers have been used in ecological studies of predator-prey interactions. Both methods give information on recent and specific predator-prey interactions.

4.1 Immunological markers

Immunological techniques are based on the principle that contacts between organisms can lead to production of antibodies capable of reacting with a specific antigen. This immunological reaction can be used in the lab to decipher prior predator-prey relations. This is mostly done in identification of invertebrate prey in suspected predators (*e.g.* Feller *et al.*, 1979, 1985; Feller & Gallagher, 1982; Ohman, 1992).

Immunoassay techniques can use polyclonal or monoclonal antibodies. Monoclonal antibodies can be used to identify specific prey species. Monoclonal based assays have been used widely in large-scale field analyses of predator-prey interactions, especially in pest control studies (role of invertebrate predators in biological control) (Sheppard & Harwood, 2005). Polyclonal antibodies can be used to detect prey types, *e.g.* family or order level, and sometimes species level (Venter *et al.*, 1999). Polyclonal antibodies have a greater sensitivity towards antigens that are vulnerable to denaturation.

The reaction of antibody with antigen can be detected in various ways:

- Precipitin test: this is a widely used technique in ecological studies of food webs, in which antibodies to specific prey are reacted in test tubes or in gels with the stomach contents or whole body extracts of suspected predators (*e.g.* Feller *et al.*, 1979).

- Passive haemagglutination inhibition assay: a sensitive method but restricted to situations where large quantities of target-organism extracts are readily available (*e.g.* Greenstone, 1977).
- Enzyme-linked immunosorbent assay (ELISA): a sensitive technique to identify antibody reactions. The purpose of an ELISA is to determine if a particular protein is present in a sample and if so, how much. This technique has become available more recently, and has been used to identify the diets of invertebrate predators, whereas vertebrate diets have been studied occasionally with ELISA (*e.g.* Symondson, 2002). ELISA has been used mostly in biomedical research, detecting substances such as hormones, bacterial antigens and antibodies. In freshwater research, ELISA is a popular technique to quickly determine the presence of microcystins (*e.g.* Metcalf *et al.*, 2000).

The ideal immunoassay for determining trophic links in a community would utilize a complete battery of taxon-specific antibodies. Unfortunately, the production of polyclonal or monoclonal antibodies is an expensive and time consuming process. However, once a monoclonal antibody is created, it is inexpensive to propagate, easy to apply in ELISAs and can be used to rapidly screen large numbers of field samples (Symondson, 2002).

Pros and cons of immunological markers

Pros:
- The marker can be species-specific.
- The method is sensitive

Cons (adapted from Feller et al., 1985):
- The marker is limited by the number and specificities of antisera available.
- It is a qualitative method, indicating presence/absence within the limits of antisera sensitivity. The ELISA technique can be used (semi-)quantitative.
- Actual specificity of an antiserum is only known after it is tested against all possible cross-reacting taxa.
- Sensitivity of the marker decreases as proteins of prey taxa undergo proteolysis during digestion, this results also in difficulties with quantification.
- In communities with a large number of species, it is practically impossible to prepare antisera specific to a large number of taxa.
- Preparation of antisera is expensive and timeconsuming; and requires use of laboratory mammals (usually rabbits).

4.2 DNA-based techniques

DNA experiments tracking trophic interactions in food-webs are based on the ability to differentiate between unique pieces of DNA from predator and prey species. The key step is differentiation of DNA. There are two general strategies applied to achieve this:

1. PCR amplification of total (predator + prey) DNA from tissue homogenates (*e.g.* gut sample, or faeces). This DNA is subjected to secondary analyses to distinguish the different sequences (species) it represents, such as sequence BLAST searches, high-resolution gel/capillary separation, restriction digestion, and DNA denaturation.
2. Amplification of prey DNA, with predator DNA excluded, using species-specific probes. It is relatively simple and inexpensive to design PCR primer sets that target organisms. These can then amplify extracted and purified target prey DNA from predator-prey homogenates (Sheppard & Harwood, 2005).

See Appendix 6 for visualization of different approaches.

Examples of recent developments:
A recent study showed that feeding of carnivorous copepods could be successfully assessed using specific primers and PCR (Vestheim *et al.*, 2005). The PCR method offers a sensitive and selective way to detect trace amounts of biological remnants (Vestheim *et al.*, 2005). This is a qualitative method. It can potentially be quantified using controlled predation experiments.

Pros and cons of DNA markers

Pros:
- The marker is species-specific
- The method is sensitive

Cons:
- Development of primers can be difficult and time-consuming
- To describe a whole food web needs development of species-specific primers, this may be a problem.
- Specialized equipment and expertise is needed to perform PCR and further analyses.

4.3 Examples of studies using molecular markers

Immunological markers have been extensively used to study the diet of invertebrate predators, with a focus on terrestrial arthropods in relation to biological pest control (see reviews Boreham & Ohiagu, 1978; Symondson, 2002; Sheppard & Harwood, 2005). The number of studies describing marine/estuarine predator-prey relations is limited and these studies originate mostly from the 1970s to 1990s (Appendix 7).
The use of DNA techniques to decipher predator-prey relationships is a new and emerging area of research. The number of studies for the marine environment is still small but this is expected to increase in the coming years (Appendix 8).

4.4 Ease of technique

Time needed
Both molecular techniques require specialized equipment and experienced technicians.

Substantial effort is needed to obtain antisera, and it involves the use of laboratory animals. If antisera are already available, the actual analysis using immunological markers can be done in a standard laboratory. When an ELISA kit is available, the test itself takes about 2 to 4 hours to execute, and tens of samples can be analyzed simultaneously (on a 96 well plate).

Development of DNA primers is a specialized exercise, and this can take many weeks to months of work from an experienced technician/scientist. Development and/or optimisation of a protocol to use the primers in a PCR analysis can also take several weeks to months. When DNA primers are available and the protocol is successfully optimised, the actual PCR analysis itself is relatively easy. It takes roughly three days to run multiple (~30) samples simultaneously. The analysis of DNA markers requires more specialized PCR equipment.

Precautions using necropsy samples
Molecular markers are developed for (partly) digested gut and faeces samples.
Sensitivity of the immunological markers decreases as proteins of prey taxa undergo proteolysis during digestion. DNA markers are less sensitive. Exact timescale of deterioration is not known, and should be further investigated.

5 Conclusions

The discussed techniques each have their advantages and disadvantages in their use to describe food webs. These are compared in Table 3.

In summary, the following distinctions can be made:

- Stable isotopes and fatty acids are analyzed in body tissues, and give thus a marker of assimilated food integrated over a longer time period. Choice of tissue determines the time scale of integration. Molecular techniques using immunological or DNA markers are used to analyze the gut or faeces content, and thus give a marker of recently ingested food.
- Stable isotope signatures serve as bulk indicators for dietary carbon sources ($\delta^{13}C$) and trophic level ($\delta^{15}N$) identification of consumers. $\delta^{34}S$ can be used to distinguish between benthic and pelagic food sources. Fatty acids may provide more detailed information on the source of a consumer's diet as well as its current nutritional and physiological status. Molecular techniques describe species specific trophic relations.
- The method of stable isotopes is rather easy, and it generates one value per sample. However, caution is advised in interpretation of the results. The simplicity can be misleading, and expert users advise that stable isotope results should always be combined with ecological and physiological information of the studied species/communities.
- Fatty acid analysis is more complicated, and it generates a large number of fatty acids per sample. Multivariate statistics are commonly used to interpret the amount of data. The final interpretation can be more precise than possible with SI.
- Several papers have combined the stable isotope with fatty acid approach, thus combining the strong aspects of both methods.

Recommendation for use in Omega:
Our recommendations follow a tiered approach:

1. *Stable isotopes* analyses of C and N are the best first step in describing food webs, since it gives information on both carbon source and trophic level.
2. *Fatty acids* can be used to differentiate within trophic groups, especially within the group of primary producers.
3. If specific information is required, molecular markers can be useful. The use of *DNA markers* is then suggested. For general purposes it is our estimation that the limitations of these methods (time and expenses needed to obtain specific primers or antibodies, time needed to analyze all possible interactions) outweigh the advantages of knowledge of specific predator-prey interactions.

Table 3: Comparison of different marker techniques

marker	part of food web	time period	specificity	relative/ absolute	quantitative/ qualitative	inert	ease of method	ease of interpretation
stable isotopes	whole food web	time specific (longer time period)	trophic level	in relation to other trophic levels	qualitative,	depends on (predictable) fractionation	easy	intermediate
fatty acids	limited, mainly herbivores	longer time period	taxonomic group	absolute	qualitative	less inert than SI	intermediate	intermediate
DNA	predator-prey	recent, depends on gut passage time	species specific	absolute	qualitative, can be developed for quantitative use	depends on digestion and denaturation	complicated	easy
immunological	predator-prey	recent, depends on gut passage time	species specific	absolute	qualitative, can be developed for quantitative use	depends on digestion and denaturation	complicated	easy

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Appendix 1 - Used methods of literature study

First step in the literature search was to define different search terms. The used search terms can be divided in keywords describing food web/chain (category A), and keywords describing different techniques (category B) (Table 1). These terms in category A and B are combined in all possible ways. If this resulted in a large number of hits, the search was restricted with the term marine/estuarine (category C). Three databases were used in the literature search, Biological Abstracts (1969 – present), Current Contents (1996 – present), and Zoological Records (1987 – present).

Table 1: used search terms

term	CODE
food web analysis OR food chain analysis	A1
food web OR food chain	A2
predator OR predation	A3
trophic level OR trophic position	A4
technique	B1
stable isotope*	B2
fatty acid*	B3
antiserum OR antisera OR immunoassay	B4
tracer	B5
biomarker	B6
method*	B7
marine OR estuarine	C1

The results for each used combination of terms was quickly scanned and judged on its relevance (Table 2). The relevant results are marked yellow and combined in an Endnote database (444 references). From these first results it followed that (combinations of) the following search terms resulted in the most relevant papers:

- food web analysis OR food chain analysis
- trophic level OR trophic position
- stable isotope*
- fatty acid*
- biomarker

These terms are further used in searches using Google Scholar and in specific journals: Marine Ecology Progress Series, Marine Biology, Oecologia and Limnology & Oceanography. These journals returned relevant literature in the first literature searches. Lastly, we used the follow-up method, searching for used references in relevant papers. The final Endnote database contains 482 references.

Table 2: Overview of number of hits and assessment of relevance for each search result for each database. The combination of grey marked search terms in the three databases combined returned 444 references.

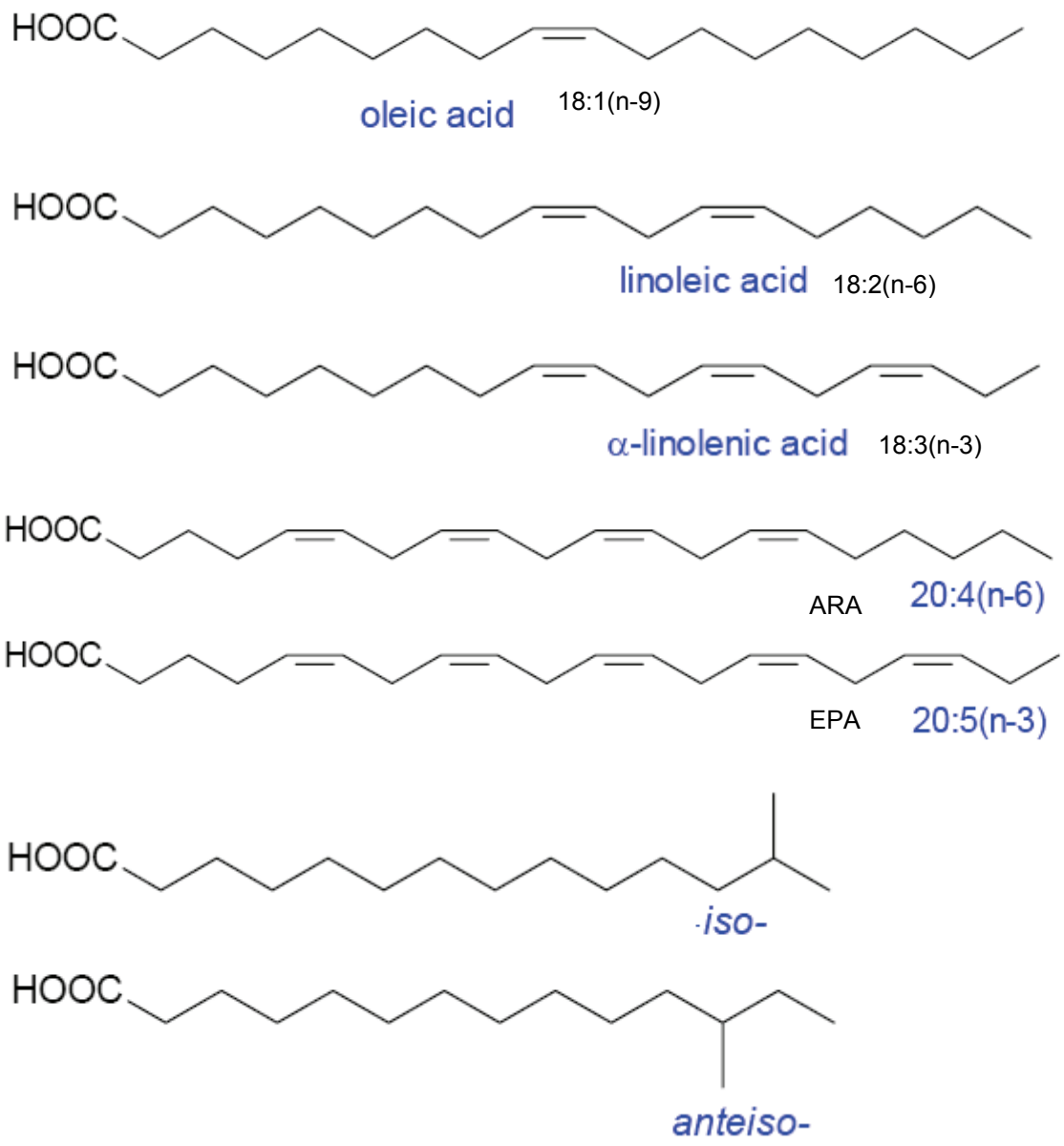
search terms	Biological Abstracts	Current Contents	Zoological Records
A1	47 (++)	32 (++)	175 (++)
A2	7566	6319	4017
A3	47322	23529	52757
A4	2007	1261	1006
A2 AND B(1 - 7) AND C1	1188 (-)	502	225 (+)
A3 AND B(1 - 7)	21032 (-)	2031	2053
A4 AND B(1 - 7) AND C1	349 (-)	145 (0/+)	105 (+)
A4 AND B2 AND C1	95 (+)	112 (+)	76 (+)
A4 AND B3	10 (+)	13 (+)	9 (+)
A(1 - 4) AND B4	14 (+)	31 (0/+)	26 (0/+)
(B2 OR B3) AND C1	6343	5995	2739
(B2 OR B3) AND C1 AND (A1 - 4)	342 (+)	464 (+)	229 (+)
total	444		

Appendix 2 - Fatty acid nomenclature and examples

Shorthand notation according to IUPAC: z:yωx where z = number of C atoms in the acyl chain, y = number of double bonds, and x = the position of the first double bond counting from the terminal methyl group of the acyl chain.

abbreviation	common used trivial or systematic name	structure	not biosynthesized by animals (according to Iverson *et al.*, 2004)
FA	fatty acid		
SAFA	saturated fatty acids	no double bonds	
MUFA	mono-unsaturated fatty acid	one double bond	
PUFA	poly-unsaturated fatty acid	two or more double bonds	
	myristic acid	14:0	
	palmitic acid	16:0	
	palmitoleic acid	16:1ω7	
	hexadecadienoic acid	16:2ω4	X
	hexatrienoic acid	16:3ω4	X
	tetraenoic acid	16:4ω3	X
	stearic acid	18:0	
	oleic acid	18:1ω9	
	vaccenic acid	18:1ω7	
LIN	linoleic acid	18:2ω6	X
	γ-linolenic acid	18:3ω6	X
ALA	α-linolenic acid	18:3ω3	X
	octadecatetraenoic acid	18:4ω3	X
	octadecapentaenoic acid	18:5ω3	
	arachidic acid	20:0	
	eicosenoic acid	20:1ω9	X
ARA	arachidonic acid	20:4ω6	X
EPA	eicosapentaenoic acid	20:5ω3	X
	erucic acid	22:1ω9	
DPA	docosapentaenoic acid	22:5ω3	
DHA	docosahexaenoic acid	22:6ω3	X
	nervonic acid	24:1ω9	
EFA	essential fatty acid	LIN, ALA, ARA, EPA, DHA	X

Structure formulas of some common fatty acids:



Iso: branched fatty acids with the branch point one carbon from the end.
Anteiso: branched fatty acids with the branch point two carbons from the end.

(source: http://www.lipidlibrary.co.uk/index.html)

Appendix 3 - Examples of marine studies using fatty acids as trophic markers

Studies are ordered chronologically (FAs = fatty acids, SI = stable isotopes)

study area	part of ecosystem	comments	reference
Gulf of St. Lawrence	finback and humpback whale	blubber fatty acid analysis to differentiate in diet	Borobia *et al.*, 1995
northwest Atlantic Ocean	three species of seals	combination with stable isotopes to distinguish between freshwater and marine feeding habits	Smith *et al.*, 1996
Weddel Sea and Greenland see	2 pteropod species	FAs confirm phytoplankton-*Limacina-Cleone* food chain	Kattner *et al.*, 1998
Svalbard	white whale blubber and 12 potential prey species	FAs suggest that White whale feeds on polar cod, capelin and shrimp	Dahl *et al.*, 2000
Seto Inland Sea, Japan	jellyfish *Aurelia aurita*	variation in FAs was seasonal, changing from diatom-based food chain to detritus-based food chain	Fukuda & Naganuma, 2001
Arctic pelagic	2 hyperiid amphipods	FAs could differentiate the ecological niches of 2 closely related species	Auel *et al.*, 2002
two areas in sub-Antarctic Pacific Ocean	7 species of mesopelagic fish and mackerel icefish	the 7 species can be distinguished based on FAs. Two areas have different food chains	Lea *et al.*, 2002
coastal bays Labrador	plankton, 16 species of macroinvertebrates and sediment	echinoderms high in 20:4ω6, FAs of plankton and sediment were reflected in macroinvertebrate FAs	Copeman & Parrish, 2003
arctic fjord	three seabird species	FAs could link bird species with benthic or pelagic food chain; combination with stable isotope analysis	Dahl *et al.*, 2003
Conceptual Bay, Newfoundland	Yellowtail flounder (*Limanda ferruginea*), 4 potential prey invertebrates, plankton and sedimenting particles	FAs suggest relatively few steps in food chain to flounder. Wild fish and laboratory-reared fish were compared.	Dwyer *et al.*, 2003
NE Atlantic Ocean, Porcupine Bight and Abyssal Plain	9 species deep-sea seastars (Echinodermata: Asteroida)	species could be separated into three trophic groups: suspension feeders, predators/scavengers, and mud ingesters	Howell *et al.*, 2003
Barents Sea	harp seal and potential prey	polar cod and amphipod are important direct prey items for harp seal. In the harp seal food chain, *Calanus* copepods and dinoflagellates are important	Falk-Petersen *et al.*, 2004
coastal lakes	seston, zooplankton and fish	Essential FA signature depends on taxonomic composition of plankton	Kainz *et al.*, 2004

Ebro delta, Spain	Common tern	combination with organochlorine contaminants	Mateo *et al.*, 2004
North Water Polynya	3 species of copepods compared with seston	feeding patterns could be discerned: herbivorous and microbial loop	Stevens *et al.*, 2004
Baltic and Norwegian sea	phytoplankton and copepods	high ω3/ω6 ratio in phytoplankton contributed to high DHA/ARA ratio in copepods	Ahlgren *et al.*, 2005
North Pacific	killer whale	combination of FAs, SI, and PCB content. FA profiles and PCB could separate the individuals into 3 ecotypes: resident, transient, offshore; SI lacked sufficient resolution	Herman *et al.*, 2005
marine sediment Tasmania	sediment, total benthic fauna, and brittlestar	20:1ω13 and 24:6ω3 appear likely biomarkers for *Amphiura elandiformis*	Mansour *et al.*, 2005
Antarctic peninsula	12 amphipod species	separation into four trophic groups: suspension feeders, macro-herbivores, omnivores, and scavengers; FA analysis combined with stable isotope analysis	Nyssen *et al.*, 2005

Appendix 4 - Calculations of isotopic δ values

In ecological studies isotopic compositions are usually expressed in terms of δ values, which are parts per thousand differences from a standard:

$\delta X = [(R_{sample}/R_{standard})-1] \times 10^3$

where X is ^{13}C, ^{15}N, or ^{34}S, and R is the corresponding ratio $^{13}C/^{12}C$, $^{15}N/^{14}N$, or $^{34}S/^{32}S$.
Standard reference materials are carbon in the PeeDee limestone, nitrogen gas in the atmosphere, and sulfur from the Canyon Diablo meteorite.
Increases in these δ values denote increases in the amount of the heavy isotope component, decreases in δ values denote a decrease in the heavy isotope component.

(from Peterson & Fry, 1987)

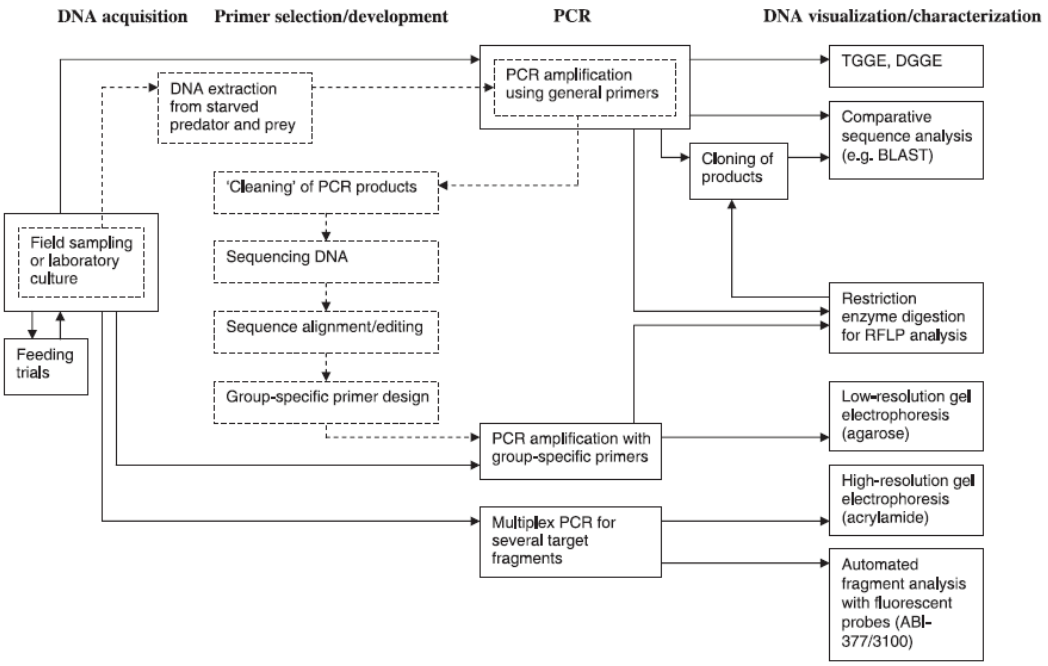
Appendix 5 - Examples of marine studies using stable isotopes as trophic markers

Studies are ordered chronologically.

study area	measured isotopes	part of ecosystem studied	comments	reference
North Pacific Ocean	N	phytoplankton, zooplankton, fish	average trophic fractionation of N = 3.4 ‰	Minagawa & Wada, 1984
northeast Pacific Ocean	C, N	22 species of seabirds	δC separates inshore and offshore feeding habits, δN defines trophic level	Hobson *et al.*, 1994
Gulf of the Farallones, California	C, N	complete marine food web	combination with metal and organochlorine contaminants; all OCs and Hg were significantly correlated with $\delta^{15}N$.	Jarman *et al.*, 1996
northwest Atlantic Ocean	C, N	three species of seals	combination with fatty acids to distinguish between freshwater and marine feeding habits	Smith *et al.*, 1996
coastal wetlands, California	C, N, S	complete food web	four trophic levels	Kwak & Zedler, 1997
Lake Baikal	C, N	complete pelagic food web	four trophic levels	Yoshii *et al.*, 1999
Prince Edward Islands, Southern Ocean	C, N	four assemblages: zooplankton, kelp-associated species, inter-island, and nearshore benthos	authochthonous and allochthonous sources of C could be discerned	Kaehler *et al.*, 2000
Cumberland Sound, Canada	C, N	predator Greenland shark and prey turbot	combination with organochlorine contaminants, no complete agreement in estimating trophic position between SIs and OCs..	Fisk *et al.*, 2002
North Water Polynya	C, N	complete food web, from POM to polar bear	highest trophic level of 5.5 for polar bear	Hobson *et al.*, 2002
central North Sea	N	invertebrates and fish	linking body size with δN	Jennings *et al.*, 2002a
central North Sea	N	invertebrates and fish	linking body size and δN with production and trophic efficiency	Jennings *et al.*, 2002b
Weddel Sea, Antarctica	C, N	90 invertebrate species	focus on eight amphipod species covering 3 trophic levels	Nyssen *et al.*, 2002
arctic marine food web Greenland	C, N	complete food web	combination with organohalogens, accumulating in invertebrate – fish - seabird foodweb	Tittlemier *et al.*, 2002

arctic fjord	C, N	three species of seabirds	combination with fatty acids	Dahl *et al.*, 2003
northeast Atlantic Ocean	C, N	6 marine mammals (apex predator)	combination with trace metal concentration, Cd in liver and kidney was significantly correlated to SI values; Hg, Zn and Cu not.	Das *et al.*, 2003
kelp forest, Norway	C, N	complete food web	kelp is carbon source for marine animals	Fredriksen, 2003
arctic marine food web	C, N	complete food web	combination with persistent organochlorine contaminants	Hoekstra *et al.*, 2003
northeast Atlantic Ocean	N	scallop, dab, and whiting	environmental correlates with spatial variation of δN	Jennings & Warr, 2003
North Sea	C, N	marine mammals	combination with perfluorinated organochemicals, correlated significantly with SI	Van de Vijver *et al.*, 2003
estuary Cape Cod	C, N	horseshoe crabs, prey species and organic matter		Carmichael *et al.*, 2004
Argentinean Patagonian coast	C, N	14 species of seabirds and selected prey	overlap in trophic level between seabirds, average TL = 4.1	Forero *et al.*, 2004
estuarine seagrass community	C, N	three autotrops: two species of seagrass and epiphyton		Guest *et al.*, 2004
estuarine seagrass community	S	seagrass *Zostera capricorni*	δS varied between 12.7 – 17.6 ‰, related with sediment OM content and height on tidal gradient	Oakes & Connolly, 2004
Scheldt estuary	C	suspended matter in surface water	combination with fatty acids	Boschker *et al.*, 2005
estuary, Australia	C	invertebrates	transects across two habitats were studied, mudflats and seagrass	Connolly *et al.*, 2005
North Pacific Ocean	C, N	eight species of pelagic seabirds	combination with chlorinated hydrocarbon contaminants in seabirds, trophic level (SI signature) explained ~50% of the variation in CHCs.	Elliott, 2005
arctic deep Canada basin	C, N	POM and invertebrates	four trophic levels	Iken *et al.*, 2005
Antarctic peninsula	C, N	twelve amphipod species	combination with fatty acids	Nyssen *et al.*, 2005
Newfoundland & Labrador continental shelf	C, N	17 fish and 16 invertebrate species, focus on Atlantic cod and its prey	significant differences between near-shore and offshore	Sherwood & Rose, 2005

Appendix 6 - Principal approaches to molecular detection of prey using DNA techniques



(from Sheppard & Harwood, 2005)

Appendix 7 - Examples of marine studies using immunological markers as trophic markers

Studies ordered chronologically.

study area	part of ecosystem	comments	reference
intertidal, Puget Sound	soft-bottom benthic community	polyclonal antisera, using double immunodiffusion precipitin tests, showed trophic relationships among 20 invertebrate taxa	Feller *et al.*, 1979
deep-sea North Atlantic	grenadier fish and 32 potential prey antisera	polyclonal antisera	Feller *et al.*, 1985
marine	penguin and 3 potential prey	identification of vertebrate and invertebrate prey species using ELISA	Walter *et al.*, 1986
North Sea	Predator species dolphin, grey seal and common seal, and prey species salmon, cod and herring	polyclonal antisera, using immunoelectrophoresis, showed that salmon antisera were strong enough to identify salmon proteins in predator faeces; however cod and herring antisera were less strong.	Pierce *et al.*, 1990
	Northern anchovy larvae and ciliate prey	polyclonal antisera, using dot blot immunoassays	Ohman *et al.*, 1991
Antarctica	squid and potential prey krill	polyclonal antisera, using Laurel Fused Rocket Immunoelectrophoresis method, identified squid species that prey on euphausiids	Kear, 1992
southern California coastal waters	oligotrich ciliate *Strombidium* as prey	polyclonal antisera, using dot blot technique	Ohman, 1992
Biscayne Bay, Florida	prey Red drum and suspected predators Great barracuda and Redfin needlefish	ELISA was applied, after resolving technical difficulties, and prey was identified in gut contents of suspected predator	Arnold *et al.*, 1996
Pacific Ocean	pelagic nemertean	polyclonal antibodies to 13 taxa of potential prey, using precipitin test	Feller *et al.*, 1998
Agulhas Bank, South Africa	squid and 5 potential zooplankton prey species	polyclonal antisera, using ELISA.	Venter *et al.*, 1999
estuary	harpacticoid copepod as prey and grass shrimp as predator	polyclonal antibodies, detection using immunodiffusion and Western blot techniques	Hoyt *et al.*, 2000
South Africa west coast	rock lobster and 26 invertebrate prey species	polyclonal antisera showed high sensitivity to undigested prey, but low sensitivity to digested prey	Mayfield *et al.*, 2000

Appendix 8 - Examples of marine studies using DNA markers as trophic markers

Studies ordered chronologically

study area	part of ecosystem	comments	reference
Sendai Bay, Japan	Stone flounder larvae as prey and shrimp as predator	PCR amplification of mtDNA was successful until 4 h after predation	Asahida *et al.*, 1997
East Antarctica	krill prey, Adelie penguin and Pygmy blue whale as predator	prey DNA in faeces or stomach could be identified	Jarman *et al.*, 2002
	calanoid copepod as predator and alga as prey	algal 18S ribosomal DNA could be identified in whole DNA extracts and faecal pallets	Nejstgaard *et al.*, 2003
Oslofjord, Norway	carnivorous copepod and prey copepod	using PCR, *Calanus* DNA was amplified from faecal pallets of predator *Pareuchaeta*	Vestheim *et al.*, 2005