

You are what you eat plus a few *per mill*: utilisation des isotopes stables en Ecologie Marine



Credit photo: Oceanus

Traçage des polluants chez les mammifères et autres vertébrés marins

Krishna Das et Gilles Lepoint
Chercheurs Qualifiés F.R.S. - FNRS
Laboratoire d'Océanologie- MARE Center
Université de Liège



Mes axes de recherche

Niveaux en
polluants?

Effets?

Toxicocinétique
et transfert à la
progéniture





Qu'est ce qu'un polluant?

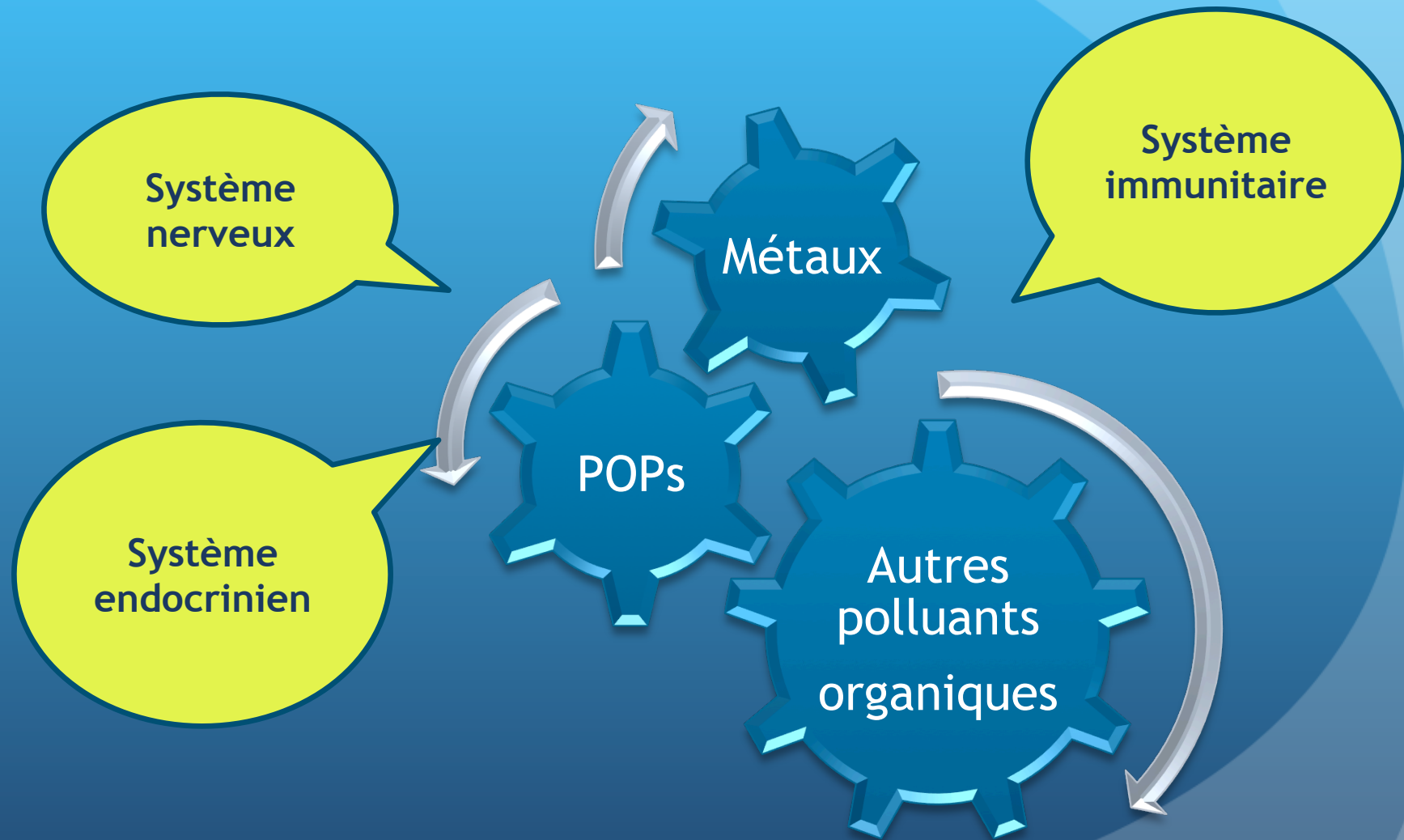
Un polluant est une substance naturelle ou d'origine anthropique

- que l'homme introduit dans un biotope donné dont elle était absente ou encore
- dont il modifie ou augmente la teneur (dans l'eau, l'air ou les sols selon le biotope) lorsqu'elle y est spontanément présente.

Peut agir comme un polluant:

- **Toute modification d'un processus physique** qui conduit à accroître les flux d'énergie ou les niveaux de radiation dans l'environnement
- **Une espèce allochtone** introduite dans un écosystème éloigné de son aire d'origine

Toxicité des polluants



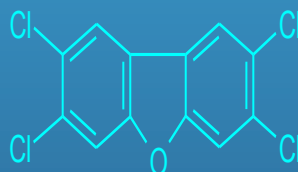
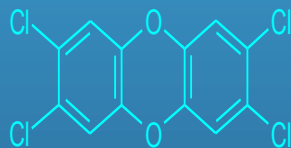
Polluants Organiques Persistants

POPs: Convention de Stockholm

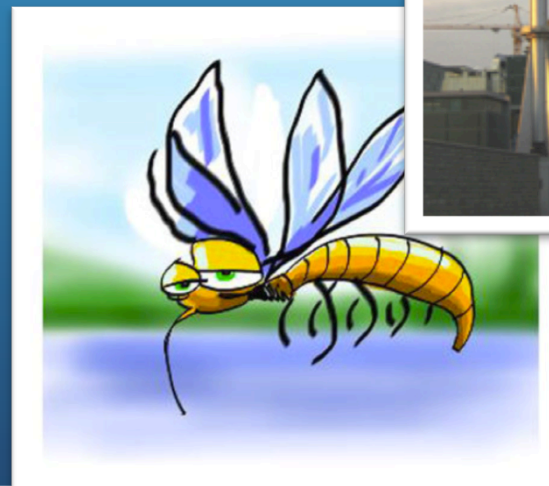
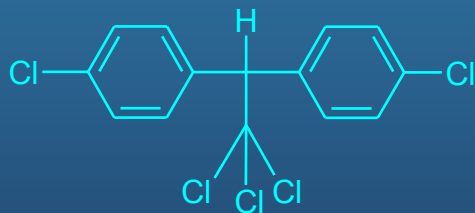
PCBss



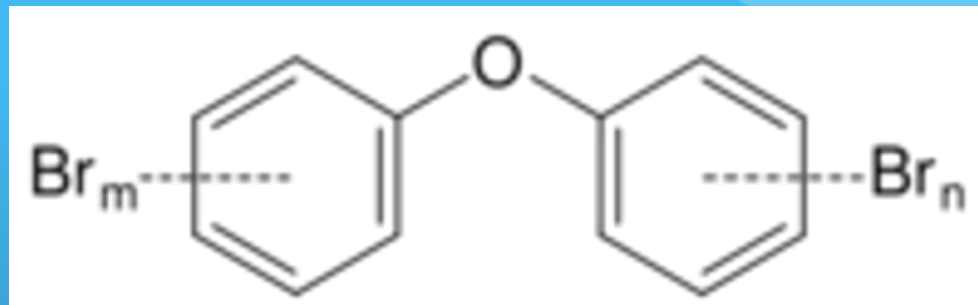
Dioxines et furannes



DDTs



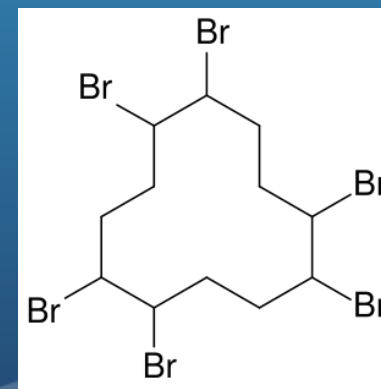
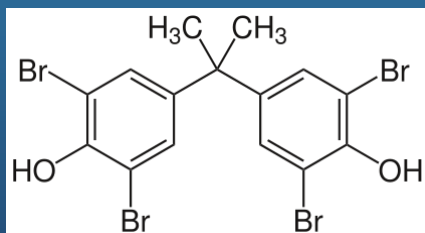
De nouvelles molécules persistantes



Polybromodiphenyléthers: PBDEs

Mais bien d'autres composés polybromés problématiques:

- Tetrabromobisphenol A: TBBPA
- Hexabromocyclododecane: HBCD



Retardateurs de flamme

Usage domestique mais pas seulement!







Des points communs!

- Stables
- Lipophiles
- Transport sur de longues distances

Et transfert à la descendance...



Concentrations décuplées dans le foie des fétus entre 1998 et 2006



Persistent Organic Pollutant Residues in Human Fetal Liver and Placenta from Greater Montreal, Quebec: A Longitudinal Study from 1998 through 2006

Josée Doucet,¹ Brett Tague,¹ Douglas L. Arnold,¹ Gerard M. Cooke,¹ Stephen Hayward,¹ and Cynthia G. Goodyer²

¹Health Canada, Health Products and Food Branch, Ottawa, Ontario, Canada; ²McGill University Health Centre—Montreal Children's Hospital Research Institute, Montreal, Quebec, Canada

BACKGROUND: There is general concern that persistent organic pollutants (POPs) found in the environment, wildlife, food, water, house dust, human tissues, and fluids may alter normal human physiologic activities (e.g., fetal development, immune and endocrine systems). Although the levels of some POPs [polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs)] in these matrices have decreased after their ban, others [polybrominated diphenyl ethers (PBDEs)] have increased in recent years.

OBJECTIVE: To determine the longitudinal trend of specific POPs in human fetal tissues for risk assessment purposes.

METHODS: We analyzed early to mid-gestation fetal liver (n = 52) and placental (n = 60) tissues, obtained after elective abortions during 1998–2006, for selected PBDEs, PCBs, and OCs using gas chromatography–mass spectroscopy.

RESULTS: Total PBDEs in fetal liver increased over time (mean ± SE: 1998, 284.4 ± 229.8 ng/g lipid; 2006, 1,607.7 ± 605.9; p < 0.03), whereas placental levels were generally lower, with no clear trend. Low levels of PCBs and OCs varied yearly, with no evident trend. The major analytes in 1998 were OCs (liver, 49%; placenta, 71%), whereas the major analytes in 2006 were PBDEs (liver, 89%; placenta, 98%). The 1998–2006 tissue PBDE congener profile is similar to that of DE-71, a commercial primarily pentabrominated diphenyl ether mixture manufactured in North America.

CONCLUSIONS: Although commercial production of penta- and octa-brominated diphenyl ethers in North America was halted in 2004, their concentrations in fetal liver and placenta are now greater than the tissue burdens for the analyzed OCs and PCBs. Our findings also demonstrate that PBDEs accumulate within the fetal compartment at a very early stage in gestation.

KEY WORDS: fetus, human, liver, placenta, OCs, PBDEs, PCBs, POPs. *Environ Health Perspect* 117:605–610 (2009). doi:10.1289/ehp.0800205 available via <http://dx.doi.org/> [Online 10 December 2008]

Persistent organic pollutants (POPs), such as organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs), contaminate many environmental matrices and can be found in human blood, adipose tissue, and breast milk (Gruenewald et al. 2003; Solomon and Weiss 2002). In addition, these POPs cross the placenta and have been reported to reduce gestational length and birth weight and to affect fetal development (Ando et al. 1985; Gruenewald et al. 2003; Lanting et al. 1998; Rogan et al. 1986; Shen et al. 2007; Taylor et al. 1984). Furthermore, some of these chemicals may induce endocrine effects, and others are classified as “reasonably anticipated to be” or “known” human carcinogens (Carpenter 2006). Multiple studies have shown that the levels of several POPs found in the environment, human fat, maternal and cord bloods, and breast milk have decreased after being banned (Bates et al. 2002; Craan and Haines 1998; Norén and Meironyte 2000; Pereg et al. 2003; Schade and Heinzow 1998; Solomon and Weiss 2002). However, this is not universal; for example, concentrations of PCBs in Great Lakes fish have continued to oscillate (Bhavsar et al. 2007).

A more recently recognized class of environmental contaminant, whose chemical structure is similar to PCBs, is the brominated

flame retardants, which includes the polybrominated diphenyl ether (PBDE) congeners (Gill et al. 2004; Hites 2004). For the past two to three decades, they have been added to plastics, polyurethane foam, textiles, and electronic equipment to prevent ignition or to slow the burn rate if a fire occurs. In animal studies, PBDEs have been found to affect neural and reproductive development as well as thyroid function (Birnbaum and Staskal 2004; Gill et al. 2004; Legler 2008; McDonald 2002; Talsness 2008). Although many of these investigations used pharmacologic doses of the PBDEs, more recent studies have demonstrated that exposure at doses relevant for humans at critical times during development can have effects on the endocrine and neural systems (reviewed in Legler 2008; Talsness 2008). Only two clinical epidemiologic studies have been published to date on the potential effects of *in utero* exposure and fetal outcome. Chao et al. (2007) recently reported that elevated levels of PBDEs in breast milk correlated significantly with lower newborn birth weight, birth length, chest circumference, and body mass index. In addition, Main et al. (2007) have found significantly higher PBDE levels in the breast milk of mothers whose newborn sons had cryptorchidism. One study of young adult males has reported a significant negative

association between serum brominated diphenyl ether congener BDE-153 levels and testis size as well as sperm concentration (Akutsu et al. 2008).

In contrast to OCs and PCBs, many studies worldwide have reported that the amount of PBDEs found in the environment, human serum, and breast milk has been increasing steadily since the 1980s (Akutsu et al. 2003; de Wit et al. 2006; Li et al. 2006; Lorber 2008; Norén and Meironyte 2000; Pereg et al. 2003; Ryan et al. 2002; Schecter et al. 2004; Solomon and Weiss 2002). A few investigators in Europe have found that the PBDE levels in European samples peaked in 1997–1998 and subsequently have plateaued or declined, likely due to the earlier limitations on the production and use of penta- and octa-BDEs within the European Union versus North America (Damerud et al. 2002; Law et al. 2006, 2008).

Human breast milk is only one source of environmental contaminants for a developing infant, and from a toxicology and regulatory perspective, it is the infant's total body burden that is of greatest interest. Very little is known about when *in utero* exposure begins to contribute significantly to an infant's body burden of PBDEs and other POPs. To examine this question, we analyzed the levels of multiple OCs, *p,p'*-DDE (1,1,1-trichloro-2-[*p*-chlorophenyl]-2-[*p*-chlorophenyl] ethane), *p,p'*-DDE (1,1-dichloro-2,2-bis[*p*-chlorophenyl] ethylene), *trans*-nonachlor, and hexachlorobenzene (HCB), PCB congeners (49, 99, 118, 137, 138, 153, and 180), and BDE congeners (47, 85, 99, 100, 153, 154, and 183) in early to mid-gestation fetal liver and placental specimens obtained between

Address correspondence to C.G. Goodyer, Endocrine Research Laboratory, McGill University Health Centre—Montreal Children's Hospital Research Institute, 4060 St. Catherine St. West, Room 415/1, Montreal, Quebec, Canada H3Z 2Z3. Telephone: (514) 412-4400 ext. 22481. Fax: (514) 412-4478. E-mail: cindy.goodyer@muhs.mcgill.ca

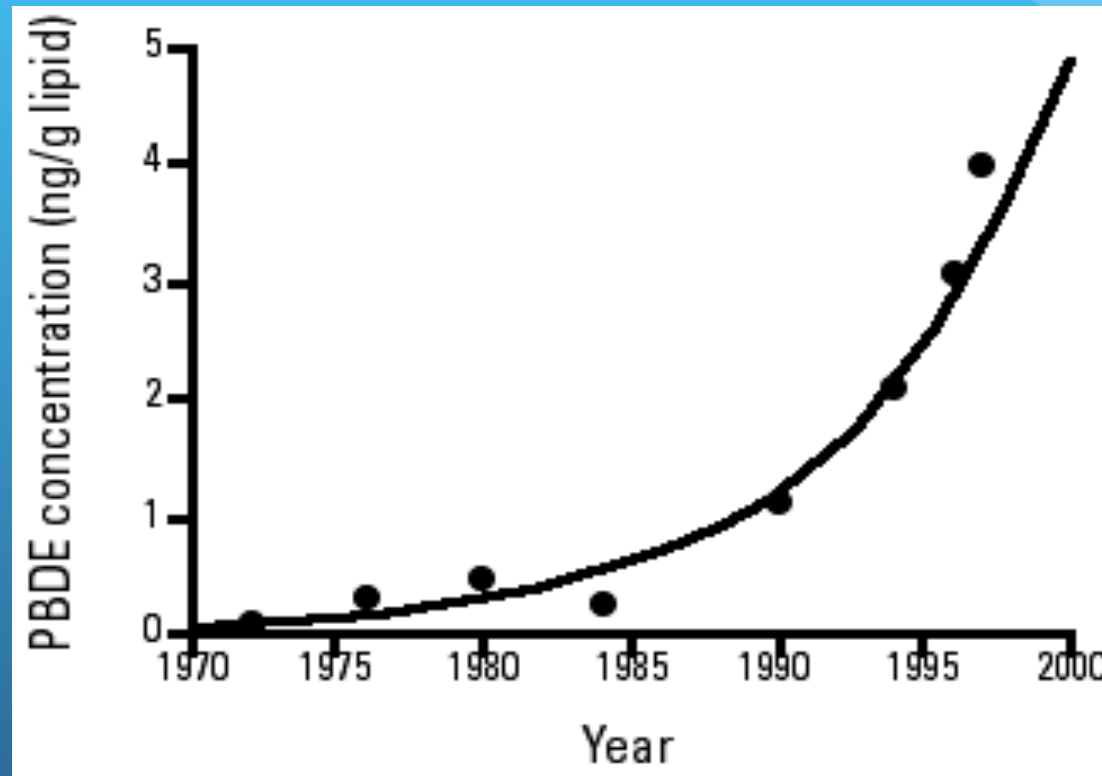
Supplemental Material is available online at <http://www.ehponline.org/members/2008/0800205/suppl.pdf>

We thank T. Rawn for her insightful review of the manuscript, and the staff of the Royal Victoria Hospital Day Surgery clinic for their help with tissue collection.

Partial funding was provided by the McGill University Health Centre—Montreal Children's Hospital Research Institute, which is supported in part by the Fonds de Recherches en Santé du Québec. The authors declare they have no competing financial interests.

Received 18 September 2008; accepted 10 December 2008.

Augmentation des concentrations en PBDEs dans l'environnement

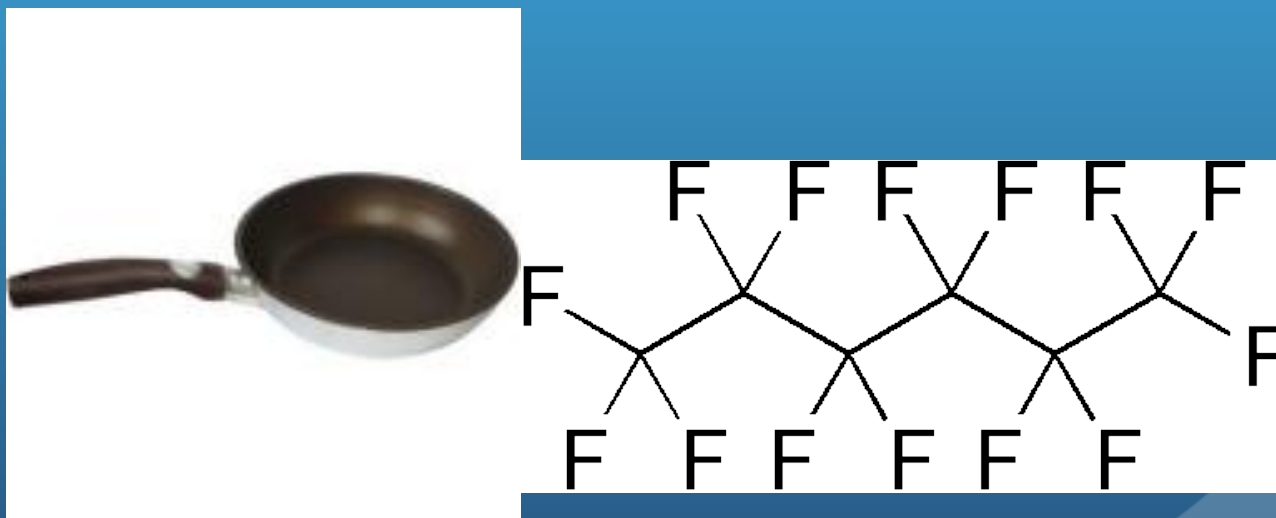


Lait maternel- Suède – (Noren and Meironyté, 1998)

concentrations doublées tous les 5 ans

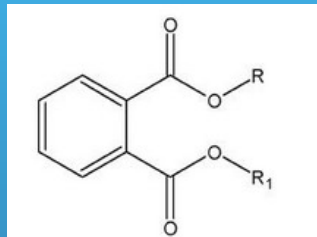
De nouvelles molécules persistantes

Composés perfluorés (PFAs)

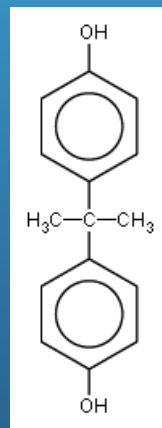


De nouvelles molécules non persistantes

- Phtalates



- Bisphenol A



Non persistantes mais une exposition chronique mène à une augmentation des teneurs chez les humains

Mer = réceptacle final de toute pollution



Produits d'origine anthropique arrivant en mer



Marées noires

Armement et munitions diverses

Déchets en provenances de bateaux

Déchets industriels et nucléaires

Epaves

Stations de forage pétrolier

Précipitations atmosphériques (métaux lourds et hydrocarbures)

Déchets urbains et routiers

Eaux d'égouts

Gaz d'échappement via atmosphère

Engrais et pesticides

Eaux de refroidissement

...

L'impact d'un polluant dépend

De la contamination environnementale

Mais également **d'évènements clés**

- Migration
- Gestation
- Lactation
- Ponte
- Jeune (lié à la lactation, la mue et les migrations)



Et ...du régime alimentaire

Etudier le régime alimentaire des vertébrés marins

Que mangent-ils?



Pourquoi l'étudier

- Prélèvement important par les mammifères marins
- Régulation des chaînes trophiques
- Alimentation = source de contamination

Comportement alimentaire



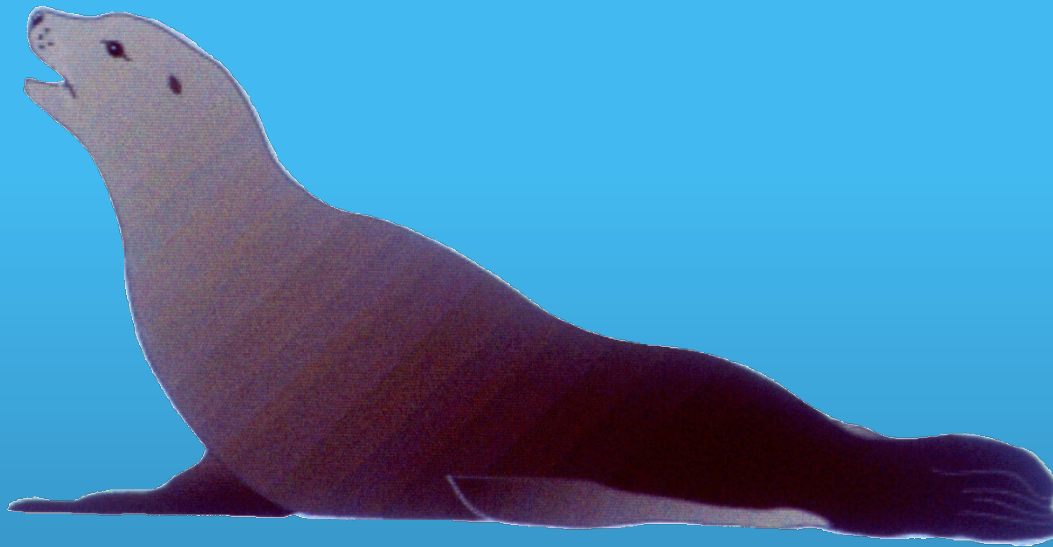
- Peu applicable pour ces espèces marines
- Protocole conventionnel difficilement transposable

Contenus stomacaux

Individus échoués ou capturés dans les filets



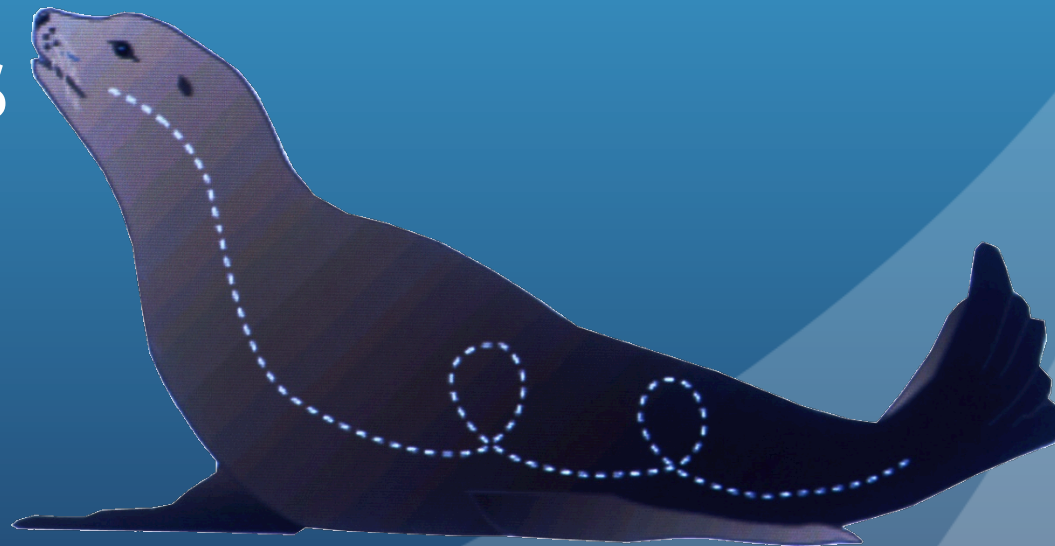
Credit: U. Siebert et T. Jauniaux



Fèces

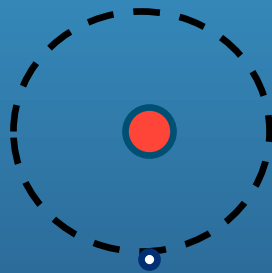


Acides gras
et isotopes
stables

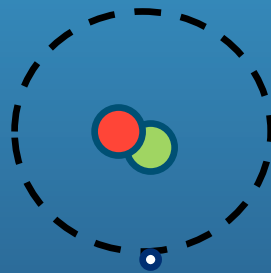


Qu'est-ce qu'un isotope stable?

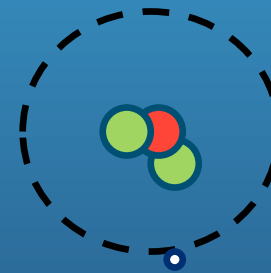
Configurations atomiques (ou atomes) qui diffèrent par leur nombre de neutrons.



Protium



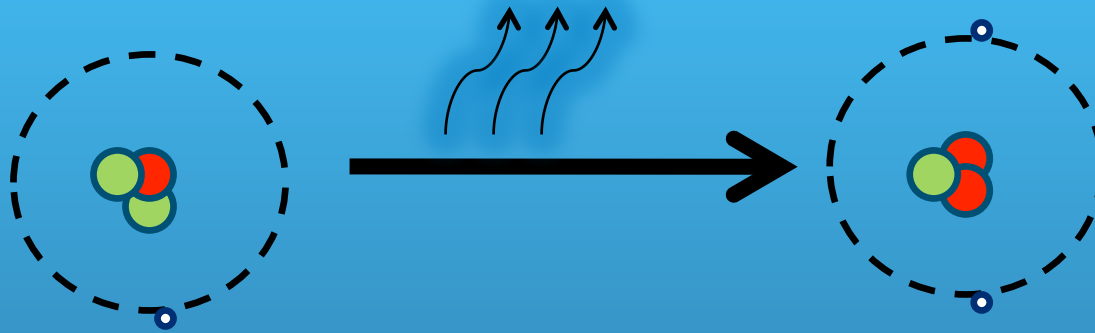
Deuterium



Tritium

Hydrogen

Isotope radioactif *versus* isotope stable



Tritium *non stable*: transformation en ${}^3\text{He}$ (Radioisotope)



Deuterium *stable*: pas de transformation (isotope stable)

Elements d'intérêt

^1H = isotope stable léger de l'hydrogène (~99.985%)

^2H (D) = isotope stable lourd de l'hydrogène (~.015%)

^{12}C = isotope stable léger du carbone (~98.9%)

^{13}C = isotope stable lourd du carbone (~1.1%)

^{14}N = isotope stable léger de l'azote (~99.63%)

^{15}N = isotope stable lourd de l'azote (0.37%)

$\delta^{13}\text{C}$ et $\delta^{15}\text{N}$

Les rapports isotopiques sont exprimés relativement à un standard international en parts pour mille

$$\delta X(\text{‰}) = \left[\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right] \times 1000$$

X est le ^{13}C ou ^{15}N et R est le rapport correspondant $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$



Hypothèses



1. Les rapports isotopiques des consommateurs sont **proportionnels** à ceux de leur régime alimentaire
2. Les rapports isotopiques **varient** selon les sources alimentaires accessibles aux consommateurs

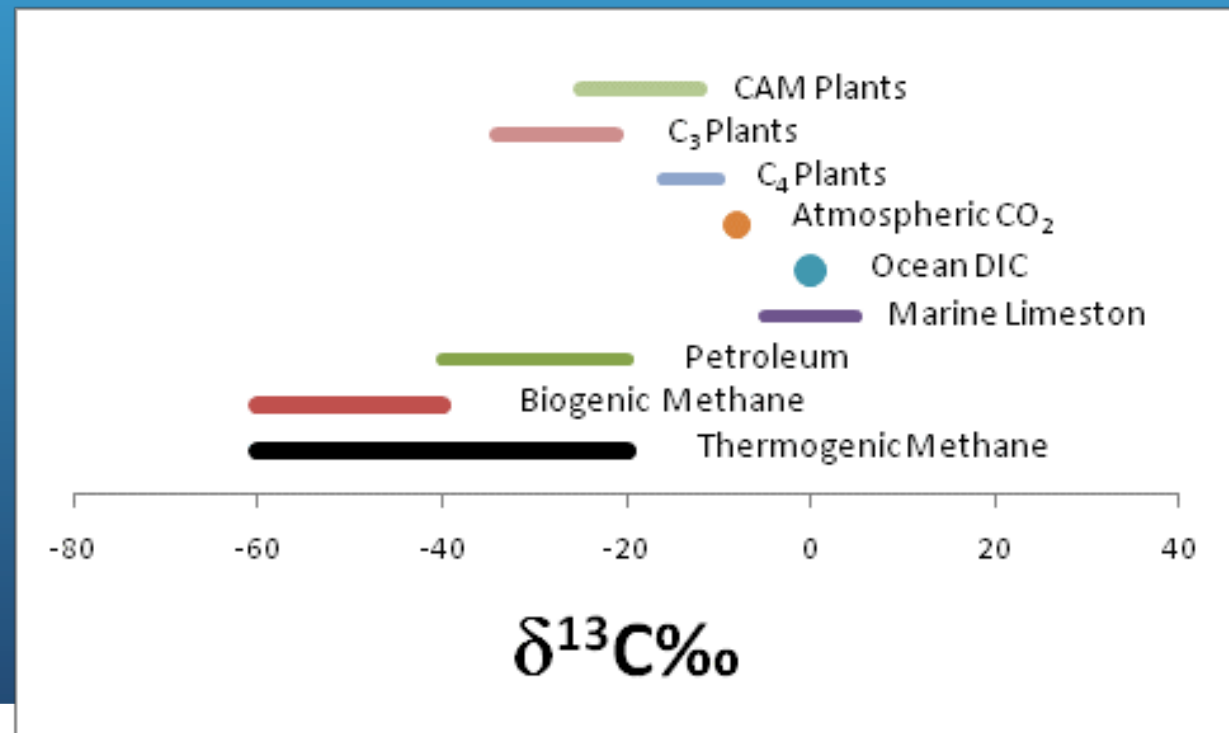
Variation des valeurs en $\delta^{13}\text{C}$

-3 -2 -1 0 +1 +2 +3

$R_{\text{sample}} < R_{\text{standard}}$

$R_{\text{sample}} = R_{\text{standard}}$

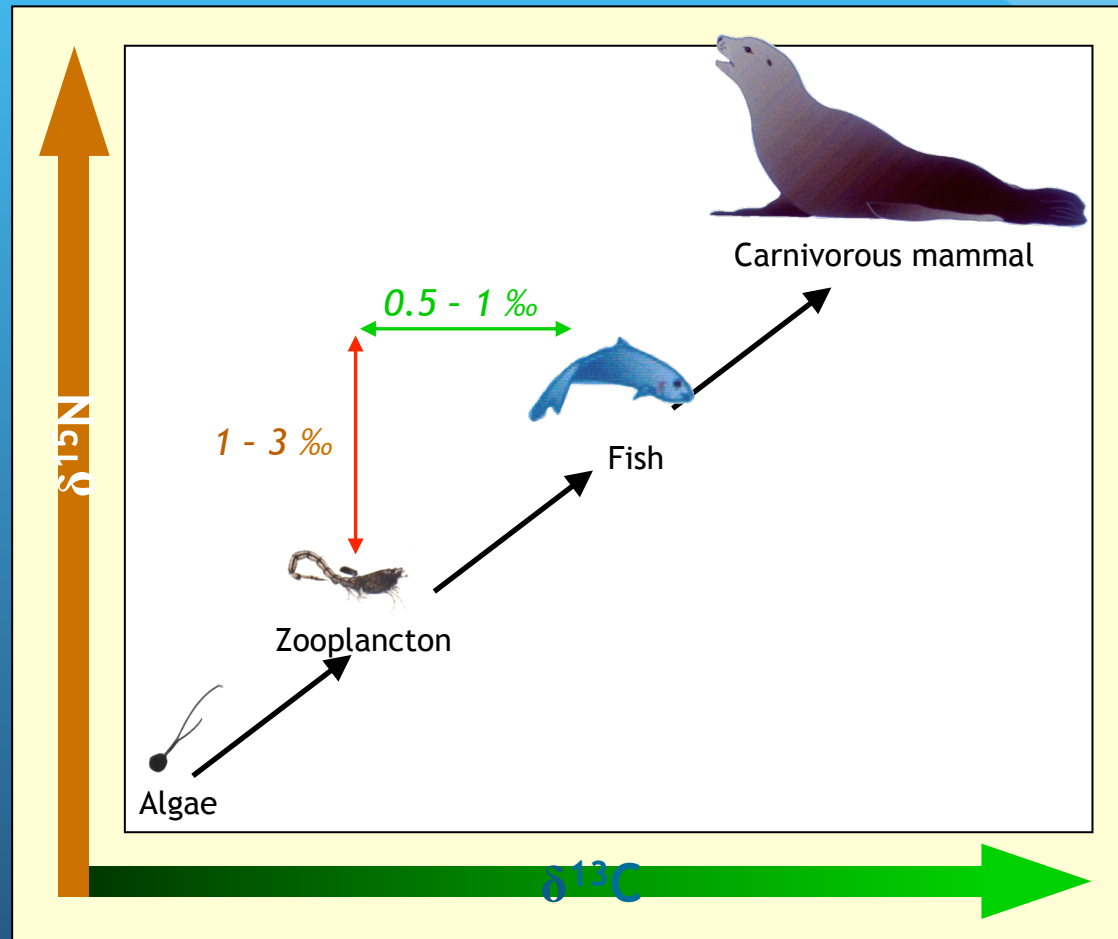
$R_{\text{sample}} > R_{\text{standard}}$



“You are what you eat ...plus a few per mille”

De Niro and Epstein. 1976. Geol. Soc. Am. Abs. Prog. 8 : 834-835

position trophique



Source de la productivité primaire

Avantages des isotopes stables

- Intégration du régime alimentaire sur une période de temps dépendant du tissu analysé
 - pas uniquement les derniers repas.
 - turn-over du tissu analysé (tissus osseux *versus* sérum)
- Mesure continue de la position trophique

1979 : une des premières études sur les isotopes stables chez les mammifères marins

McConnaughey, T. and C.P. McRoy. 1979. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. Marine Biology 53: 257-262



And 30 years later, more than 250 published papers visible on ISI
488 for marine fish, 285 for seabirds and 56 for turtles

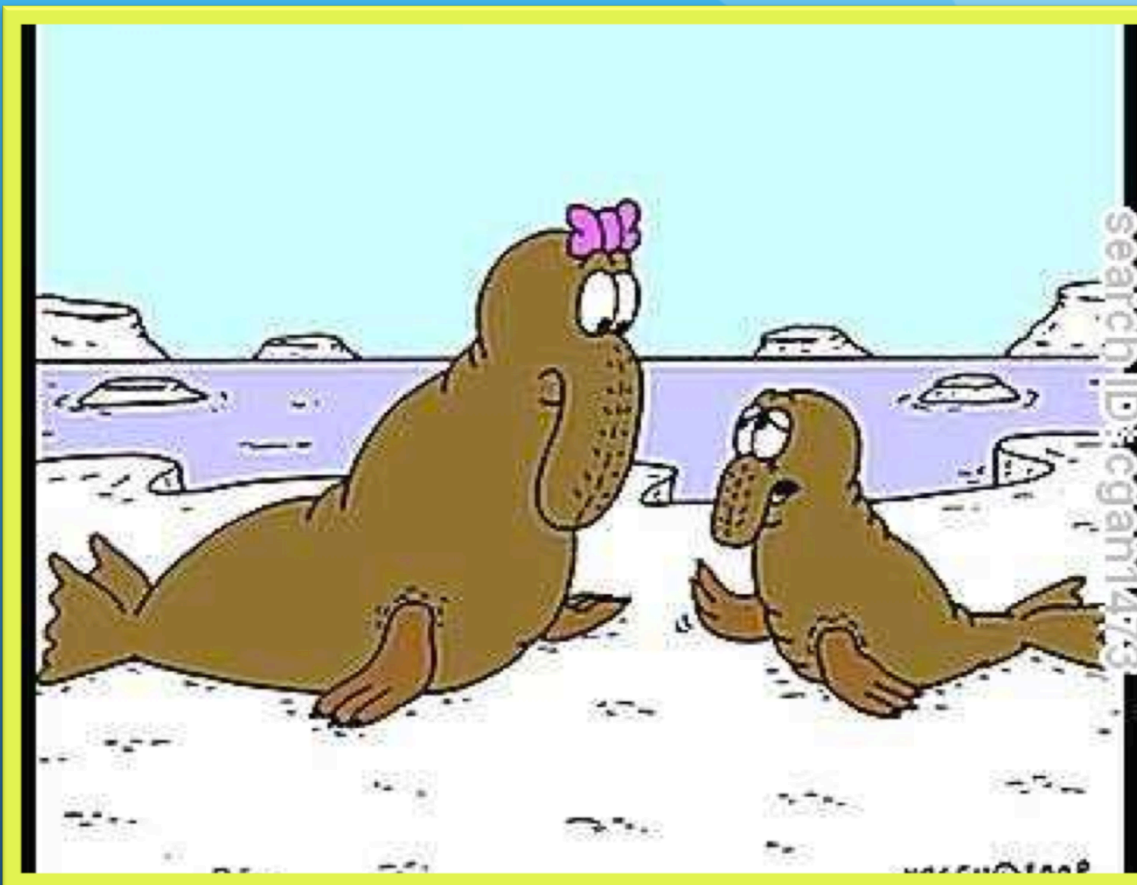
Les isotopes stables chez les vertébrés marins

1. **Ecologie trophique**
2. Physiologie
3. Contaminants



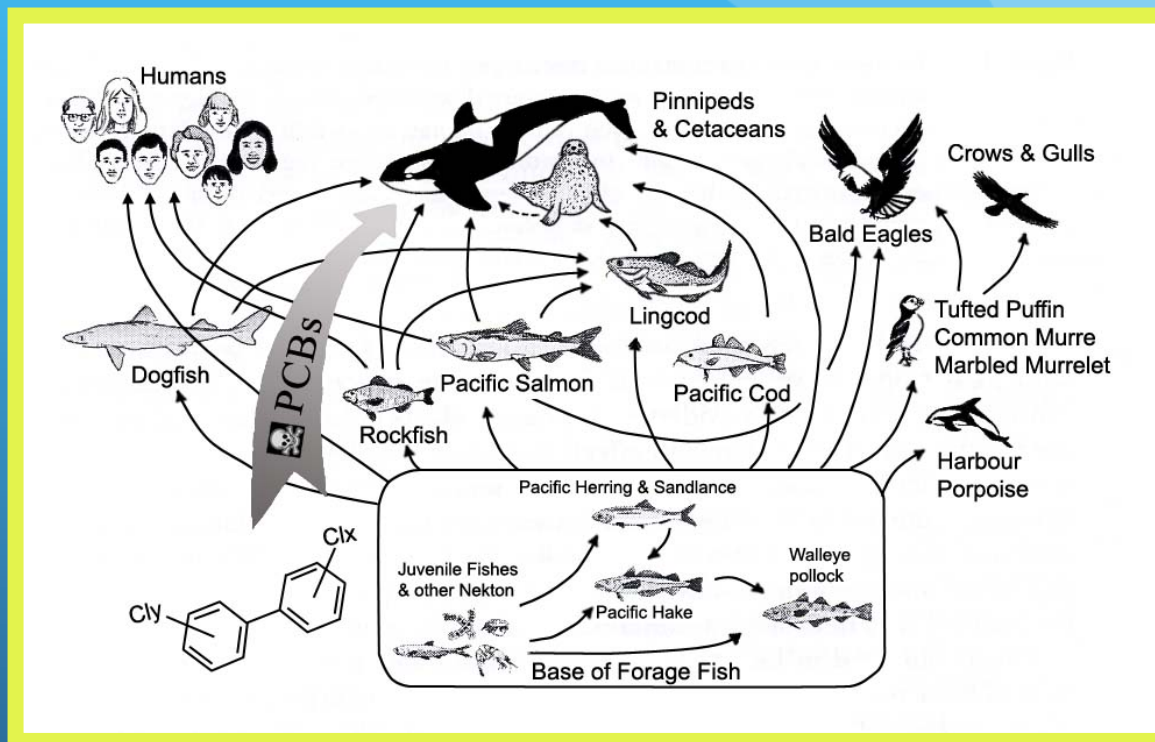
Les isotopes stables chez les vertébrés marins

1. Ecologie trophique
2. **Physiologie**
3. Contaminants



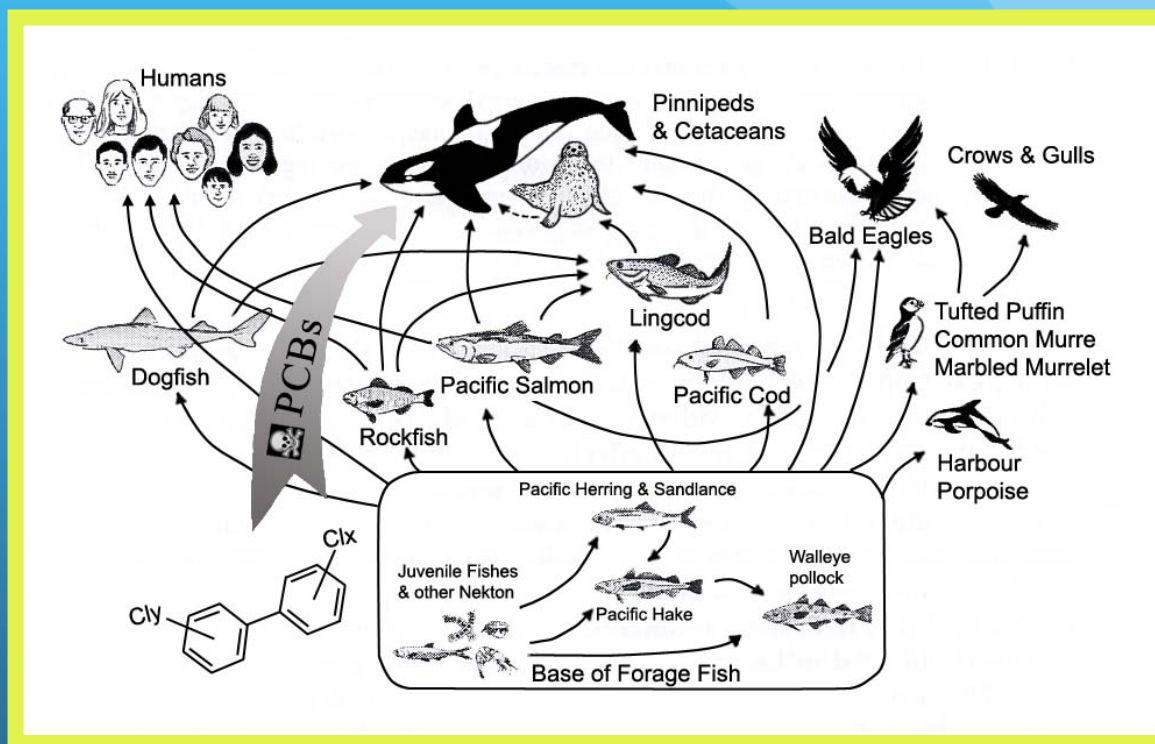
Les isotopes stables chez les vertébrés marins

1. Ecologie trophique
2. Physiologie
3. Contaminants



Les isotopes stables chez les vertébrés marins

1. Ecologie trophique
2. Physiologie
3. Contaminants



- ✓ Transfert trophique
- ✓ Structure des populations
- ✓ Variation de niches écologiques

Mais comment récolter les échantillons?

- Animaux morts



Photo: J. Haelters, MUMM



Photo: E. Donnay

Mais comment récolter les échantillons?

- Animaux en ponte



Crédit: E. Guirlet

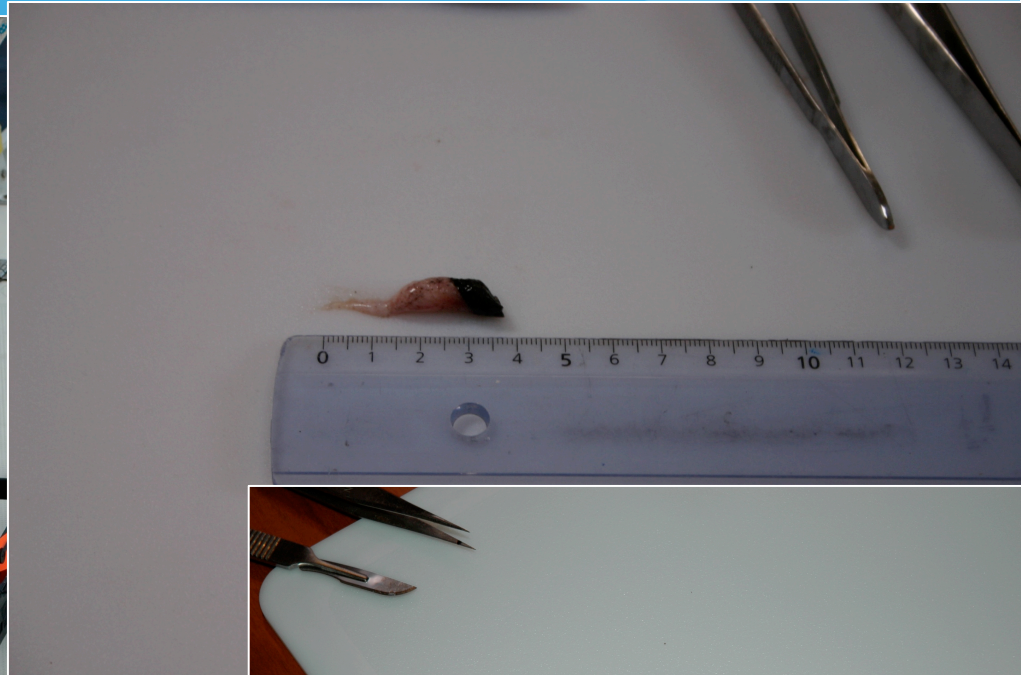
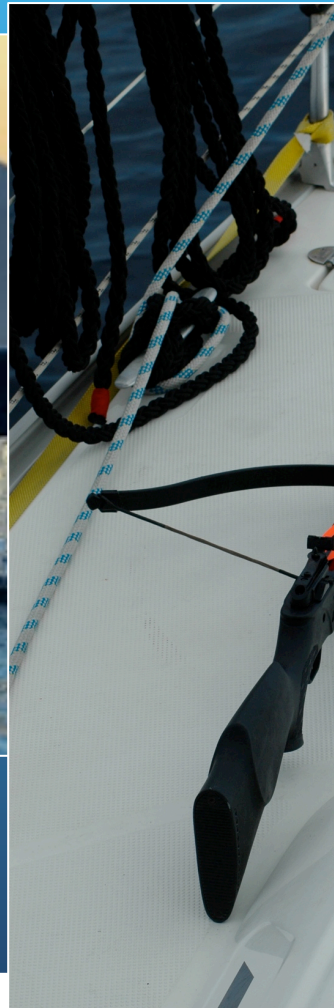
Mais comment récolter les échantillons?

- Biopsies de peau et de lard chez les cétacés

Crédit: Dr. E. Praca



<http://www.oceanus.uk.com/>



Les isotopes stables chez les vertébrés marins

1. **Ecologie trophique**
2. Physiologie
3. Contaminants



Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis*

Keith A. Hobson^{1,2}, Harold E. Welch²

¹ Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0

² Department of Fisheries and Oceans, Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba, Canada R3T 2

ABSTRACT: We measured stable-carbon ($^{13}\text{C}/^{12}\text{C}$) and/or nitrogen ($^{15}\text{N}/^{14}\text{N}$) isotope ratios in 322 tissue samples (minus lipids) representing 43 species from primary producers through polar bears *Ursus maritimus* in the Barrow Strait-Lancaster Sound marine food web during July–August, 1988 to 1990. $\delta^{13}\text{C}$ ranged from $-21.6 \pm 0.3\%$ for particulate organic matter (POM) to $-15.0 \pm 0.7\%$ for the predatory amphipod *Stegocephalus inflatus*. $\delta^{15}\text{N}$ was least enriched for POM ($5.4 \pm 0.8\%$), most enriched for polar bears ($21.1 \pm 0.6\%$), and showed a step-wise enrichment with trophic level of $+3.8\%$. We used this enrichment value to construct a simple isotopic food-web model to establish trophic relationships within this marine ecosystem. This model confirms a food web consisting primarily of 5 trophic levels. $\delta^{13}\text{C}$ showed no discernible pattern of enrichment after the first 2 trophic levels, an effect that could not be attributed to differential lipid concentrations in food-web components. Although Arctic cod *Boreogadus saida* is an important link between primary producers and higher trophic-level vertebrates during late summer, our isotopic model generally predicts closer links between lower trophic-level invertebrates and several species of seabirds and marine mammals than previously established.

Régime alimentaire et position trophique



- Augmentation des valeurs en $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ avec le niveau trophique
- 5 niveaux trophiques dans l'Arctique



Et en mer du Nord?



Vol. 263: 287–298, 2003	MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser	Published November 28
-------------------------	-----------------------------------------------------	-----------------------

Marine mammals from the southern North Sea: feeding ecology data from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements

K. Das^{1,2,*}, G. Lepoint¹, Y. Leroy¹, J. M. Bouqueneau¹

¹Marine Research Center (MARE), Laboratory for Oceanology, University of Liège, B6c, Sart-Tilman, 4000 Liège, Belgium
²Forschung- und Technologiezentrum Westküste, Werftstrasse 6, 25761 Büsum, Germany

ABSTRACT: The harbour porpoise *Phocoena phocoena*, grey seal *Halichoerus grypus*, harbour seal *Phoca vitulina* and white-beaked dolphin *Lagenorhynchus albirostris* are regularly found stranded along southern North Sea coasts. Occasionally, offshore species such as the fin whale *Balaenoptera physalus*, the white-sided dolphin *L. acutus* and the sperm whale *Physeter macrocephalus* are also found stranded. In order to trace their diet, we measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in their muscles as well as in 49 invertebrate and fish species collected from the southern North Sea. The $\delta^{15}\text{N}$ data indicate that the harbour seal, grey seal and white-beaked dolphin occupy the highest trophic position, along with ichthyophageous fishes such as the cod *Gadus morhua* (mean muscle values of 18.7, 17.9, 18.8 and 19.2‰ respectively). The harbour porpoise occupies a slightly lower trophic position (mean $\delta^{15}\text{N}$ value of 16.2‰), reflecting a higher amount of zooplanktivorous fishes in its diet (mean $\delta^{15}\text{N}$ of 14.7‰); 2 suckling harbour porpoises displayed a significant $\delta^{15}\text{N}$ enrichment of 2.2‰ compared to adult females. Adult females are $\delta^{15}\text{N}$ -enriched compared to adult male harbour porpoises. Fin whales, sperm whales and white-sided dolphins are ^{13}C -depleted compared to southern North Sea particulate organic matter and species, suggesting that despite regular sightings, they do not feed within the southern North Sea area.

KEY WORDS: North Sea · Marine mammals · Stable isotopes · Food web

The harbour porpoise,
Phocoena phocoena



The harbour seal,
Phoca vitulina



The grey seal,
Halichoerus grypus



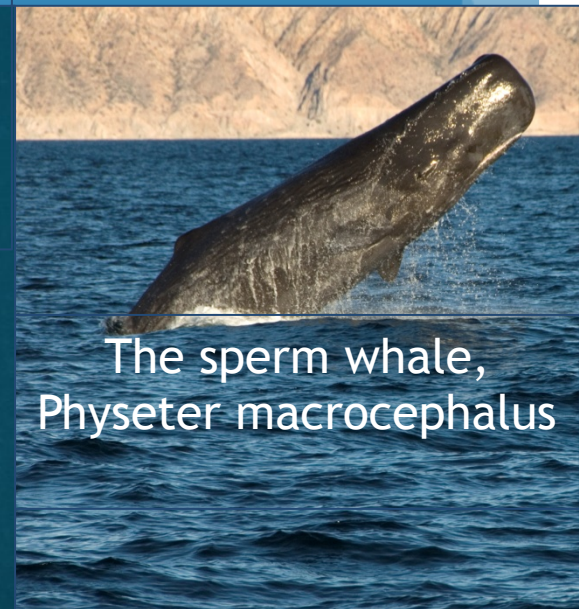
The white-sided
dolphin,
*Lagenorhynchus
acutus*



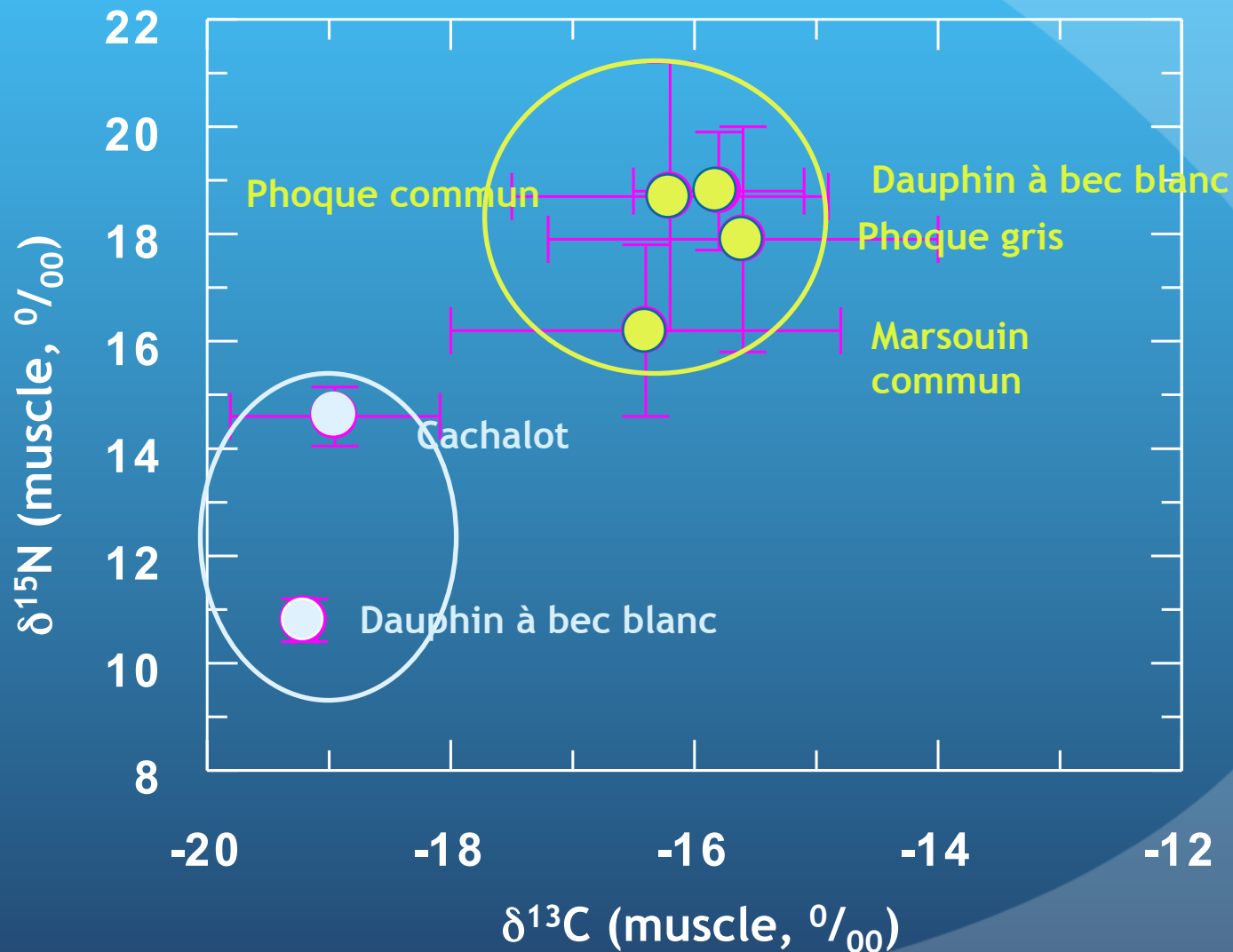
The white-
beaked dolphin,
*Lagenorhynchus
albirostris*



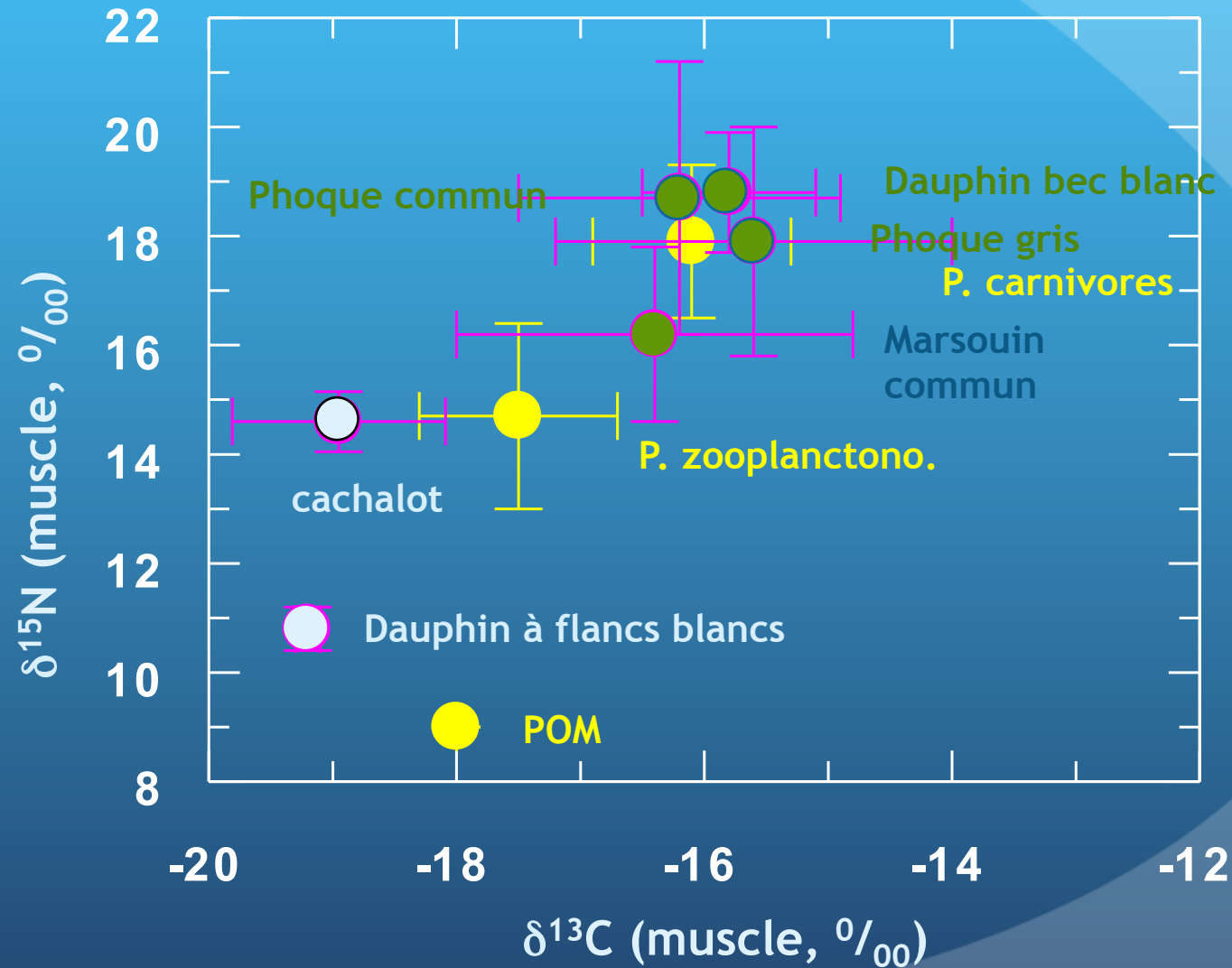
The sperm whale,
Physeter macrocephalus



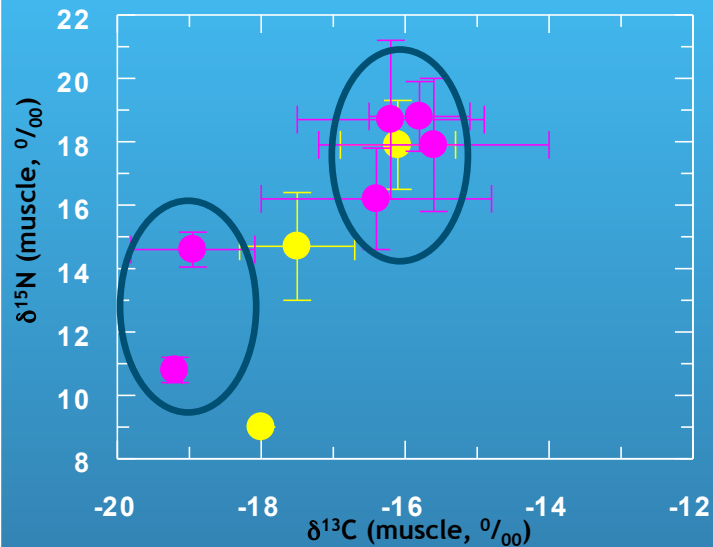
Position trophique des mammifères marins



Position trophique des mammifères marins

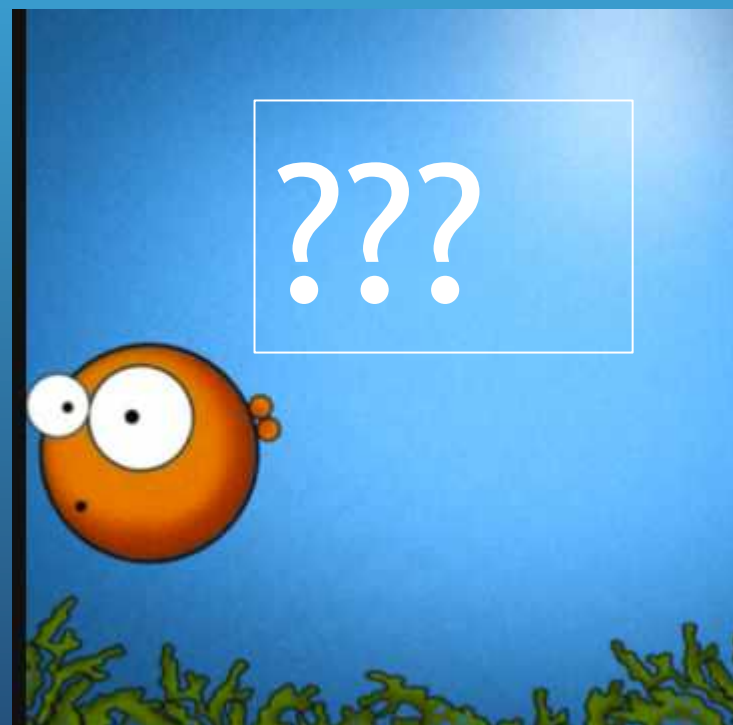
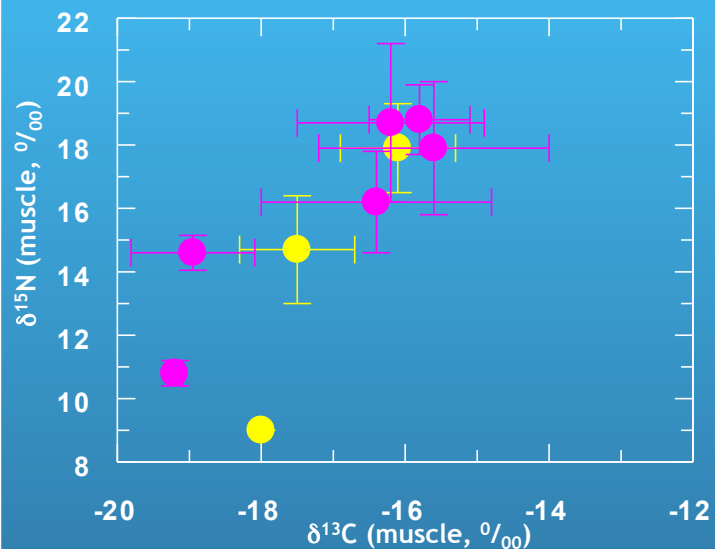


Position trophique des mammifères marins



Zone d'alimentation	SI signature
Océanique	valeurs faibles en $\delta^{13}\text{C}$ et $\delta^{15}\text{N}$
Mer du Nord	Valeurs élevées en $\delta^{13}\text{C}$ et $\delta^{15}\text{N}$

Poissons carnivores et phoques: structure des chaînes trophiques en mer du Nord



Deux aires d'alimentation pour la tortue Luth?

OPEN ACCESS Freely available online



Isotope Analysis Reveals Foraging Area Dichotomy for Atlantic Leatherback Turtles

Stéphane Caut^{1,3*}, Elodie Guirlet^{2,3*}, Elena Angulo², Krishna Das¹, Marc Girondot^{2,3}

1 Laboratory for Oceanology, MARE Center, B6C Liège University, Sart-Tilman, Liège, Belgique, **2** Laboratoire d'Ecologie, Systématique et Evolution, Université Paris Sud, Orsay, France, **3** Département Evolution et Systématique, Muséum National d'Histoire Naturelle de Paris, Paris, France

Abstract

Background: The leatherback turtle (*Dermochelys coriacea*) has undergone a dramatic decline over the last century; this is believed to be primarily the result of mortality associated with fisheries bycatch followed by egg and nest harvest. Atlantic leatherback turtles undertake long migrations across ocean basins from subtropical and tropical beaches to productive frontal areas. Migration between two nesting seasons can last 2 or 3 years, a time period referred to as the remigration interval (RI). Recent satellite transmitter data revealed that Atlantic leatherbacks follow two major migration patterns after nesting season, through the North Gulf Stream area or more eastward across the North Equatorial Current. However, information on the whole RI is lacking, precluding the accurate identification of feeding areas where measures may need to be applied.

Methodology/Principal Findings: Using stable isotopes as dietary tracers we determined the characteristic feeding grounds of leatherback females nesting in French Guiana. During migration, 3-year RI females differed from 1-year RI females in their isotope values, implying differences in their choice of feeding habitats (offshore vs. nearshore). Egg-yolk and blood isotope values are useful for assessing isotope values in these turtles, indicating that egg analysis is a useful tool for assessing isotope values in these turtles, in addition to blood analysis when not available.

Conclusions/Significance: Our results complement previous data on turtle movements during the first year of nesting season, integrating the diet consumed during the year before nesting. We suggest that the French Guiana leatherback population segregates into two distinct isotopic groupings, and highlight the urgent need to identify feeding habitats of the turtle in the Atlantic in order to protect this species from incidental take by commercial fisheries. Our results also emphasize the use of eggs, a less-invasive sampling material than blood, to assess isotopic data for adult female leatherbacks.

Citation: Caut S, Guirlet E, Angulo E, Das K, Girondot M (2008) Isotope Analysis Reveals Foraging Area Dichotomy for Atlantic Leatherback Turtles. *PLoS ONE* 3(3): e1845. doi:10.1371/journal.pone.0001845

Editor: Michael Somers, University of Pretoria, South Africa

Received: November 27, 2007; **Accepted:** February 8, 2008; **Published:** March 26, 2008

Copyright: © 2008 Caut et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by DIREN-Guyane. Krishna Das received a grant from FNRS-FRS and Stéphane Caut received a post-doctoral fellowship from the University of Liège. Elena Angulo acknowledges funding by the ANR-Biodiversité project.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: stephane.caut@u-psud.fr (SC); elodie.guirlet@u-psud.fr (EG)

† These authors contributed equally to this work.



Deux aires d'alimentation pour la tortue Luth?

Open Access Peer-reviewed article



Isotope Analysis Reveals Foraging Area Dichotomy for Atlantic Leatherback Turtles

Stéphane Caill^{1*}, Eodis Guirlet^{2*}, Elena Angulo³, Krishna Das⁴, Marc Ginouard^{1,5*}

1 Laboratoire d'Océanographie, 9180 Courcouronnes, France; **2** Centre National de la Recherche Scientifique, Institut Français de Recherche pour l'Exploitation de la Mer, 29100 Brest, France; **3** Département d'Ecologie et d'Évolution, Université Paris-Saclay, 91190 Evry, France; **4** Département d'Ecologie et d'Évolution, Université de la Nouvelle-Guinée, 6000 Port Moresby, Nouvelle-Guinée; **5** Centre National de la Recherche Scientifique, Institut Français de Recherche pour l'Exploitation de la Mer, 29100 Brest, France

Abstract

Background: The leatherback turtle (*Dermochelys coriacea*) has undergone a dramatic decline over the last 25 years, and this is believed to be primarily the result of mortality associated with fisheries bycatch followed by egg and nesting habitat losses. Atlantic leatherback turtles undertake long migrations across ocean basins from subtropical and tropical nesting beaches to productive foraging areas. Migrations between nesting seasons can last 2 or 3 years, a time period termed the migration interval (MI). Recent satellite transmitter data revealed that Atlantic leatherbacks follow two major dispersion patterns after nesting success through the North Gulf Stream area or more easterly across the North Equatorial Current. However, information on the whole MI is lacking, precluding the accurate identification of feeding areas where conservation measures may need to be applied.

Methodology/Principal Findings: Using stable isotopes in dietary tissues we determined the characteristics of feeding grounds of leatherback females nesting in French Guiana. During migration, 2-year MI females differed from 2-year MI females in their isotopic values, implying differences in their choice of feeding habitats offshore or more coastal and foraging habitats (North Atlantic or West African coasts, respectively). Egg and blood isotopic values are consistent in nesting females, indicating that egg analysis is a useful tool for assessing isotopic values in these turtles, including adults whose sex is unknown.

Conclusions/Significance: Our results complement previous data on turtle movements during the first year following the nesting season, integrating the diet consumed during the post-nesting period. We suggest that the French Guiana leatherback population segregates into two distinct isotopic groupings, and highlight the urgent need to determine the feeding habitats of the turtles in the Atlantic in order to protect the species from incidental loss by commercial fisheries. Our results also emphasize the use of eggs, a less-invasive sampling method than blood, to assess isotopic data and feeding habits for adult female leatherbacks.

Citation: Caill S, Guirlet E, Angulo E, Das K, Ginouard M (2018) Isotope Analysis Reveals Foraging Area Dichotomy for Atlantic Leatherback Turtles. PLoS ONE 13(2): e0191600. doi:10.1371/journal.pone.0191600

Editor: John Sponberg, University of Wyoming, UNITED STATES

Received: November 27, 2017; **Accepted:** February 9, 2018; **Published:** March 26, 2018

Copyright: © 2018 Caill et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by CNRS-Copernic, CNRS-ANR, the Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER), and the Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER).

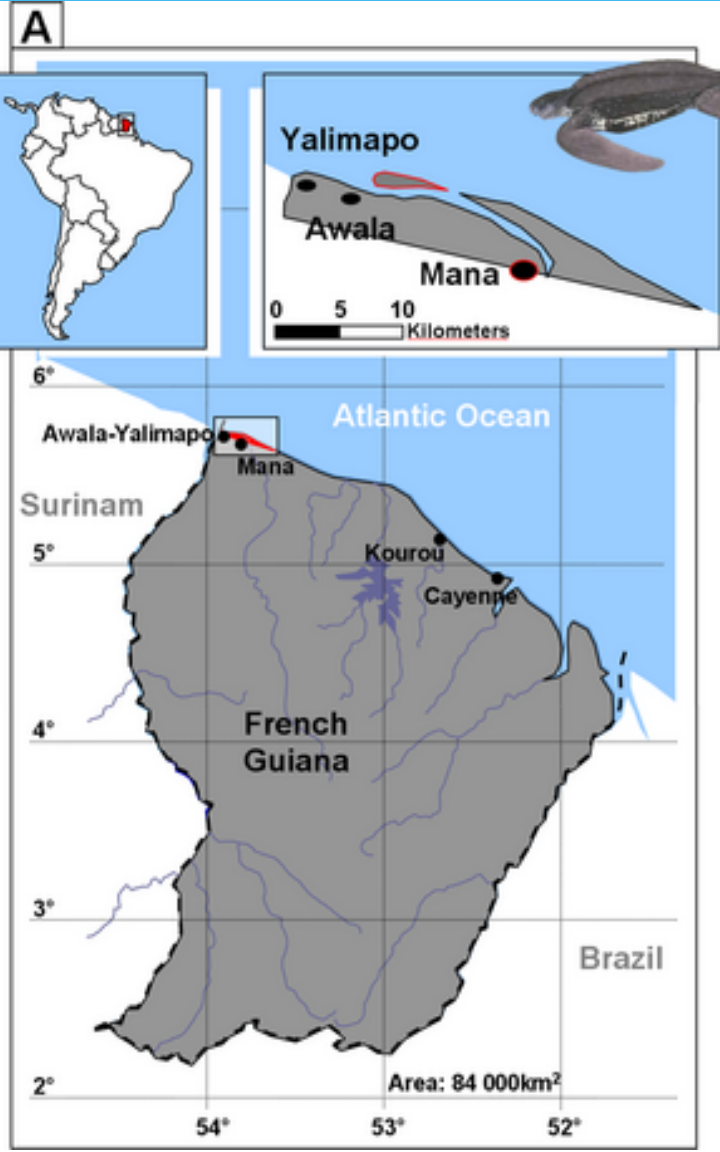
Competing Interests: The authors have declared that no competing interests exist.

* stcaill@ifremer.fr; guirlet@ifremer.fr; ginouard@ifremer.fr

† These authors contributed equally to this work.

Introduction
 Yalimapo beach in French Guiana, South America, is one of the main nesting grounds for the largest of the sea turtles, the leatherback (*Dermochelys coriacea*), valued by up to 30–40% of the world's population of nesting females [1]. The major nesting season at Yalimapo extends from March to the end of August, including a peak in June, with some procreant nesting outside this period [2]. During the nesting season a female may lay up to six or seven 140–150 g clutches, with an average interval between nesting events of 15 days [3]. Due to the separation of breeding and foraging sites at the end of the reproductive season leatherbacks engage several thousand kilometers [4,5] across ocean basins in patchy foraging areas where their prey, large jellyfish and other gelatinous organisms, is more abundant than on regional coasts [6–7]. The leatherback is the only marine turtle that feeds on gelatinous organisms throughout its life. This diet is extremely poor in lipid and energy, and consequently very little energy and nutrient is extracted from a given mass of prey [8]. Consequently [9] constant diet maintenance in such a low energy (North Atlantic) consumes a quantity of prey equivalent to at least 30% of their body mass per day.

During the breeding season, energy requirements are high because of egg production, nest construction, and nest-guarding activities between nesting events [9]. However, leatherbacks appear to adopt different strategies during their inter-nesting intervals, depending on their nesting site [9]. In the Eastern Pacific Ocean and at Costa Rica and Mexico, leatherbacks may maintain energy expenditure to maximize the amount of energy allocated to egg production and egg production [10,11]. In the Atlantic Ocean and Western Pacific Ocean, leatherbacks probably cover great distances at high speed and spend consecutive, probably for



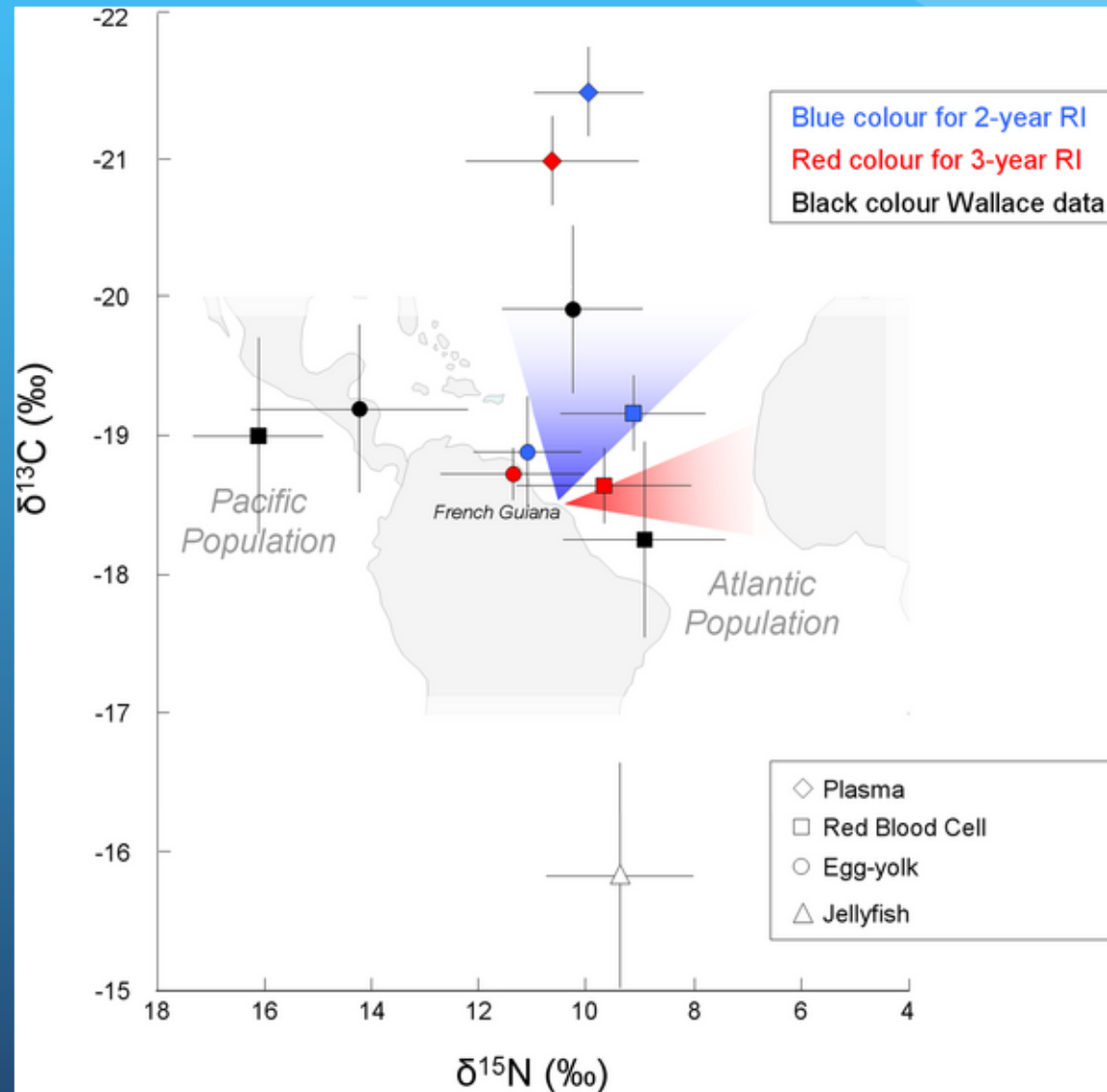
Intervalle de Remigration:

-2 ans

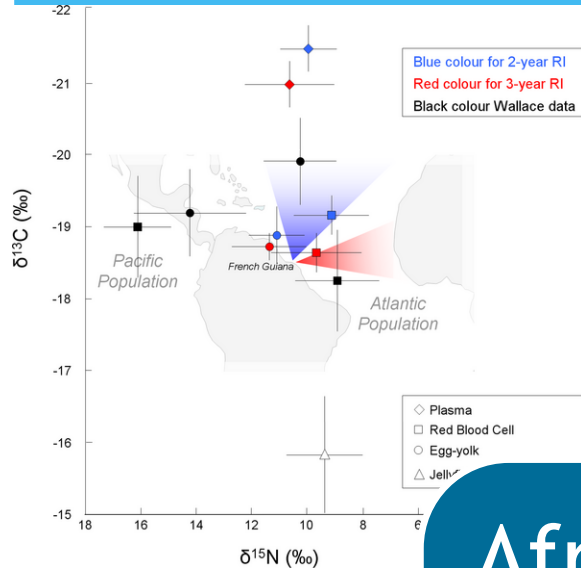
-3 ans



Deux aires d'alimentation pour la tortue Luth?



Deux aires d'alimentation pour la tortue Luth?



Afrique de
l'Ouest

Atlantique
Nord

- Afrique: Retard?
- Impact de la pêche côtière?

???

Traceurs oui mais...?



Credit: Oceanus

Les isotopes stables chez les vertébrés marins

1. Ecologie trophique
2. **Physiologie**
3. Contaminants



Physiologie et Fractionnement

1978: Michael J. DeNiro and Samuel Epstein. Influence of diet on the distribution of carbon isotopes in animals
Geochimica and Cosmochimica Acta 42: 495-506

1. Les valeurs en $\delta^{13}\text{C}$ d'un consommateur sont directement proportionnelles à celles de leur diète: régime contrôlé en laboratoire sur 8 espèces d'invertébrés et de souris.
2. Cet enrichissement en ^{13}C est minime (moins de 2‰).
3. Cette différence proie-diète dépend du type d'aliment ingéré et du tissu considéré.

Processus physiologiques

- ▶ Gestation
- ▶ Lactation
- ▶ Jeûne
- ▶ Mue





Assessment of gestation, lactation and fasting
on stable isotope ratios in northern elephant seals
(*Mirounga angustirostris*)

SARAH HABRAN
MARE Centre,
Laboratory for Oceanology B6c,
University of Liège, B-4000 Liège, Belgium
E-mail: s.habran@ulg.ac.be

CATHY DEBIER
Unité de Biochimie de la Nutrition,
Institut des Sciences de la vie,
Université catholique de Louvain,
Place Croix du Sud 2/8, B-1348 Louvain-la-Neuve, Belgium

DANIEL E. CROCKER
Department of Biology,
Sonoma State University,
Rohnert Park, California 94928, U.S.A.

DORIAN S. HOUSER
BIOMIMETICA,
7951 Shantung Drive,
Santee, California 92071, U.S.A.

GILLES LEPOINT
JEAN-MARIE BOUQUEGNEAU
KRISHNA DAS
MARE Centre,
Laboratory for Oceanology B6c,
University of Liège, B-4000 Liège, Belgium

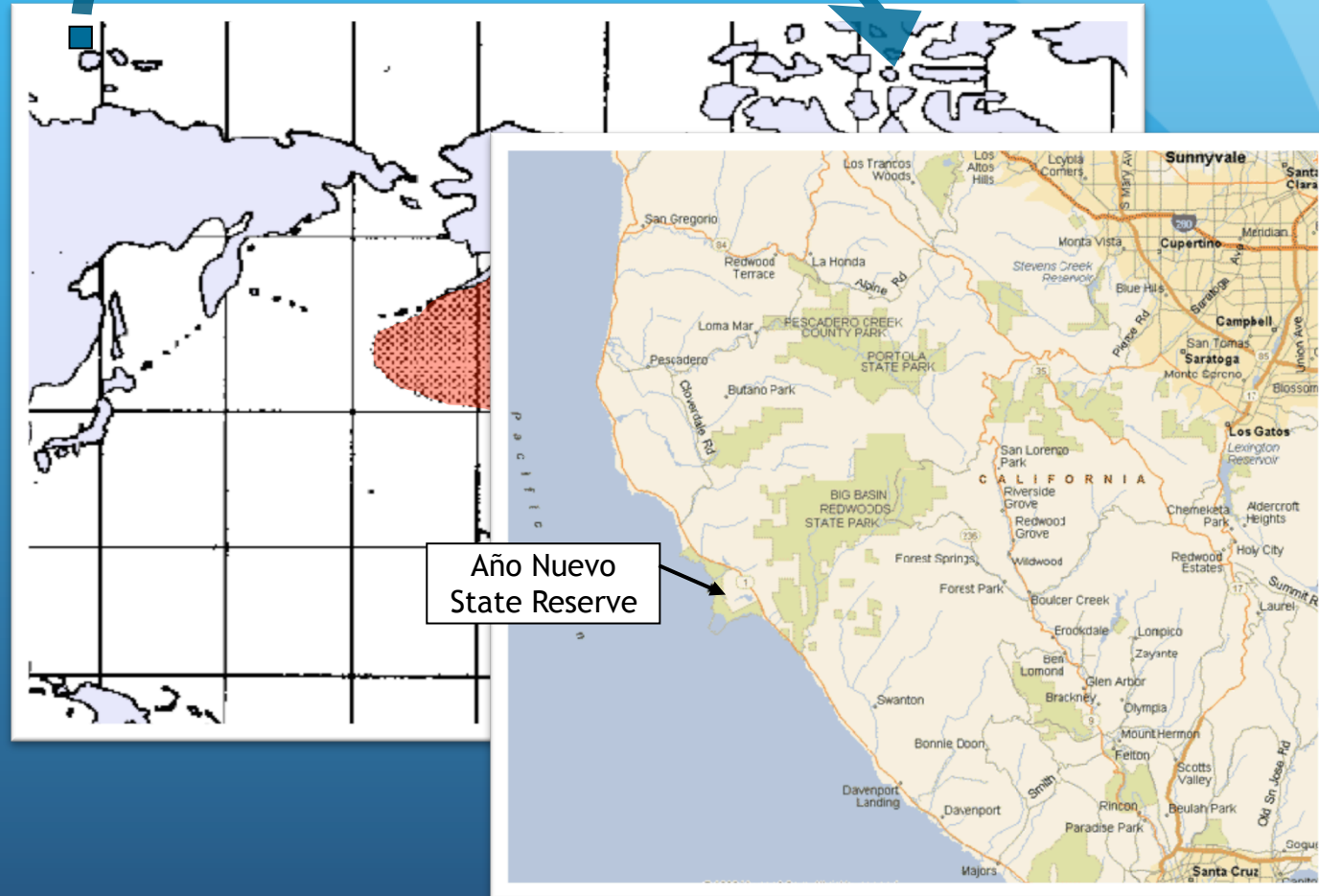
ABSTRACT

Effects of physiological processes such as gestation, lactation and nutritional stress on stable isotope ratios remain poorly understood. To determine their impact, we investigated these processes in simultaneously fasting and lactating northern elephant seals (*Mirounga angustirostris*). Stable carbon and nitrogen isotope values were measured in blood and milk of 10 mother-pup pairs on days 5 and 22 of lactation. As long- and short-term integrators of diet, blood cells and serum may reflect foraging data or energy reserves from late gestation and lactation, respectively. Limited changes in isotopic signatures of maternal blood over the lactating period were highlighted. Nitrogen isotope fractionation associated with mother-to-offspring

Influence de la gestation, de la lactation et du jeûne sur les rapports isotopiques chez l'éléphant de mer



Distribution de l'éléphant de mer septentrional



La lactation est un processus intense chez les femelles

~ 25 days

Début



x 1/3

x 4

Fin



Un peu moins chez les mâles!

Physiologie



@Sarah Habran



Plages rocheuse



sang



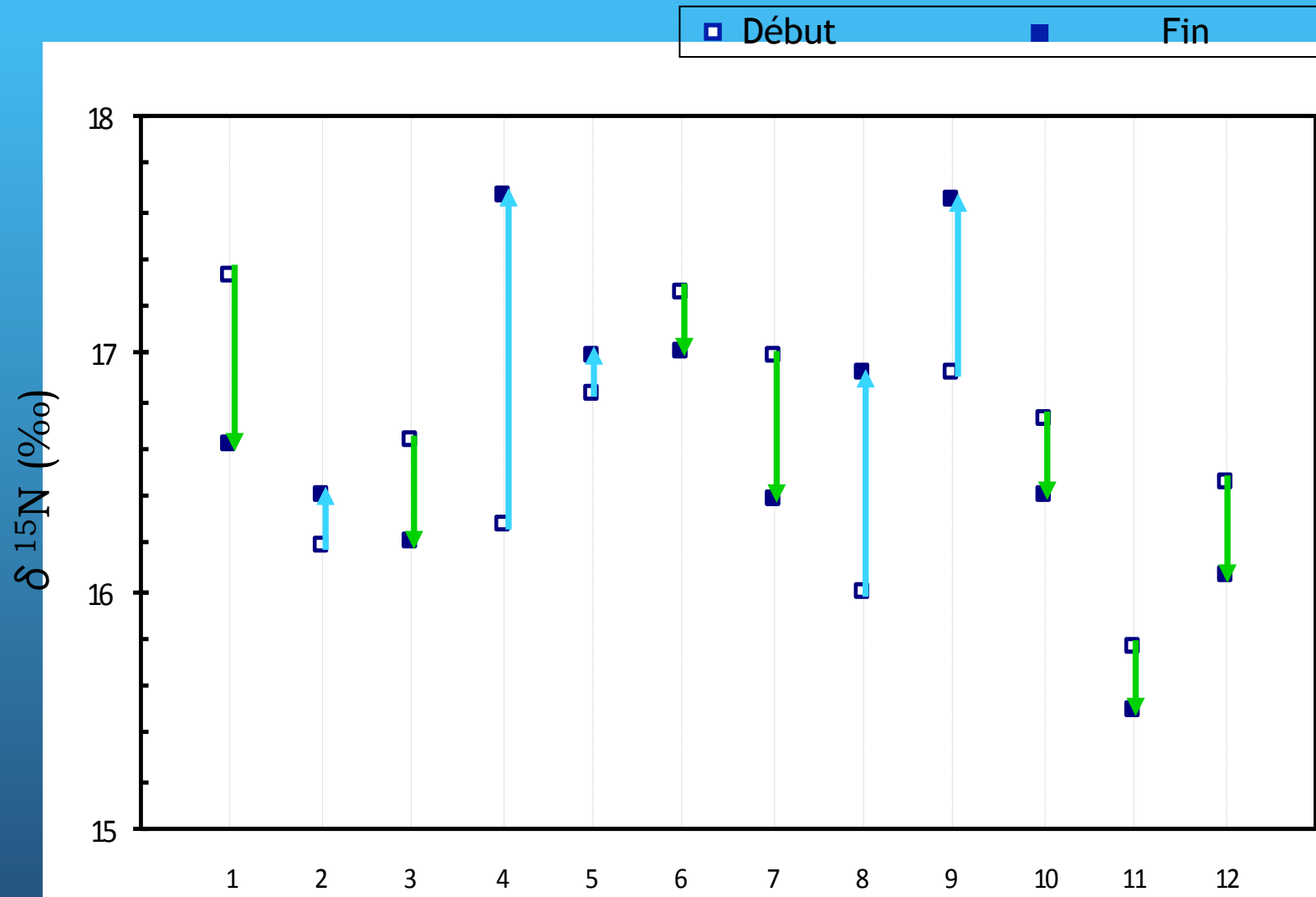
lait



lard

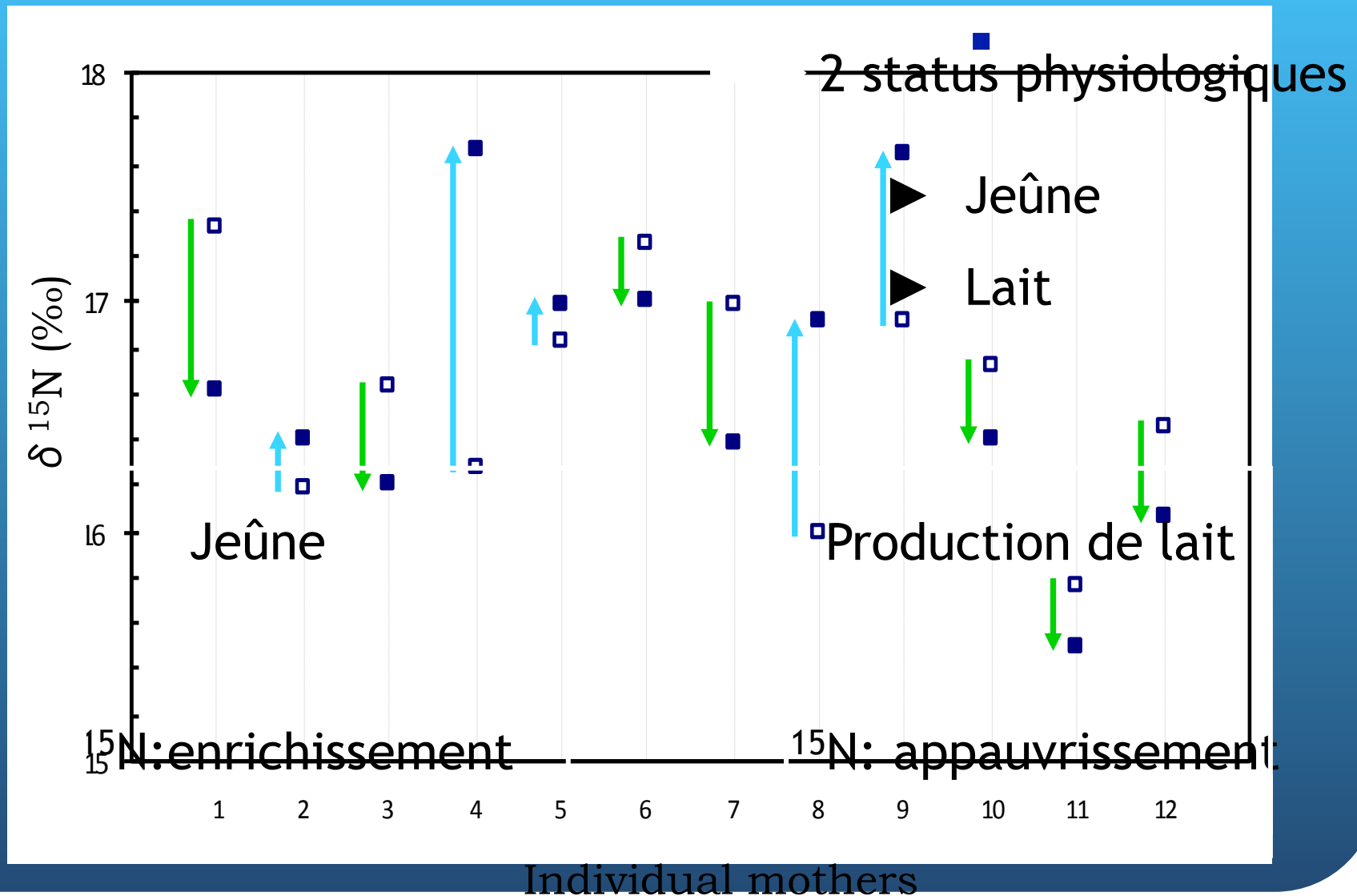
- ▶ Animaux sauvages
 - Representatifs de la population
 - Techniques peu invasives
- ▶ accessible

Variabilité dans le sérum

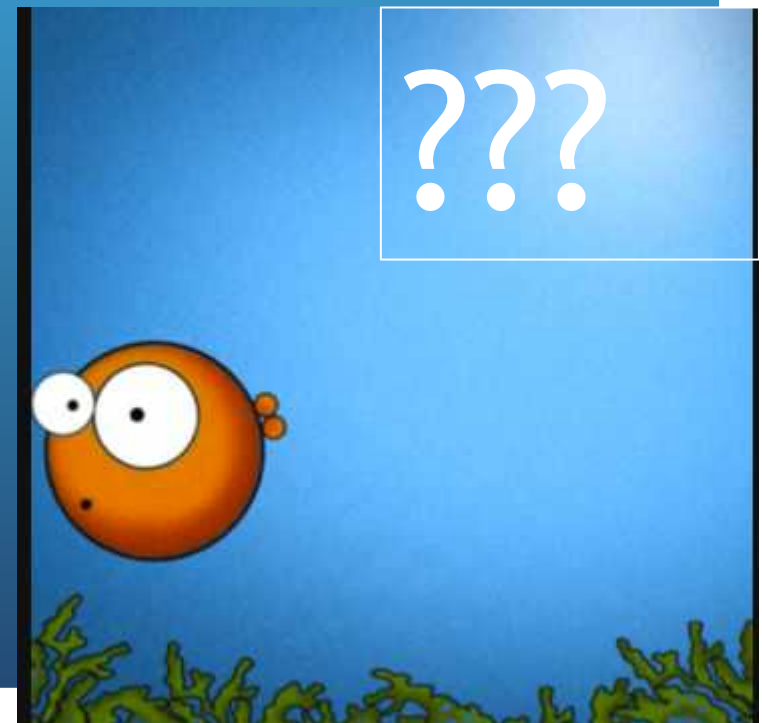


Mères éléphant de mer

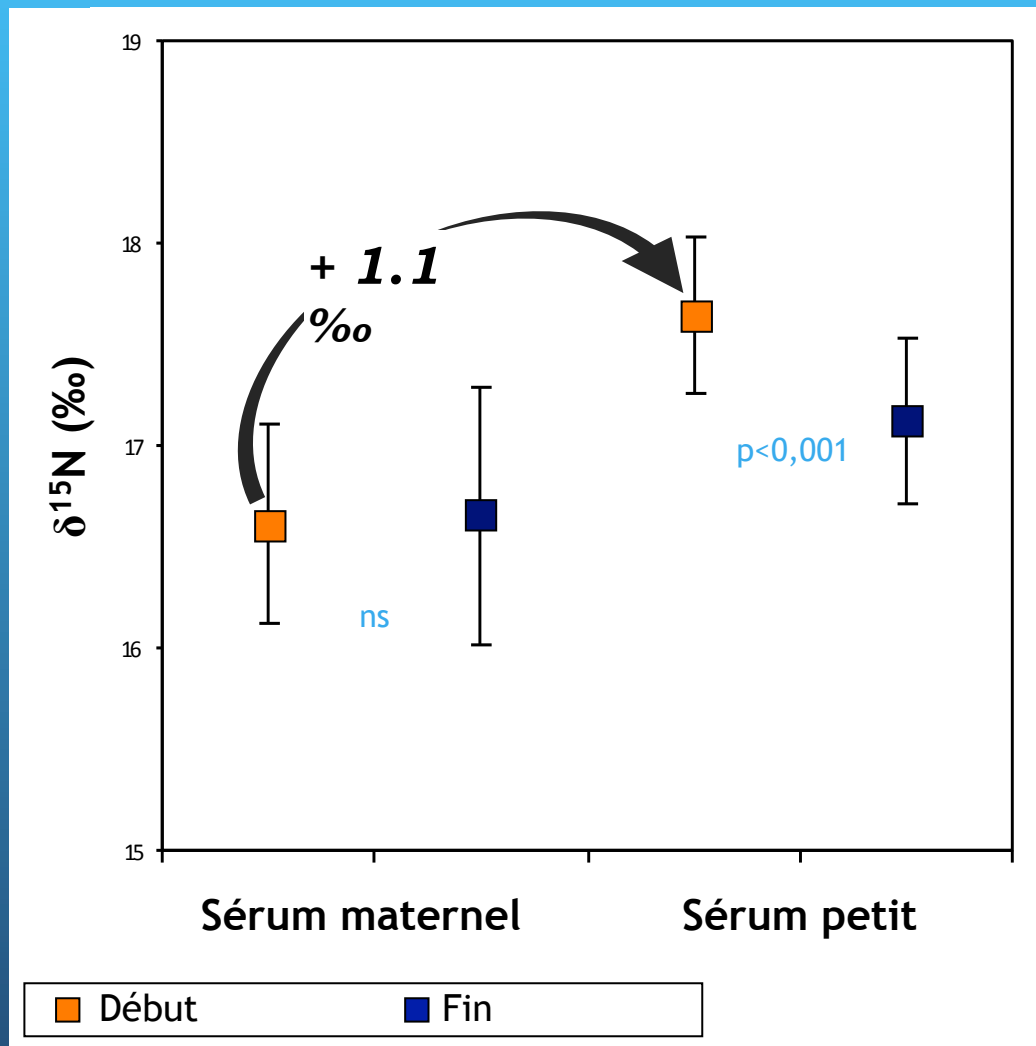
Variabilité dans le sérum



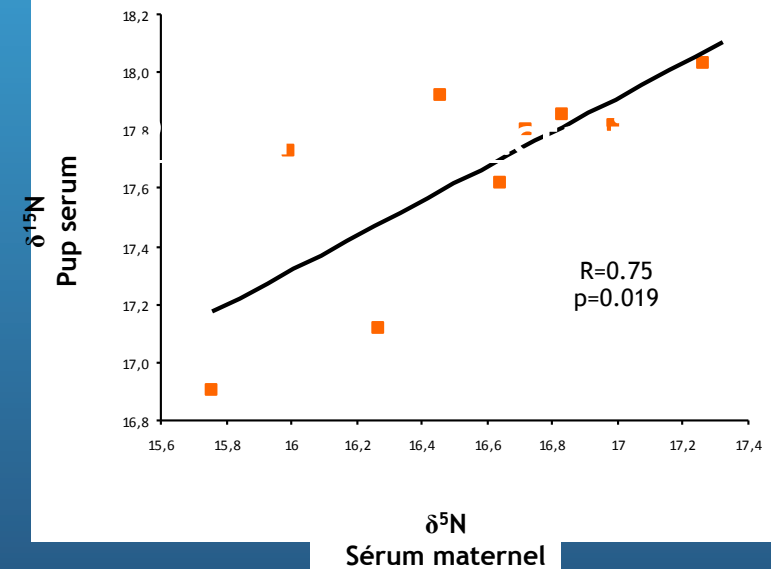
- Le sérum réagit de manière non prévisible lors des processus de jeûne et de production de lait
- Turn-over très rapide
- Temps de demi-vie des valeurs en $\delta^{13}\text{C}$ et $\delta^{15}\text{N}$ lors de ces processus?



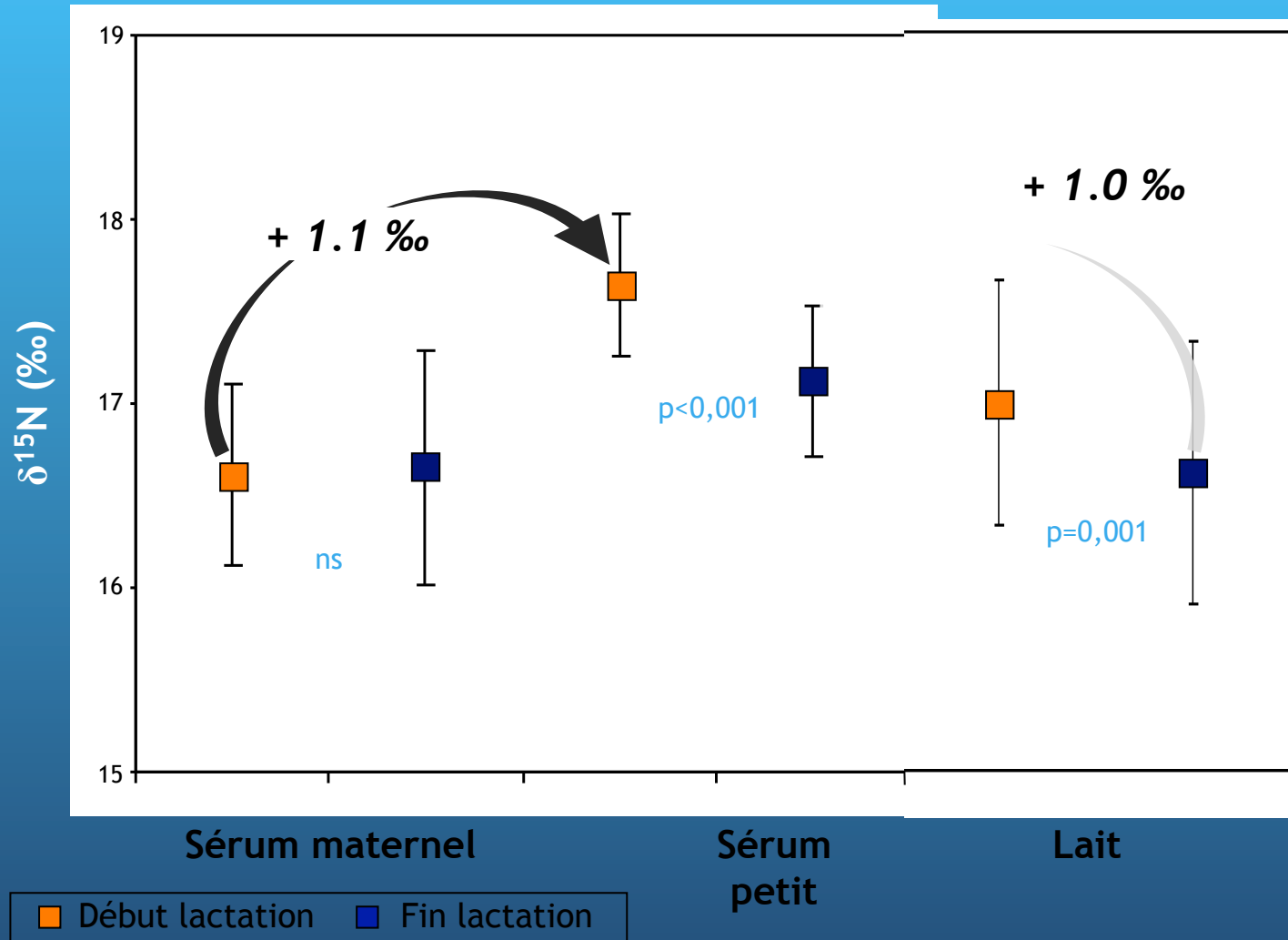
Fractionnement mère- petit



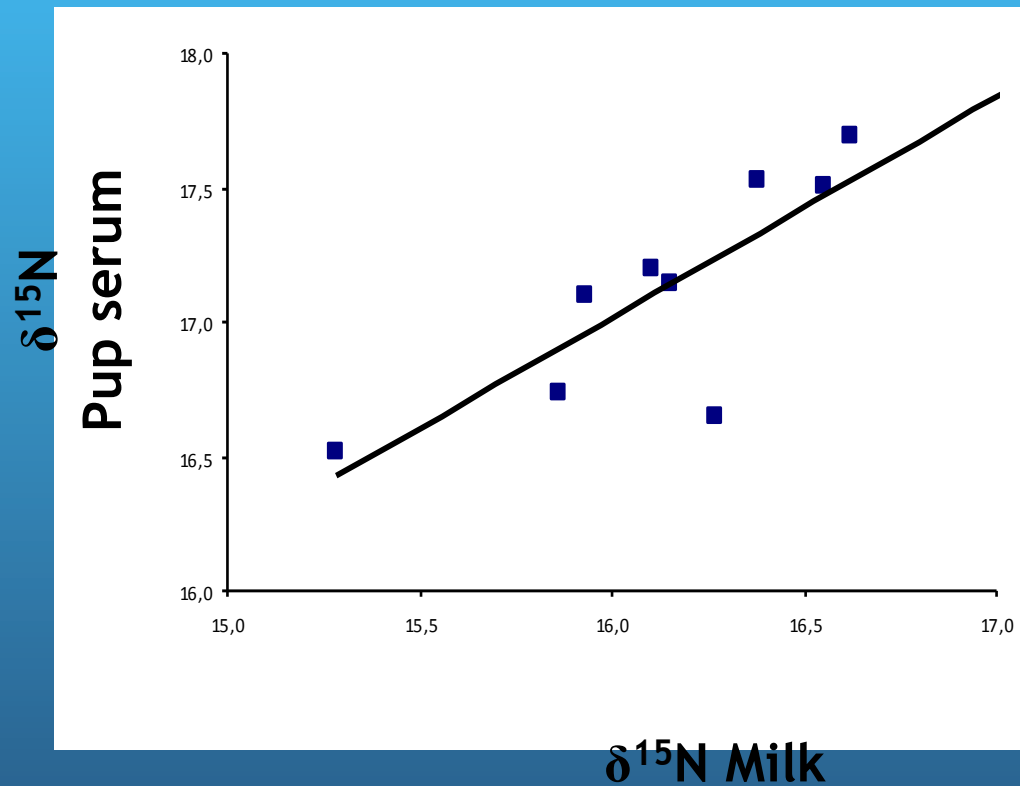
► Sérum du nouveau-né lié au sérum maternel



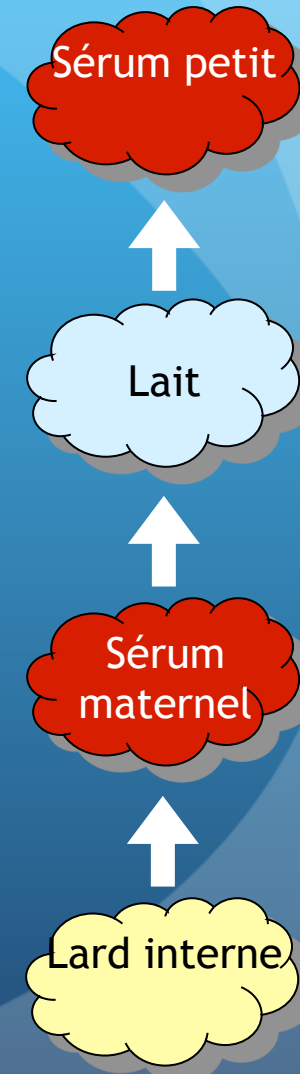
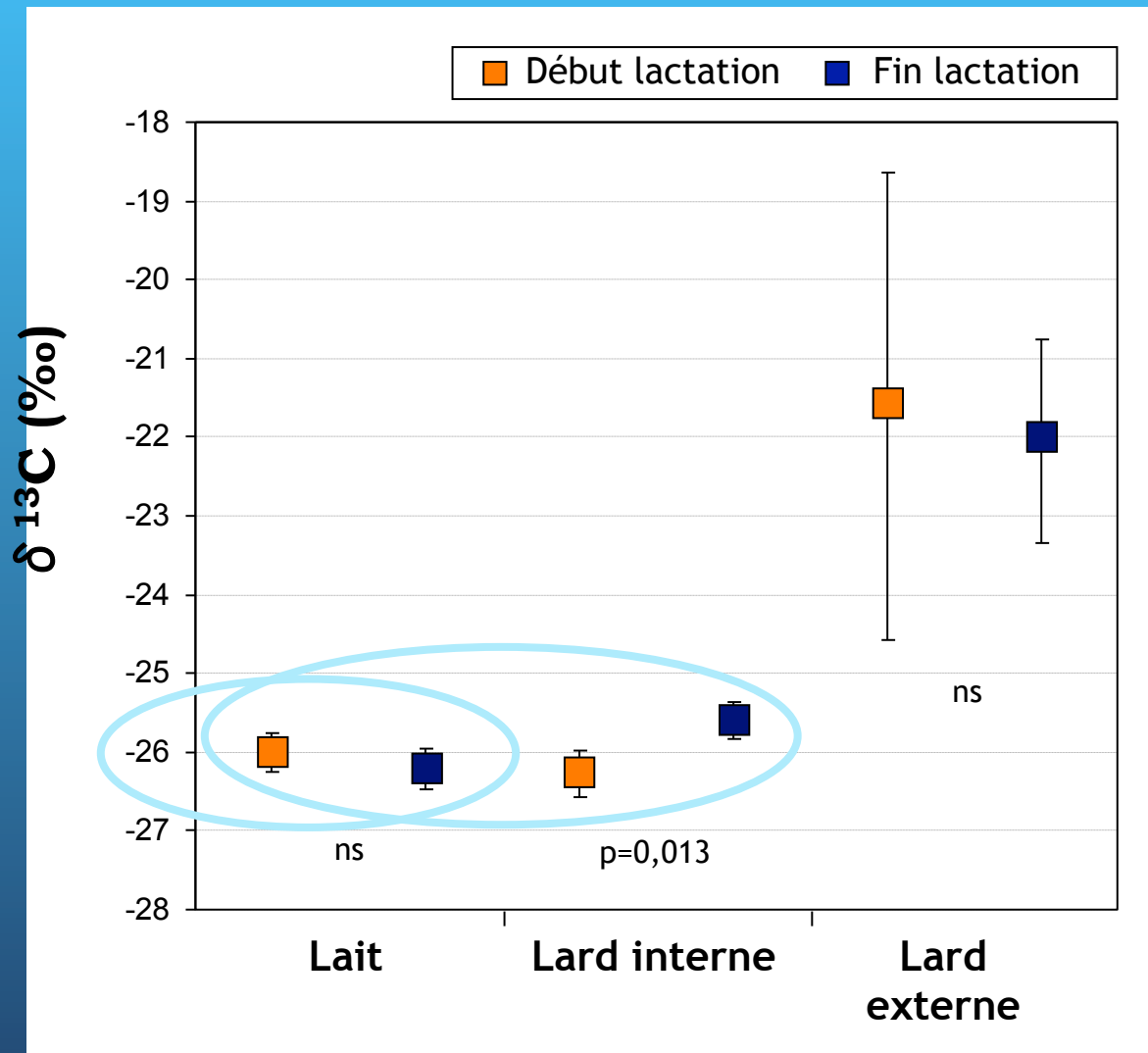
Fractionnement lait- petit



Sérum du petit lié au lait en fin de lactation

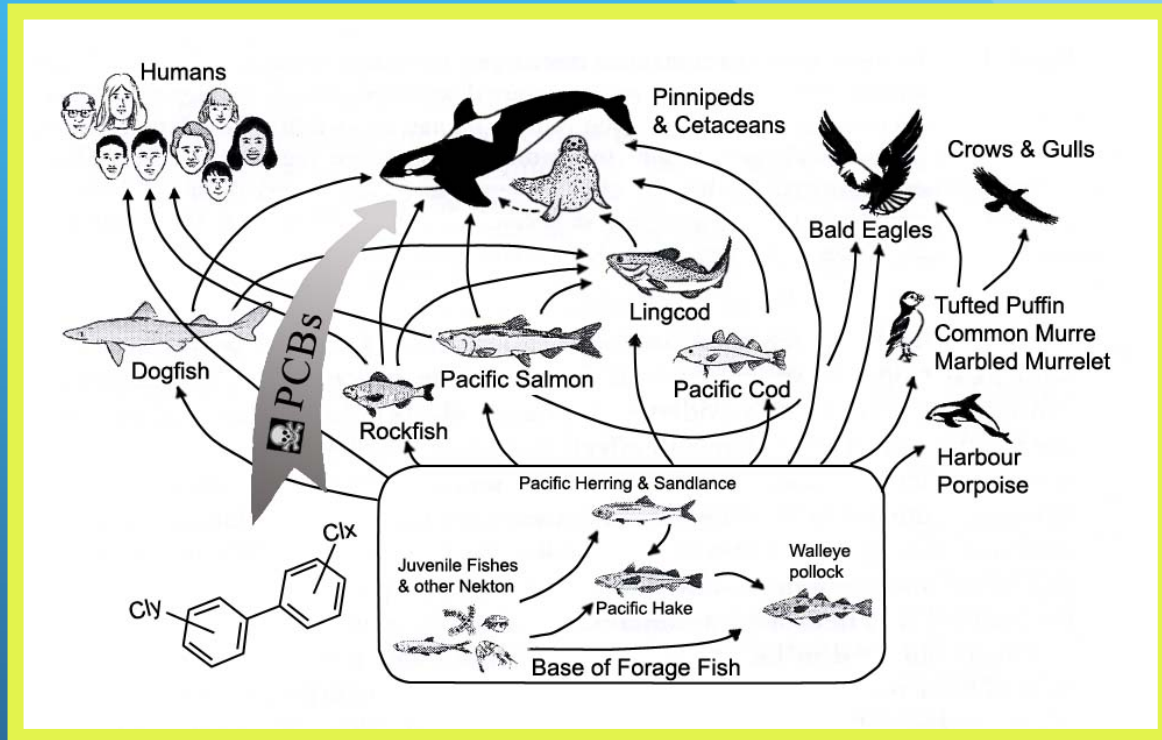


Production de lait à partir du lard interne



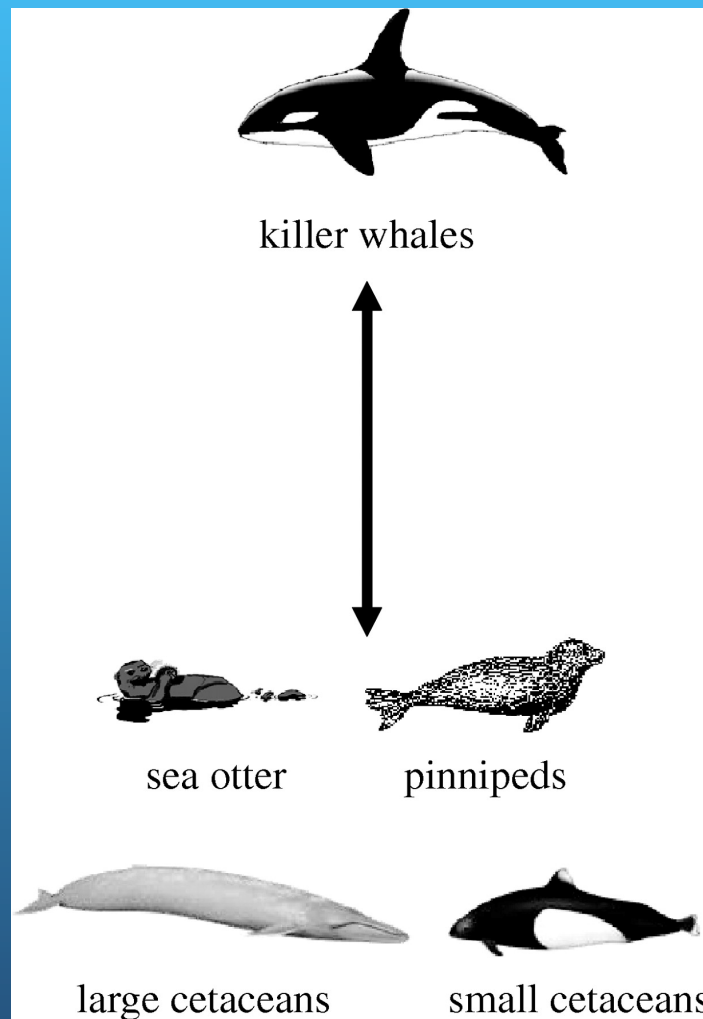
Les isotopes stables chez les vertébrés marins

1. Ecologie trophique
2. Physiologie
3. Contaminants



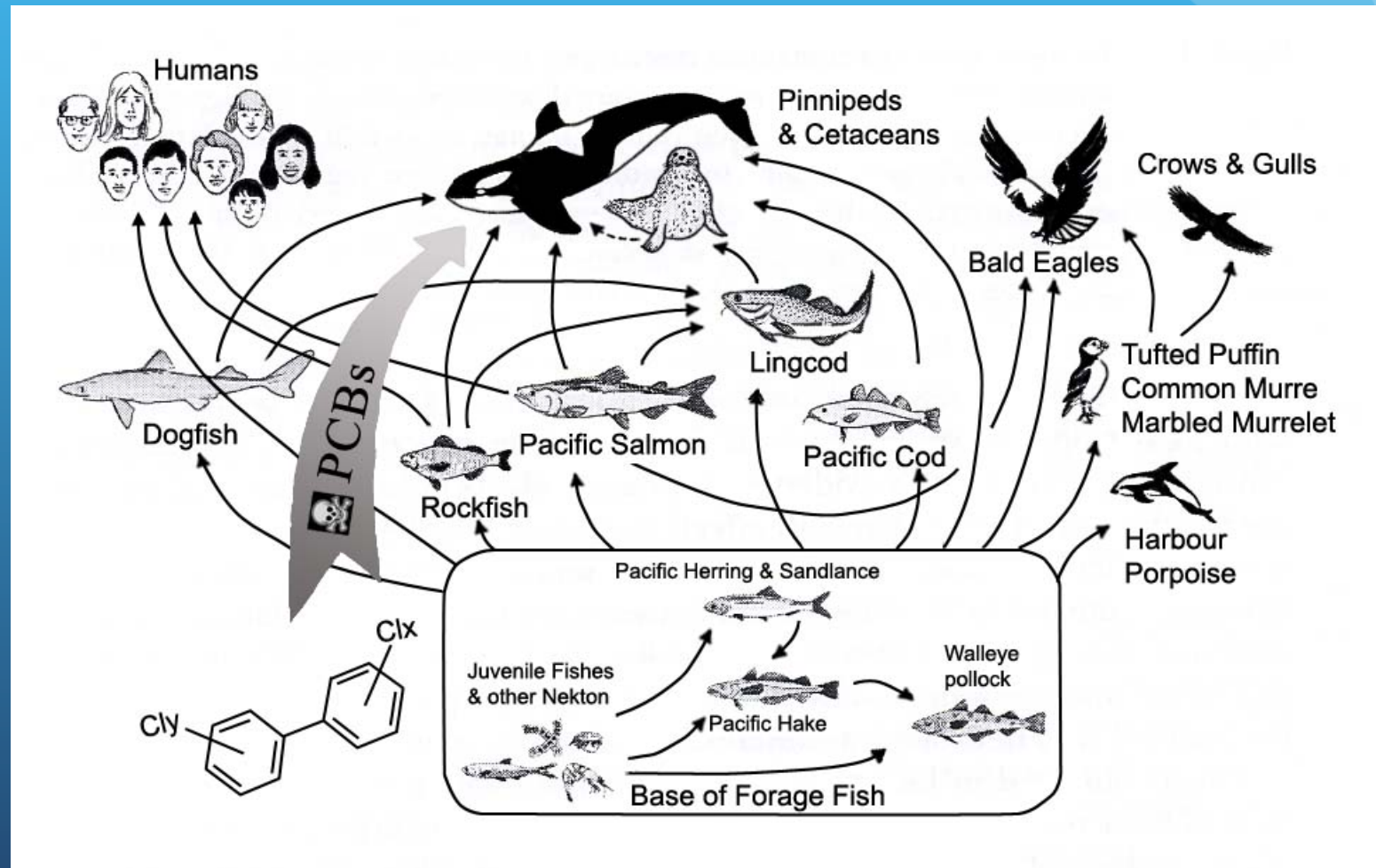
- ✓ Transfert trophique
- ✓ Structure des populations
- ✓ Variation de niches écologiques

Les mammifères marins occupent le haut des chaînes trophiques



Estes J et al. Phil. Trans. R. Soc. B 2009;364:1647-1658

Biomagnification des polluants organiques en milieu marin!



L'épaulard, *Orcinus orca*, au sommet des chaînes trophiques

- > 35 ans
- Entre 5 et 7 mètres
- Mâles: 8 tonnes
- Femelles: 4 tonnes



CREDIT: ARI FRIEDLAENDER



CREDIT: DAVE ELLIFRIT/NOAA ALASKA FISHERIES SCIENCE CENTER UNDER PERMIT 782-1719/NATIONAL MARINE FISHERIES

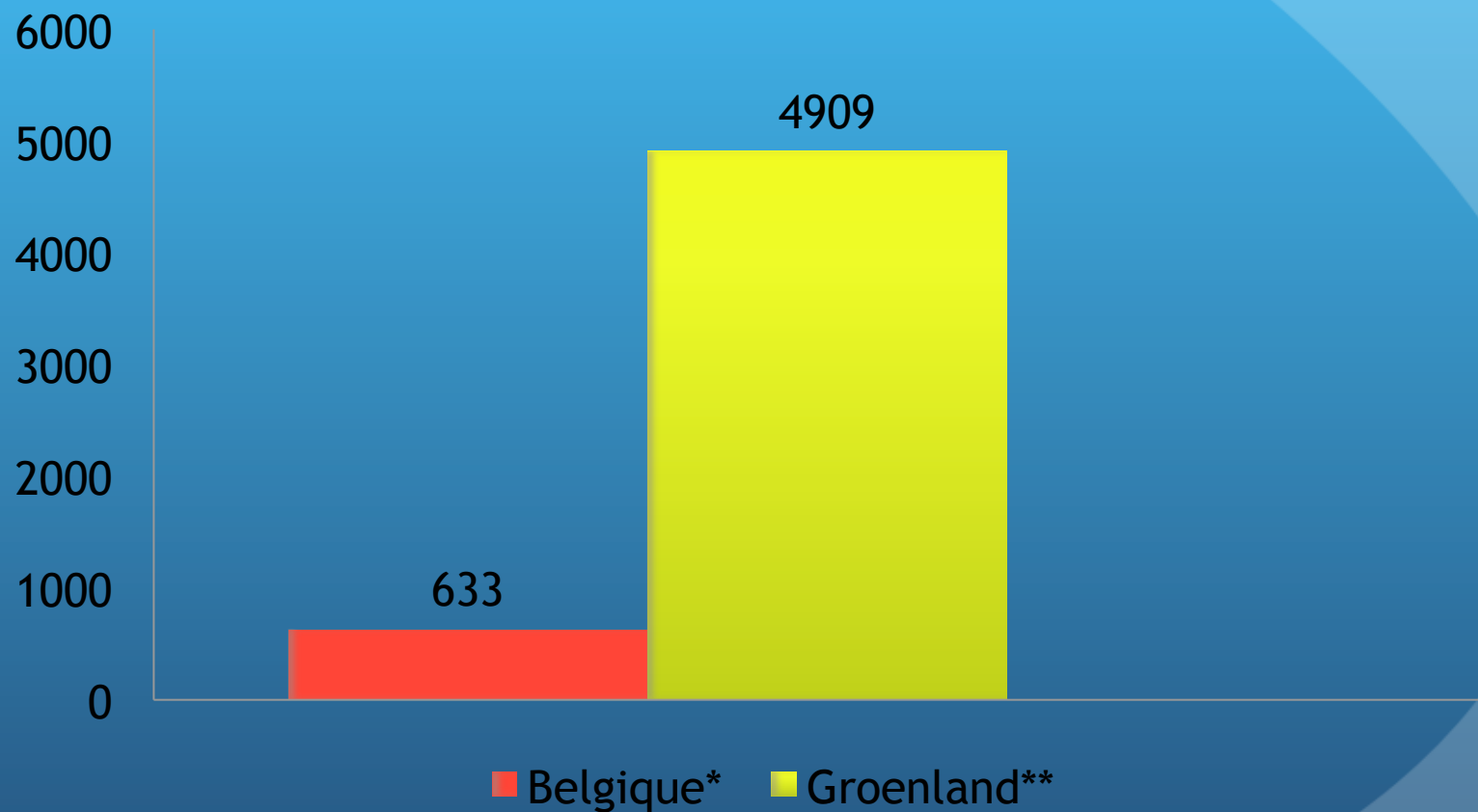


CREDIT: LANCE BARRETT-LENNARD



CREDIT: JOHN DURBAN/NORTH GULF OCEANIC SOCIETY

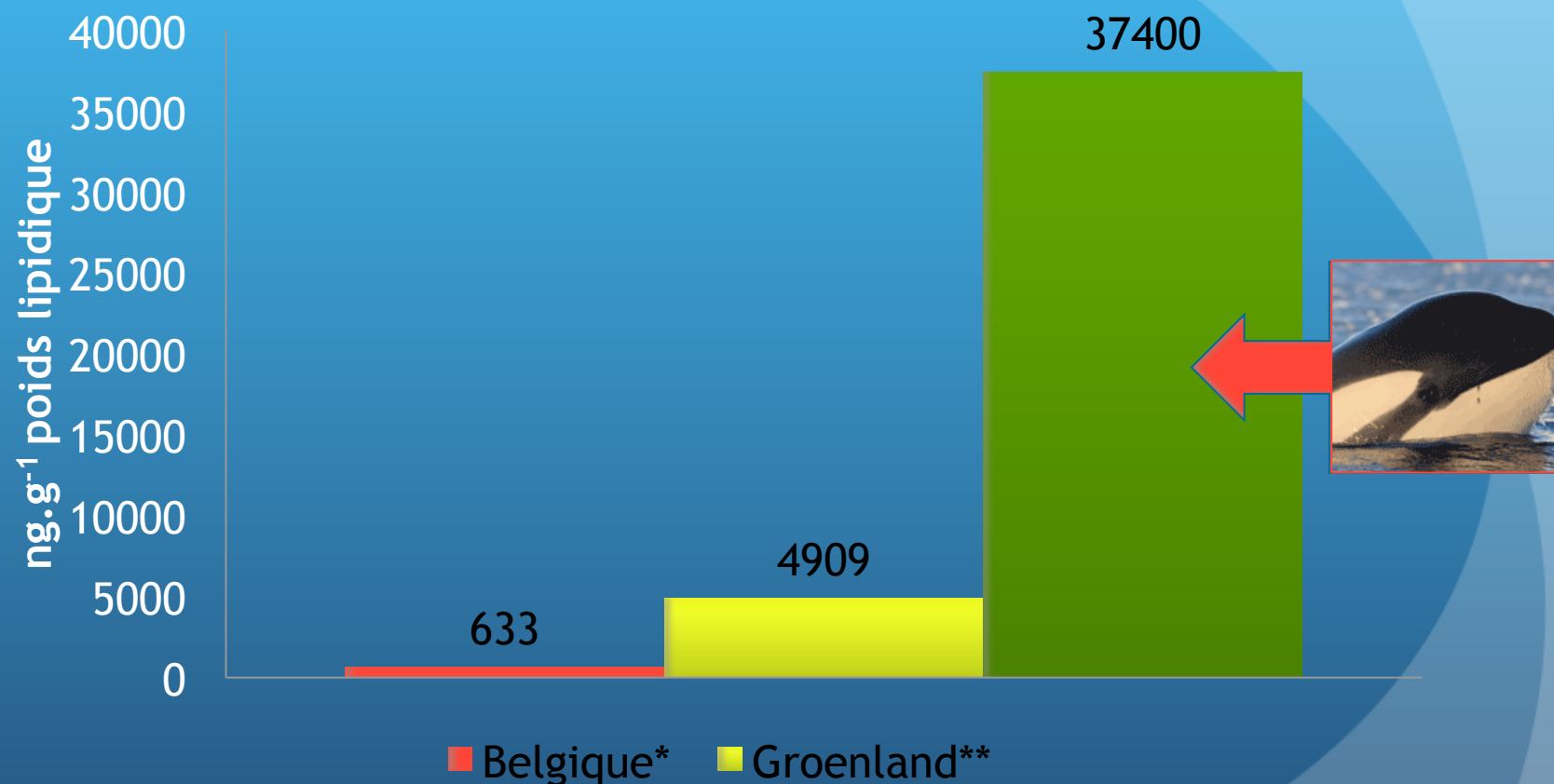
PCBs dans la graisse humaine ng par g de poids lipidique



* De Saeger et al. 2005 - Moyenne pour 57 Belges mâles (Σ PCB 28, 52, 101, 118, 138, 153 et 180)

** De wailly et al., 1999 - Moyenne for 16 Groenlandais - 41-54 ans - (Σ 14 PCBs)

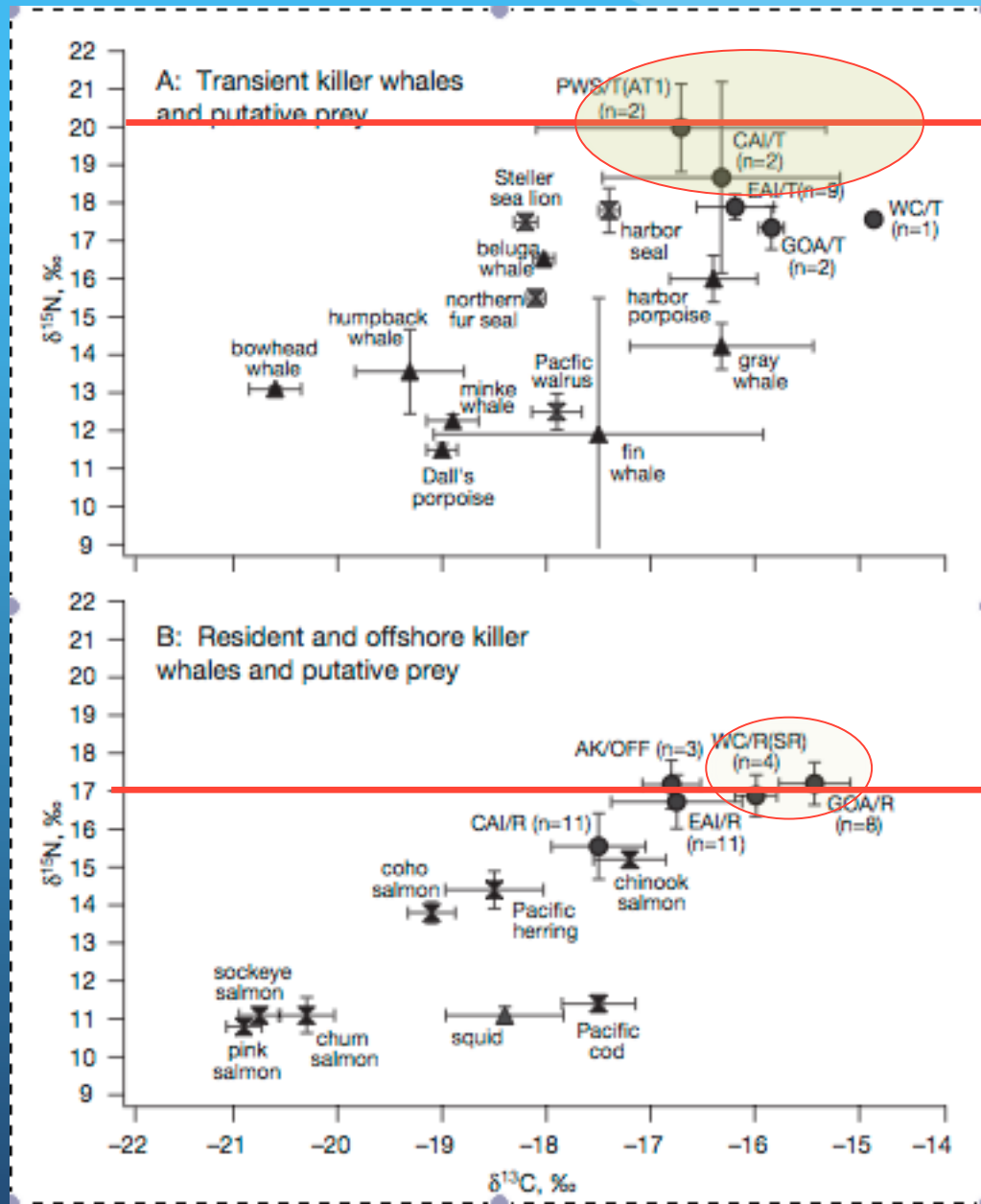
L'épaulard détient un triste record



* De Saeger et al. 2005 - Moyenne pour 57 Belges mâles (Σ PCB 28, 52, 101, 118, 138, 153 et 180)

** De wailly et al., 1999 - Moyenne for 16 Groenlandais - 41-54 ans - (Σ 14 PCBs)

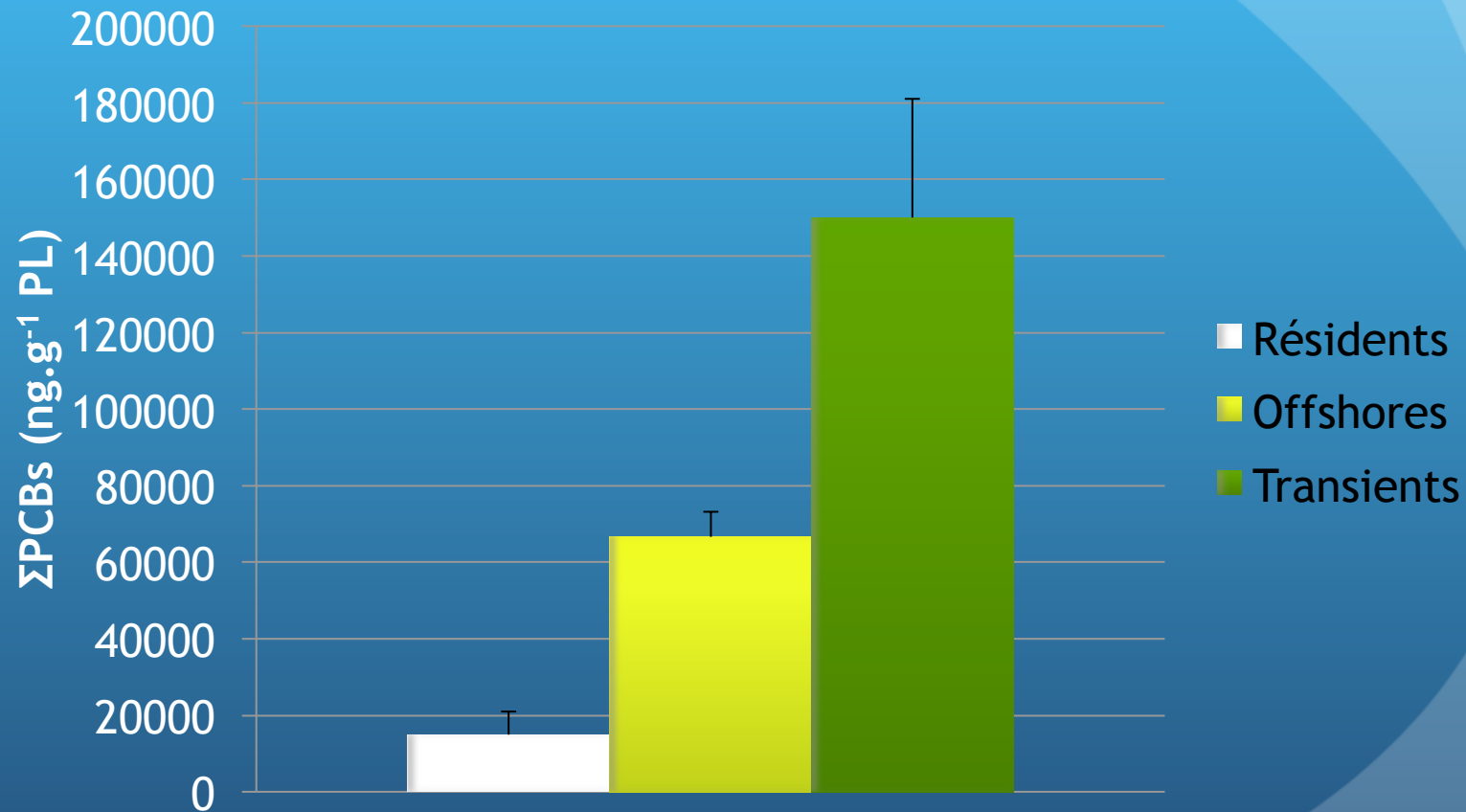
*** Ross et al. 2000 in adult males (Σ 136 PCBs)



Transient

Résidents

Concentrations en PCBs* chez les épaulards de l'Alaska



From Herman, D. P., D. G. Burrows, et al. (2005). "Feeding ecology of eastern North Pacific killer whales *Orcinus orca* from fatty acid, stable isotope, and organochlorine analyses of blubber biopsies." *Marine Ecology Progress Series* 302: 275-291

* Somme de 40 congénères de PCBs

Et en mer du Nord?

Vol. 281: 283–295, 2004

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published November 1

Ecological and pathological factors related to trace metal concentrations in harbour porpoises *Phocoena phocoena* from the North Sea and adjacent areas

Krishna Das^{1,2,*}, Ursula Siebert², Michaël Fontaine¹, Thierry Jauniaux³, Ludo Holsbeek⁴, Jean-Marie Bouqueneau¹

¹Marine Research Center (MARE), Laboratory for Oceanology, B6c, Liège University, 4000 Liège, Belgium

²Forschungs- und Technologiezentrum Westküste, Christian-Albrechts-Universität Kiel, Werftstraße 6, 25761 Büsum, Germany

³Department of Pathology, Faculty of Veterinary Medicine, B43 Liège University, 4000 Liège, Belgium

⁴Laboratory for Ecotoxicology, Free University of Brussels, 1050 Brussels, Belgium

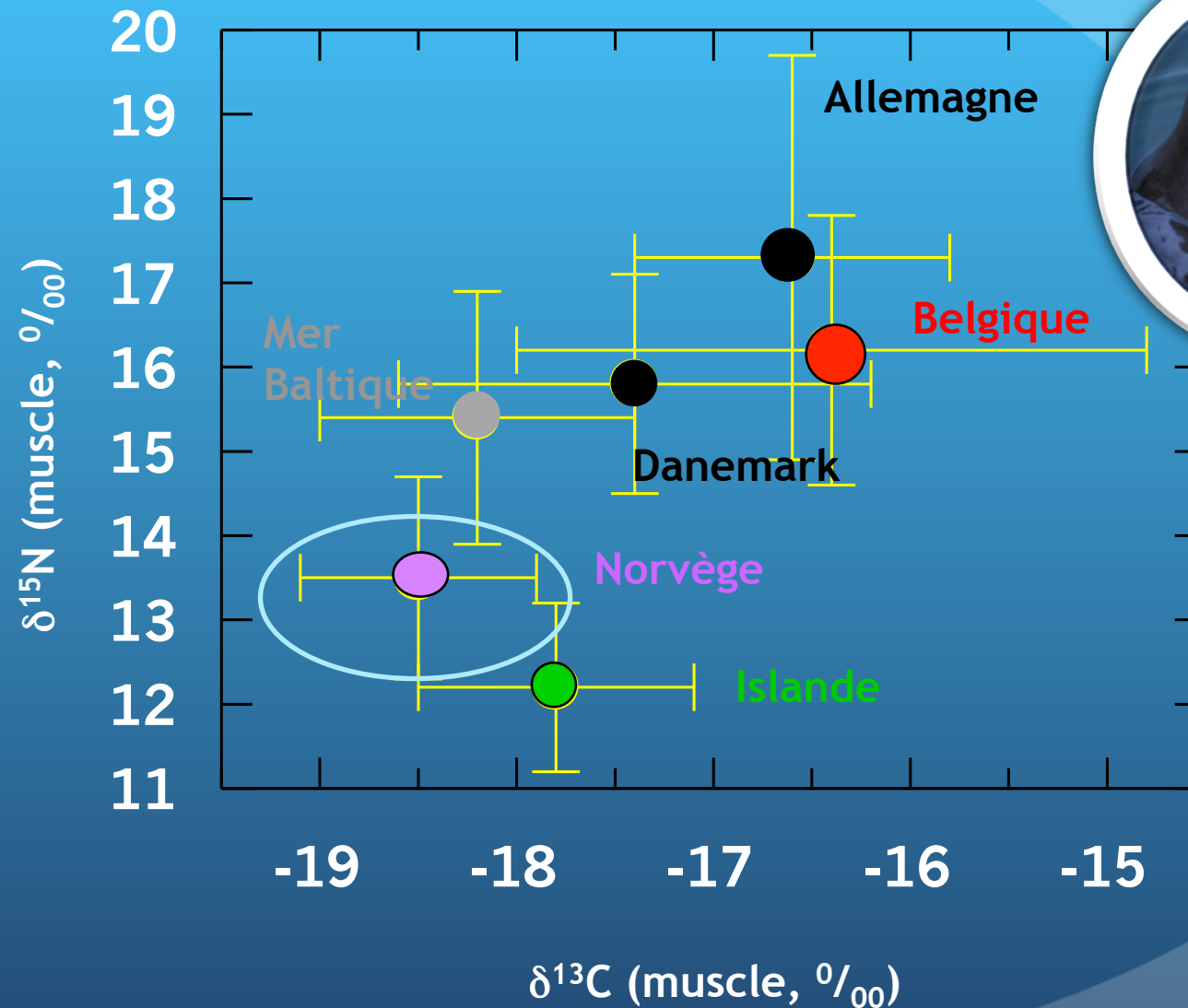
ABSTRACT: There is growing concern about the health status of the harbour porpoise *Phocoena phocoena* in the North Sea and adjacent areas. The interaction between toxicological results (Zn, Cd, Cu, Fe, Se, Hg), stable isotope data ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and the most common pathological findings, namely emaciation and lesions of the respiratory system, were investigated in 132 porpoises collected along the coasts of northern France, Belgium, Germany, Denmark, Iceland and Norway between 1994 and 2001. The body condition of harbour porpoises stranded on the French, Belgian and German coasts was poor compared to that of by-catch individuals from Iceland and Norway, as reflected by blubber thickness and hepatic to total body-mass ratio. High Zn and Hg concentrations were observed in some porpoises collected along the southern North Sea coast compared to by-catch individuals from Iceland, Norway and the Baltic Sea. Increasing Zn levels were observed with deteriorating health condition (emaciation and bronchopneumonia), while Hg increases were not significant. The increases were not related to shrinking liver mass which remained unchanged. These observations indicate a general redistribution of trace metals within the organs (muscles and blubber to liver), as a result of protein and lipid catabolism. Muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values remained unchanged with deteriorating body condition. Cd concentrations were associated only with age and low $\delta^{15}\text{N}$ values, indicating that high Cd concentrations in Iceland and Norway porpoises may be partly diet-related, i.e. a result of Cd contaminated prey.

KEY WORDS: North Sea · Marine mammals · Stable isotopes · Heavy metals · Harbour porpoise · Health status

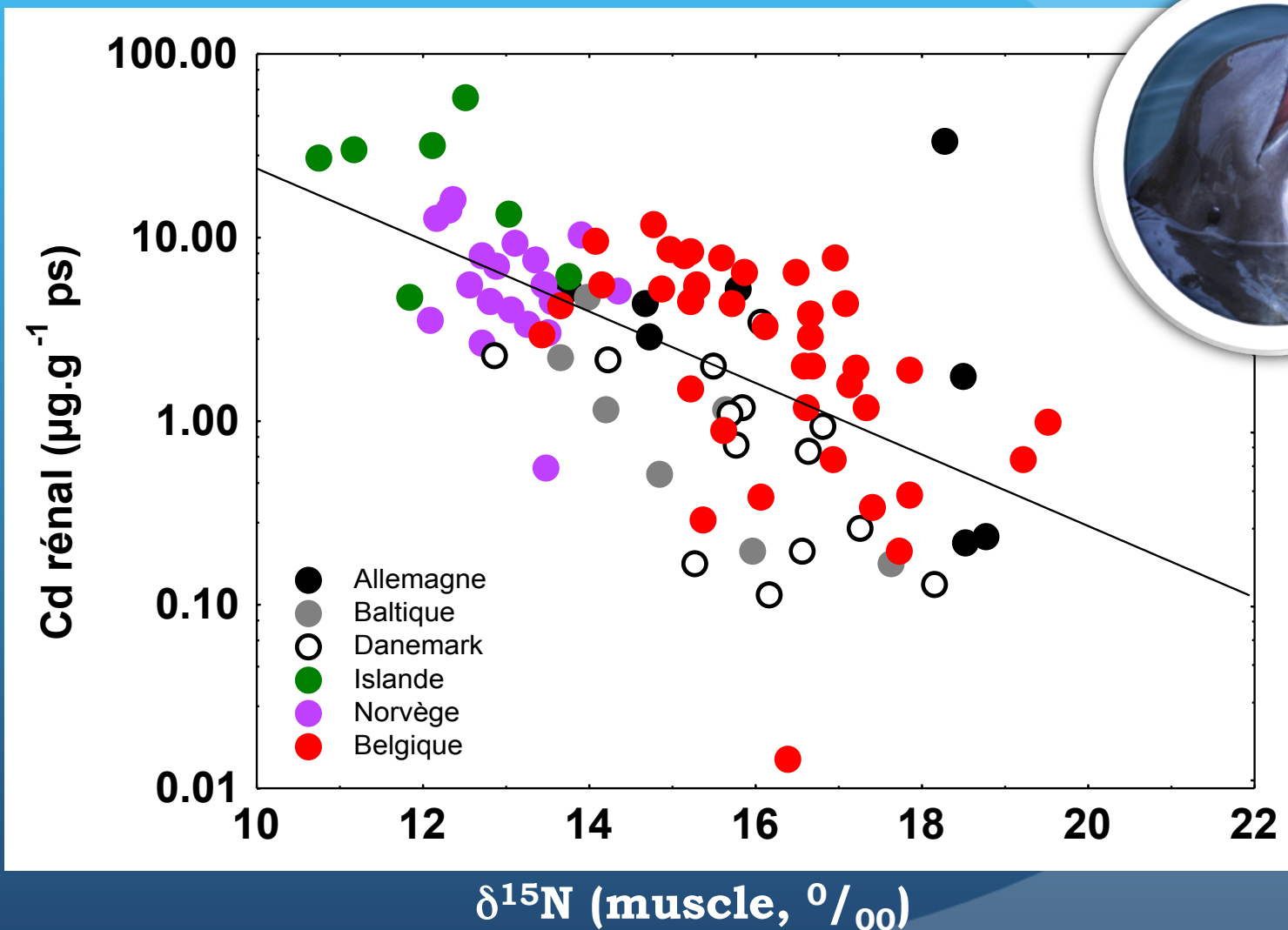
Resale or republication not permitted without written consent of the publisher



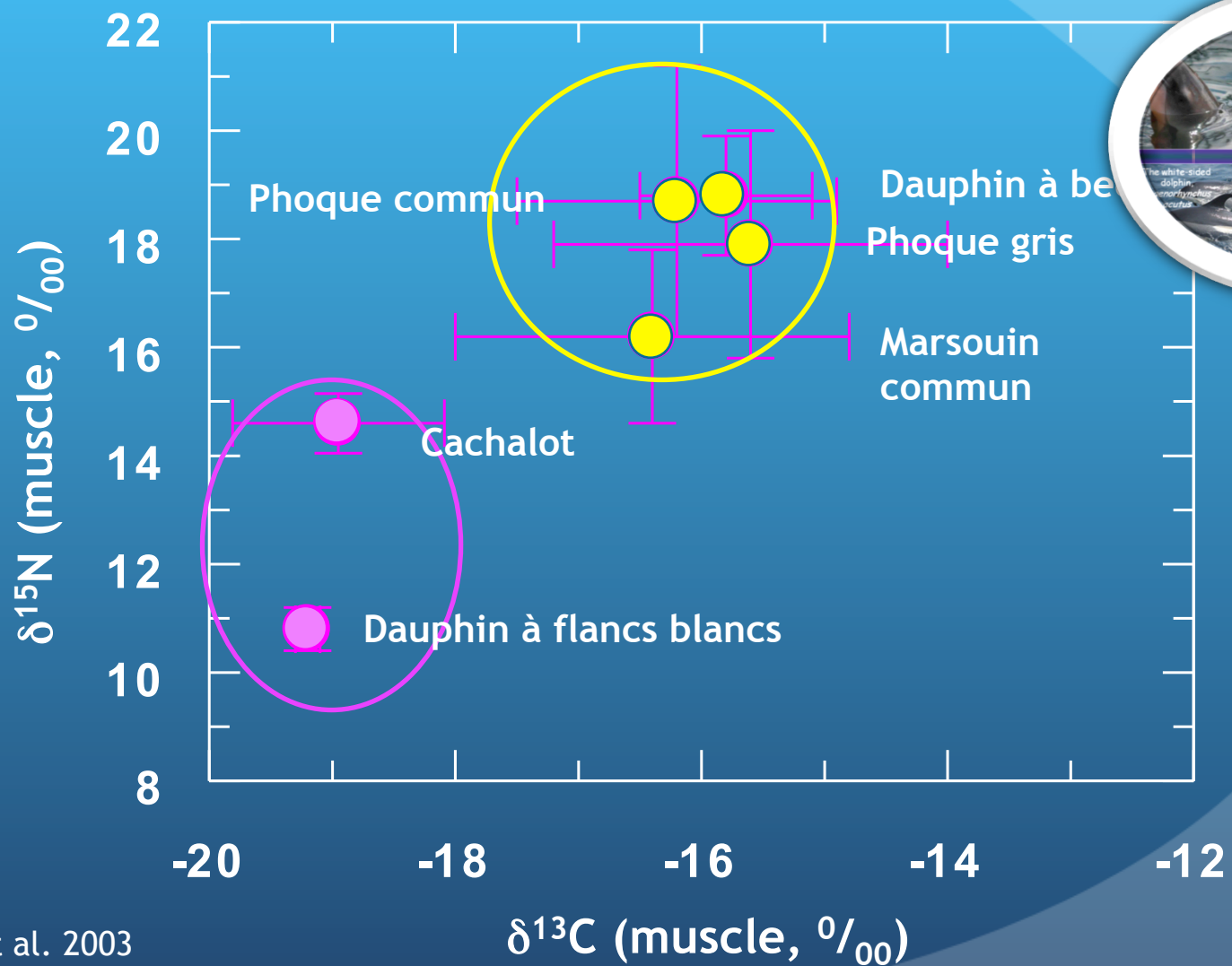
Le marsouin commun en Europe



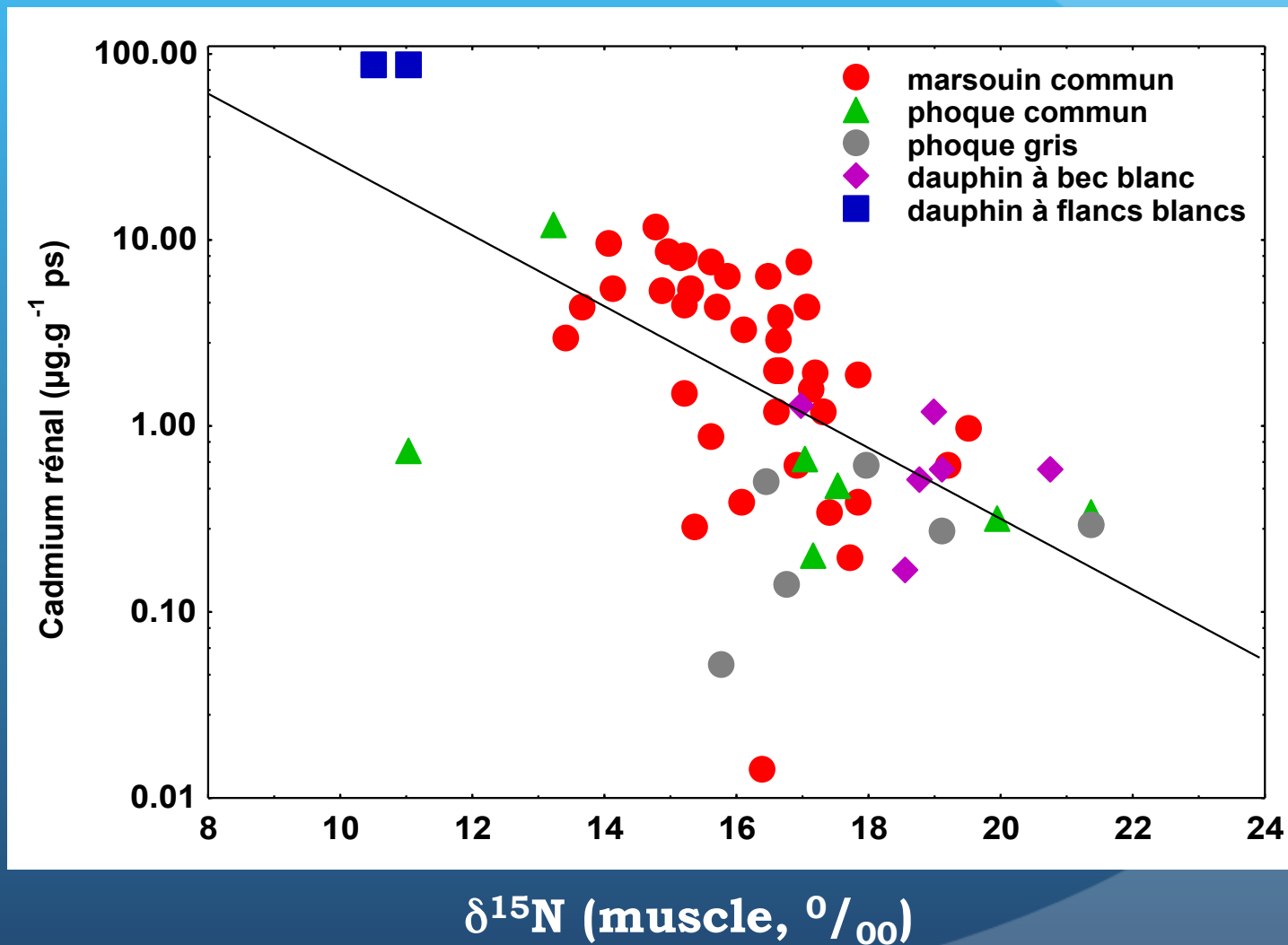
Relation entre les valeurs en $\delta^{15}\text{N}$ et les concentrations en cadmium



Et les autres mammifères marins?

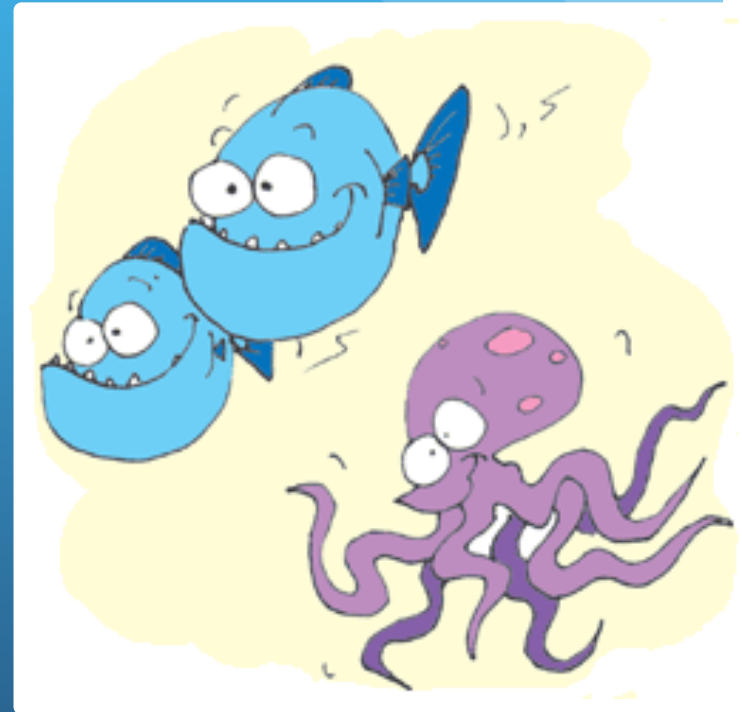


Relation entre les valeurs en $\delta^{15}\text{N}$ et les concentrations en cadmium



Le cadmium, un marqueur océanique?

- ✓ Les céphalopodes accumulent le Cd dans leur glande digestive
- ✓ L'Arctique contient des sédiments naturellement enrichis en Cd - Gradient Nord-Sud?
- ✓ Pas de relation entre les concentrations en Hg et les valeurs $\delta^{15}\text{N}$



Quid des autres polluants?

Environ. Sci. Technol. **2003**, *37*, 5545–5550

Perfluorinated Chemicals Infiltrate Ocean Waters: Link between Exposure Levels and Stable Isotope Ratios in Marine Mammals

KRISTIN INNEKE VAN DE VIJVER,^{*,†}
PHILIPPE TONY HOFF,[†] KRISHNA DAS,[‡]
WALTER VAN DONGEN,[§]
EDDY LOUIS ESMANS,[§]
THIERRY JAUNIAUX,^{||}
JEAN-MARIE BOUQUÉNEAU,[‡]
RONNY BLUST,[†] AND WIM DE COEN[†]

Department of Biology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerpen, Belgium, MARE Center, Laboratory for Oceanology, Liège University, B6, B-4000, Liège, Belgium, Nucleoside Research and Mass Spectrometry Unit, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium, and Department of Pathology, Faculty of Veterinary Medicine, Liège University, B43, B-4000 Liège, Belgium

Introduction

The global marine ecosystem is continuously under pressure due to expanding anthropogenic activities and the development and release of new chemicals. High contaminant burdens in animals from higher trophic levels have led to a need for more information on the occurrence, distribution, and fate of several hazardous compounds. Marine mammals occupy the highest trophic positions in the marine food web and may therefore be more affected by pollutants in comparison to other animals. High concentrations of persistent compounds in marine mammals are frequently reported in the literature (1–4). Polychlorinated biphenyls (PCBs), organochlorine pesticides (such as DDT and its derivatives), and polybrominated diphenyl ethers (PBDEs) are just a few chemicals occurring in tissues of marine mammals.

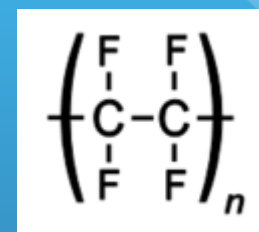
To understand the fate of contaminants and their impact on the marine ecosystem, it is essential to gain knowledge on the trophic relationships within this ecosystem. In the past, trophic position was mainly assessed using food composition analysis. However, as gut content analyses give only indirect information on trophic interactions, stable nitrogen and carbon isotope measurements have recently been used in order to quantify trophic status and dietary overlap of marine organisms (5, 6). Nitrogen isotope ratios

Le teflon®: un composé perfluoré

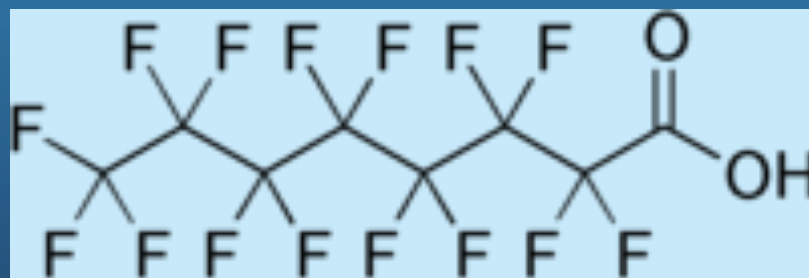
polytétrafluoroéthylène (PTFE).



C'est un polymère:



Fabriqué à partir de l'acide perfluorooctanique (C8)



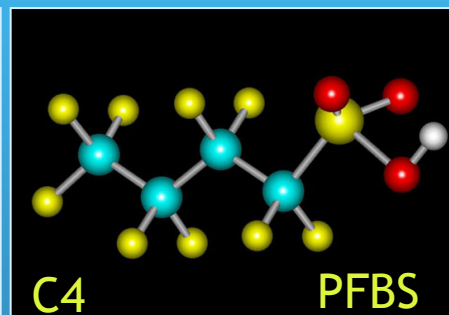
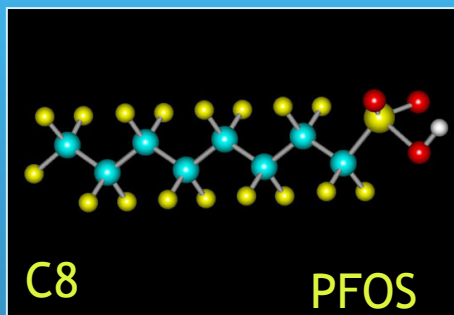
De nombreuses molécules différentes et applications très diversifiées

- Teflon
- Produits “Scotchgard”
- Emballage anti-graisse (pizza, popcorn...)
- Produits cosmétiques
- shampoings et soins dentaires
- Gore-Tex



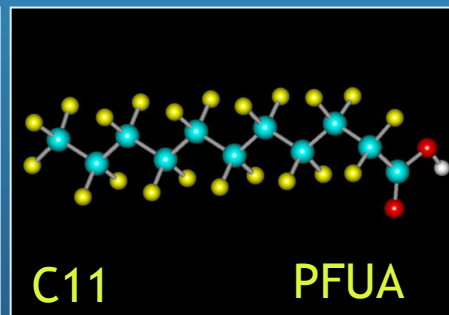
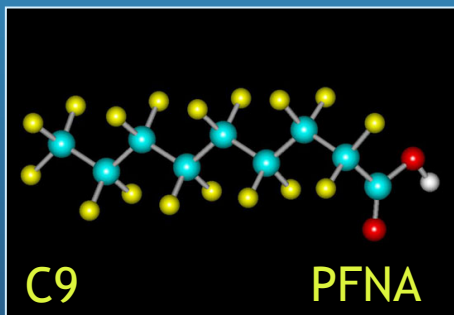
Différentes classes de PFCs

perfluorinated sulfonates



Functional group = SO_3^-

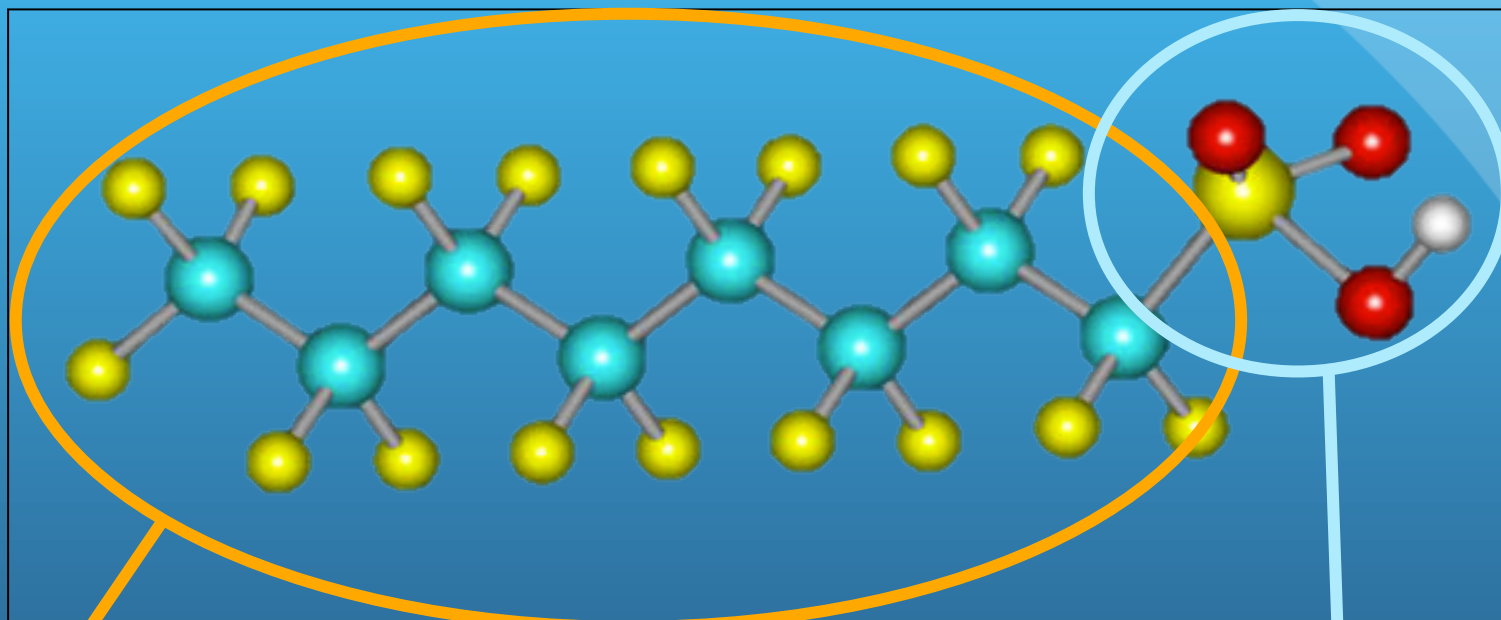
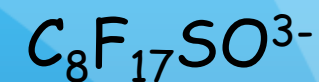
perfluorinated carboxylic acids



Functional group = COOH

Parmi les composés fluorés:

perfluorooctane sulfonate (PFOS)



carbon-fluorine chain = hydrophobe

Group fonctionnel = hydrophile

Propriétés physico-chimiques uniques

D'où viennent ces composés?

Produits depuis plus de 50 ans

Applications tant l'industrie que chez les particuliers

- revêtements de surface des tissus, cuir, tapis
- papier anti gras (burgers - pizzas)
- mousse anti-incendie
- film photographique
- Shampoing
- insecticides
- ...

De nombreuses molécules différentes et applications très diversifiées

- Teflon
- Produits “Scotchgard”
- Emballage anti-graisse (pizza, popcorn...)
- Produits cosmétiques
- shampoings et soins dentaires
- Gore-Tex

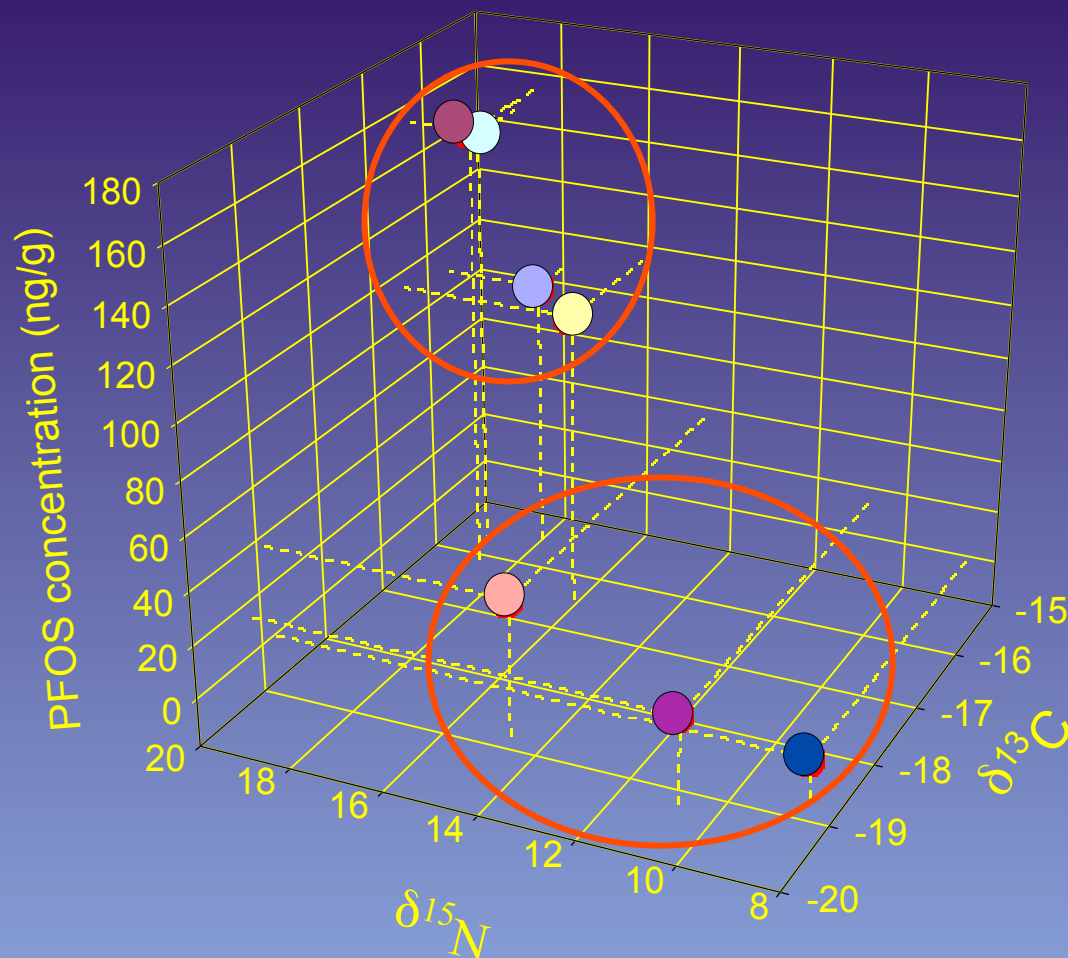




Composés perfluorés

- **Distribution à l'échelle planétaire** jusque dans les zones arctiques et antarctiques
- **Persistants**: résistent aux dégradations biotiques et abiotiques
- **Bioaccumulation**: foie, sang (se lie aux protéines)

PFOS et position trophique



● Phoque commun

● Dauphin à bec blanc

● Marsouin commun

● Phoque gris

= espèces côtières



● Cachalot

● Dauphin à flancs blancs

● Rorqual commun

= Espèces océaniques

Toxiques?

Recherches tjs en cours: mais...

Niveau cellulaire:

- Effets au niveau des membranes
- Effets sur le métabolisme des lipides

Niveau de l'organisme:

- Lésions hépatiques chez les poissons
- Cancer chez les rongeurs

Niveau de la population:

- Baisse de reproduction
- ??????????



Pas de relation claire
entre les isotopes stables
et certains polluants plus
typiques comme Hg, PCBs
or PBDEs

Author's personal copy

Environment International 35 (2009) 893–899

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Biomagnification of naturally-produced methoxylated polybrominated diphenyl ethers (MeO-PBDEs) in harbour seals and harbour porpoises from the Southern North Sea

Liesbeth Wejjs^{a,b}, Sara Losada^{b,c}, Krishna Das^d, Laurence Roosens^b, Peter J.H. Reijnders^e, Javier F. Santos^c, Hugo Neels^b, Ronny Blust^a, Adrian Covaci^{a,b,*}

^a Laboratory for Ecophysiology, Biochemistry and Toxicology, Department of Biology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium

^b Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

^c Analytical Chemistry Department, University of Barcelona, Martí i Franquès 1–11, 08028 Barcelona, Spain

^d Laboratory for Oceanology—MARE Center, University of Liège B6C, 4000 Liège, Belgium

^e IMARES—Institute for Marine Resources and Ecosystem Studies, Department of Ecology, PO Box 167, 1790 AD Den Burg, The Netherlands

ARTICLE INFO

Article history:

Received 20 January 2009

Accepted 21 March 2009

Available online 17 April 2009

Keywords:

Marine mammals

Fish

Biomagnification

MeO-PBDEs

PBDEs

North Sea

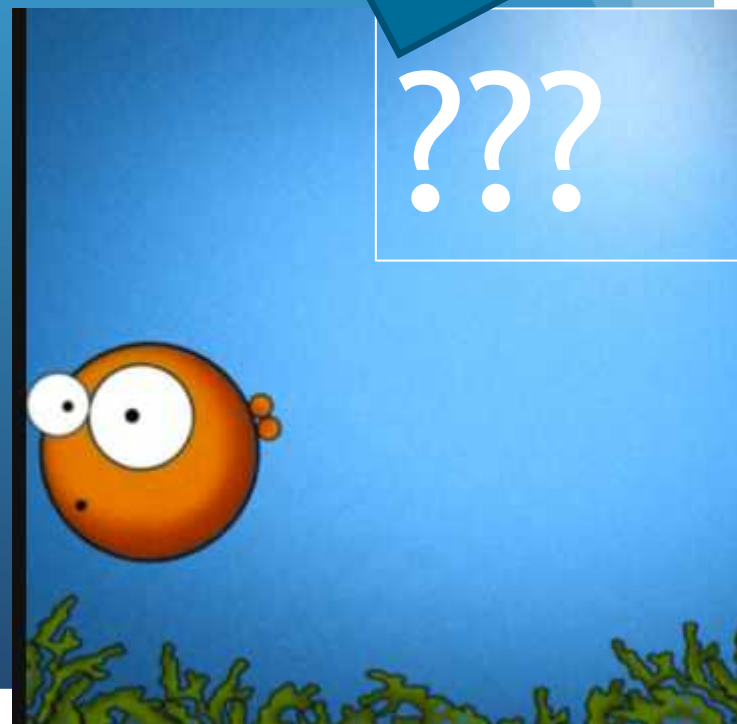
ABSTRACT

Harbour seals and harbour porpoises are top predator species from the North Sea, have long life spans and hence, are known to accumulate high levels of anthropogenic contaminants. To gain knowledge about the behaviour of naturally-produced compounds in these marine mammals, the biomagnification of naturally-produced methoxylated polybrominated diphenyl ethers (MeO-PBDEs) was assessed. The biomagnification of MeO-PBDEs (2'-MeO-BDE 68 and 6-MeO-BDE 47) was lower in harbour seals (all biomagnification factors (BMFs) <1) compared to the same age-gender groups of the harbour porpoises (all BMFs >1). This may indicate a better metabolic breakdown of MeO-PBDEs in harbour seals, as was previously suggested for polybrominated diphenyl ethers (PBDEs). In both predators, 6-MeO-BDE 47 had the highest concentrations (range: 45–483 ng/g lw and 2–38 ng/g lw for harbour porpoises and seals, respectively) compared to 2'-MeO-BDE 68 (range: 2–28 ng/g lw and 1–6 ng/g lw for harbour porpoises and seals, respectively). In general, the highest concentrations were found in juveniles, suggesting an increased biotransformation capacity with age or the influence of dilution by growth for both species. Here we show that naturally-produced brominated organic compounds can biomagnify and accumulate in North Sea top predators, although to a lesser extent than anthropogenic lipophilic contaminants, such as polychlorinated biphenyls (PCBs) or PBDEs.

© 2009 Elsevier Ltd. All rights reserved.

Autres facteurs :

- Teneurs en lipides
- Age
- Sexe

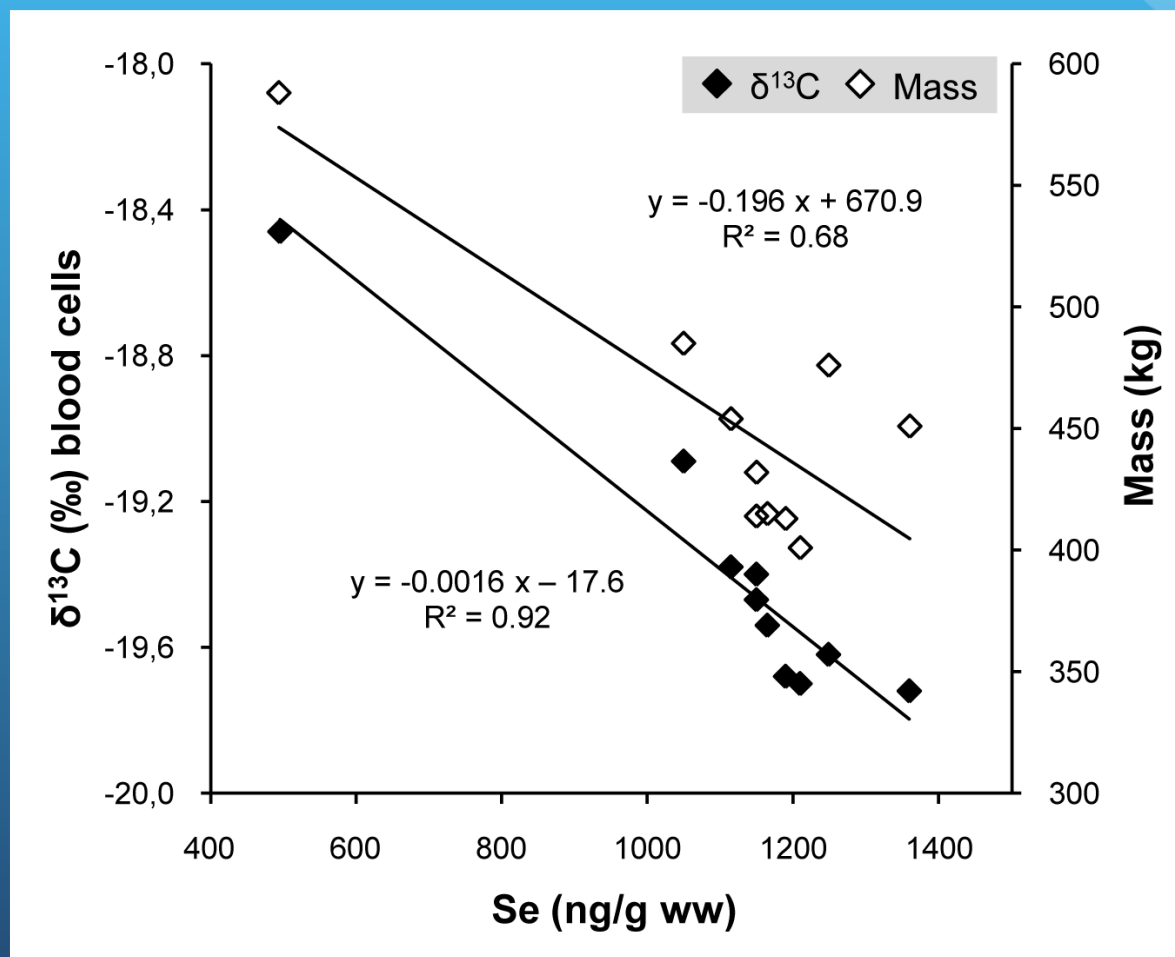


Et chez l'éléphant de mer septentrional?



Relations entre:

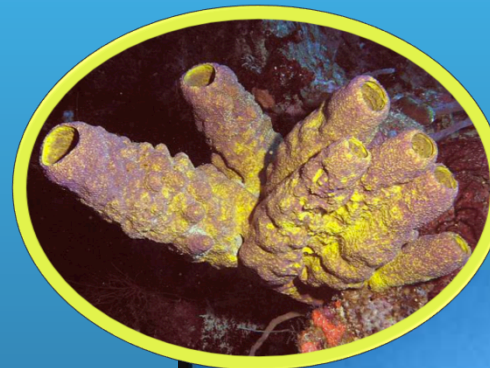
1. Se dans le sang maternel et les valeurs en $\delta^{13}\text{C}$ values dans les cellules sanguines
2. Se dans le sang maternel et la masse corporelle



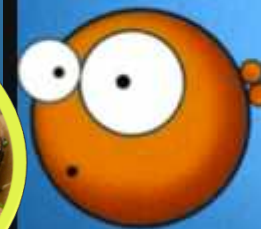
Transfert trophique du Sélénium chez les éléphants de mer?

Chez d'autres espèces de vertébrés marins, le Se était lié à l'ingestion de:

- Demospongiae
- Mollusques bivalves



???



Conclusions

Over the last decade, SIA has been used in ecotoxicology.

“Because contaminant concentrations in biota are affected by the complex interplay between several environmental processes, researchers have begun to use isotopic ratios in conjunction with physical, chemical or biological factors to understand contaminant concentrations in marine vertebrates.” Jardine, 2006.

BUT...

A faire!

Etudes expérimentales

- Régime contrôlé et turn over des tissus

Standardisation des valeurs en $\delta^{15}\text{N}$

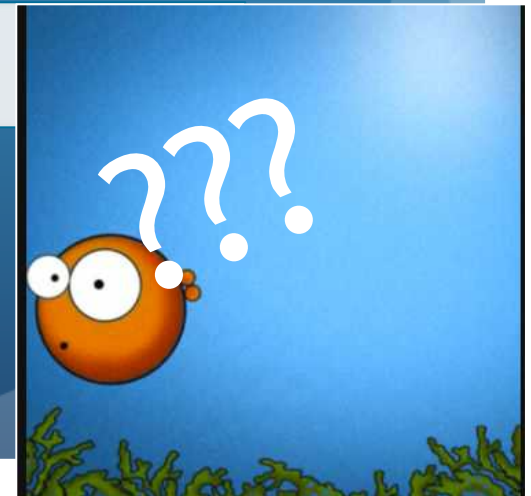
- Utilisation de mollusques pour les niveaux de base

Autres facteurs biologiques

- Physiology, animal movement pattern

CSIA: Compound-Specific Carbon Isotopic Analysis

- Sources des polluants chez les vertébrés marins





Funding agencies

F.R.S. - FNRS

Université de Liège

Politique Scientifique Fédérale



La Politique scientifique fédérale et la Présidence belge



Merci à tous



Gilles Lepoint et Loïc Michel: logistique du spectromètre de masse



Nos nombreux collaborateurs:

Ursula Siebert, Cathy Debier, Thierry Jauniaux, Patrick Dauby, Sylvie Gobert et Paulo Dorneles

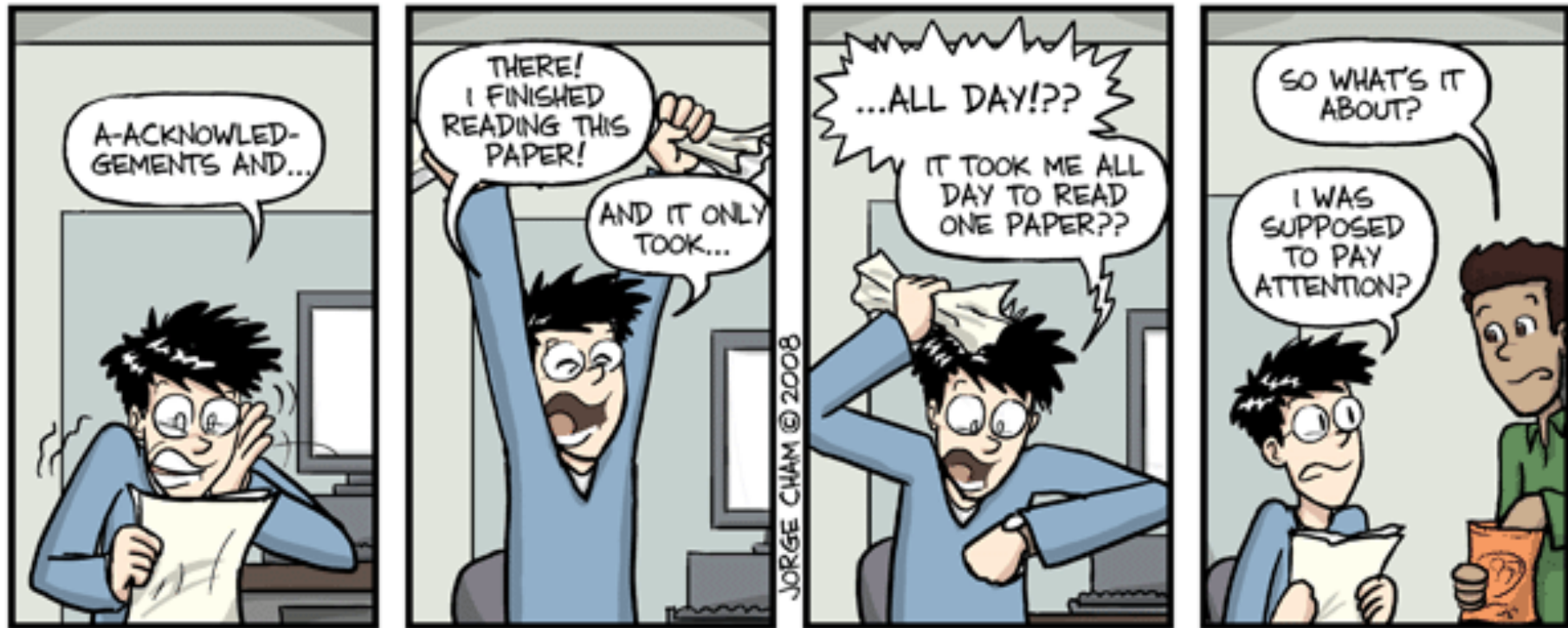


Nos mémorants, doctorants et chercheurs post-doctoraux:
Sarah Habran, Michaël Fontaine, Stéphane Caut, Elodie Guirlet, Stéphanie Gillet, Joseph Schnitzler

Collaborations *Urbi et Orbi*

- **Université of Liege**
 - CART (Prs. J-P Thomé et E. de Pauw)
- **Belgium**
 - Université de Louvain-La-Neuve (Prs. C. Debier et J-F Rees)
 - Université d'Anvers (Pr. R. Blust, Dr. A. Covaci, L. Weijs)
- **Europe**
 - Christian-Albrechts- University of Kiel FTZ, Allemagne (Pr. U. Siebert and S. Gaarte)
 - IMARES, Institute for Marine Resources & Ecosystem Studies, Pays-Bas (Pr. P. Reijnders)
 - ...
- **World**
 - Universidade do Rio de Janeiro, Brésil (Pr. P. Dorneles and co)
 - Universidad Nacional de Mar del Plata, Argentine (Dr. D. Rodriguez)
 - University of South Florida, USA (Pr. D. Mann)

Merci de votre attention!



WWW.PHDCOMICS.COM

Et pour en savoir plus...



Plus d'info sur

<http://orbi.ulg.ac.be/>

et

<http://www2.ulg.ac.be/oceanbio/>



You are what you eat plus a few *per mill*: utilisation des isotopes stables en Ecologie Marine



Credit photo: Oceanus

Traçage des polluants chez les mammifères et autres vertébrés marins

Krishna Das et Gilles Lepoint
Chercheurs Qualifiés F.R.S. - FNRS
Laboratoire d'Océanologie- MARE Center
Université de Liège

