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**The ontogeny of foraging in Weddell seal pups
and dietary behaviour in lactating females.**

Ph.D. Thesis

Roberto Palozzi

Supervisor: Prof. Valerio Sbordoni

Co-supervisors: Prof. Michele Scardi - Dott. Marino Vacchi

Coordinator: Prof. Patrizia B. Albertano

This work is dedicated to my Father, Domenico and my Mother, Liana.

Without their unshakable faith in me I would not have overcome all the difficulties I met in the research project.



**I.A.M.C. Istituto per l'Ambiente Marino Costiero
C.N.R. Napoli**

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ABSTRACT

Mammal females that fast throughout lactation are called “capital breeders” while, on the contrary, lactating females that continue to forage from the parturition to the pups’ weaning are indicated as “income breeders”.

The Weddell seal (*Leptonychotes weddellii*), the southernmost Antarctic pinniped, has been considered an extreme capital breeder species for long time, but the most recent studies on its diving behaviour and feeding habits raised many doubts with regard to the validity of this strict categorization.

Also, it was unclear if Weddell seal lactating pups begin to forage during lactation and what is their adaptive strategy to complete the transition from maternal milk to independent foraging.

Even if Weddell seal diving behaviour has been studied for a long time in Antarctica, above all in the McMurdo Sound, one aspect received less attention than others: the diving skill development in pups during lactation (from birth until about 6 weeks of life) along with the associated behaviour of lactating females and the ontogeny of their diet.

This work tried to shed light on this crucial phase for the newborn survival and on the maternal strategies during lactation and it offers a complementary sight of the mum diving behaviour with that of their own pups.

Biochemical data of lactating females, their dependent pups, milk and prey items from stable isotopes analysis were compared with data from Time Depth Recorders deployed on 16 mums and 8 pups, between the second and the third day after parturition; moreover diving data were analyzed applying a traditional approach, in which the dive profile shapes are previously fixed by the software in number and pattern (MT-Dive, Jensen Software Systems) in comparison with an unsupervised artificial neural network, the Self-Organizing Map (SOM).

Results showed that the associated diving behaviour during lactation reflects a high intra-specific variability (time in water, number of dives, max depth, duration, profile shapes); but while the traditional approach seems to suggest a clear foraging activity both in mums and pups (U-shape dives - traditionally linked with foraging activity - were predominant along the lactation and early

weaning), SOMs produced an opposite result. Larger SOMs, which can be regarded as non linear ordinations, provided a much more faded transition from V-shape to U-shape, while smaller SOMs, which act as non hierarchical classifiers, practically did not find any U-shaped dive cluster.

Analyses of the time spent in water indicated a very close association between mums and their own pups but also a wide range of the underwater space use by the mums when they leave the pups alone; results showed trophic resource exploitation strategies varying appreciably from an individual to another letting understand that the maternal strategy of the lactating females is not unique and range from capital to income breeding.

Stable isotopes analysis suggested that pups do not forage independently in a relevant and detectable manner during lactation but this methodology was not able to indicate the exact moment in which the transition to the independent foraging occurs as well as to clearly detect the passages from a nutritional status to another in the mums.

Data at a higher resolution could arrive from the isotopic analysis of the plasma aqueous fraction that this study explored for the first time.

1. INTRODUCTION



1.1 - Introduction

The Weddell Seal *Leptonychotes weddellii* is the most extensively studied Antarctic pinniped among the four species of phocid seals (Weddell seal, Leopard seal, Ross seal and Crabeater seal) living in Antarctica (Figure 1).

And, absolutely, it represents an exception to the context of generalized incompleteness of knowledge about the natural history of marine mammals (Castellini *et al.*, 1991).

Since early XXth century, scientists described in detail Weddell seal anatomy, physiology and behaviour, focusing on the feeding habits and the reproductive ecology (Barrett-Hamilton, 1901; Wilson, 1907; Tims, 1910; Brown - Bruce a and b -Haig - Hepburn - Thomson, 1915; Lindsey, 1937; Bertram, 1940) and, since 1960s, its diving behaviour began to be detected by innovative bi-logging devices: Maximum Depth Recorder - MDR - and Time Depth Recorder -TDR (De Vries and Wohlschlag, 1964; Kooyman, 1965 and 1966).



Fig. 1



Fig. 2: The ice-shelf on Ross sea. Mount Erebus, Ross Island.



Fig. 3: Weddell seal teeth undergo a heavy wear throughout the adult life (as shown by the image of the teeth condition of this very old - 21- lactating female) and it represents a considerable cause of mortality.

It is not by chance that the first TDR was designed and built in 1963 to be deployed on some individuals of freely diving Weddell seal (the first applications of a modern bio-logging device to a wild marine mammal) with the aim of obtaining environmental and behavioural information of their underwater activity as support for better understanding data produced in some physiological experiments (Kooyman, 1965).

The reason of this “anomalous” ease in working with the Weddell seals in comparison with other marine mammals (and specifically the other true seals) is on their principal habitat: a peculiar environment of the Antarctic sea ice.

The ecology of all phocid seals living around the Antarctic continent is tightly depending on the dynamics and extension of the sea ice (pack-ice), but the Weddell seal is the only one able to inhabit the fast ice area surrounding the continental shoreline and the Antarctic islands (Wilson, 1907; Lindsey, 1937; Stirling, 1969a and b; Kaufman, 1975; Siniff *et al.*, 1977; Castellini *et al.*, 1991; Davis *et al.*, 1999; Cameron and Siniff, 2004), often scores of kilometres away from the open sea (Figure 2).

While Leopard seal, Ross seal and Crabeater seal live at the edge of the ice floe (the seasonal pack-ice) with limited possibilities of navigating and penetrating under the sea ice (they need to come back in open sea at the end of the apnea), the Weddell seal is able to move under the floe for kilometres (Hindell *et al.*, 2002) using natural cracks in the ice cover formed by tidal action, glacier movements and wind. The sensorial system it uses to navigate under the ice to come back to the water entry hole after a dive, or to move from a hole to another is not yet known (Castellini, 1991); but this extraordinary ability – that no any other Antarctic pinniped possesses – allows Weddell seal to inhabit and colonize even the multi-year fast-ice, the glacier ice and the permanent ice shelf environments, taking advantage of the perennial cracks (Castellini, 1991; Cameron and Siniff, 2004) where the seals maintain the breathing holes open using their incisors and canine teeth (Figure 3).

Land-fast-ice, usually called fast-ice, is a particular expression of the sea ice that differs from the pack-ice (the most common frozen seawater) both in its development and form (Baroni, 2001; Manzoni, 2001; Thomas, 2004).

Basically, the main and fundamental difference is in the fact that pack-ice is free



Fig. 4: The “mummified” carcass of a very young pup (see the umbilical cord still attached) evidences the stability of the land fast-ice environment.



Fig. 5



Fig. 6

floating and directly affected in its kinematics by the action of winds and water currents, while fast-ice is a static structure firmly anchored to the shoreline.

This “coastal floe” differs from the pack-ice also because can come through the summer season without completely melting and giving life to multi-year fast-ice that represents a very stable environment (Figure 4) on which, not only Weddell seals breed, but also humans may travel by heavy means of transporting

and even create landing strips for huge aircrafts (Figure 5).

Consequently, scientists can easily and safely reach the animals in the breeding colonies (above all those on multi-year fast-ice and permanent ice shelf) and since the Weddell seal normally does not show fear of human beings and high aggressiveness toward them, it is easy to catch for any kind of scientific sampling and measurement (Stirling, 1969b; Kaufman *et al.*, 1975; Castellini *et al.*, 1991) (Figure 6).

One of the most significant multi-year fast-ice concentration of all Antarctica is located in the Ross sea region where an estimated population of about 50.000 adult Weddell seals is thought to be widely spread (Stirling, 1969a; Burns and Kooyman, 2001). The most numerous colonies in this area are those along the Ross Island south-western coastline (McMurdo Sound) in which adult females gather along the tidal cracks to bring forth their young in October/November, forming aggregations of mum-pup pairs that range from few to more than 200 individuals (Wilson, 1907; Stirling, 1969b; Siniff *et al.* 1977; Cameron and Siniff, 2004; Palozzi's personal observation, 2007).

Adult males are not welcome in the colonies during lactation: until weaning females react aggressively to their attempt of approaching them and the numerical presence of adult males on the ice is marginal. During this period males are engaged in conquering or defending underwater territories and vicious fights occur in the water (Stirling, 1969b), even if sub-aerial clashes have been observed as well (personal observation, 2007) (Figure 7a-b).

The main bases of the US and New Zealand Antarctic Program (McMurdo Station and Scott Base) are sited in the southern part of Ross Island (Hut Point Peninsula) and they provide a complete logistical support to the scientists working on the Weddell seal colonies of the island.

This is why the great majority of the research projects on the Weddell seal were carried out along the western coast of the Hut Point Peninsula and over the Erebus Glacier Tongue (Erebus Bay), although these seal aggregations are, probably, the southernmost breeding colonies in the world of a mammal and are not ecologically representative of the typical circumpolar distribution of the species *Leptonychotes weddellii*, normally occurring in the areas of annual sea ice all around the continent.

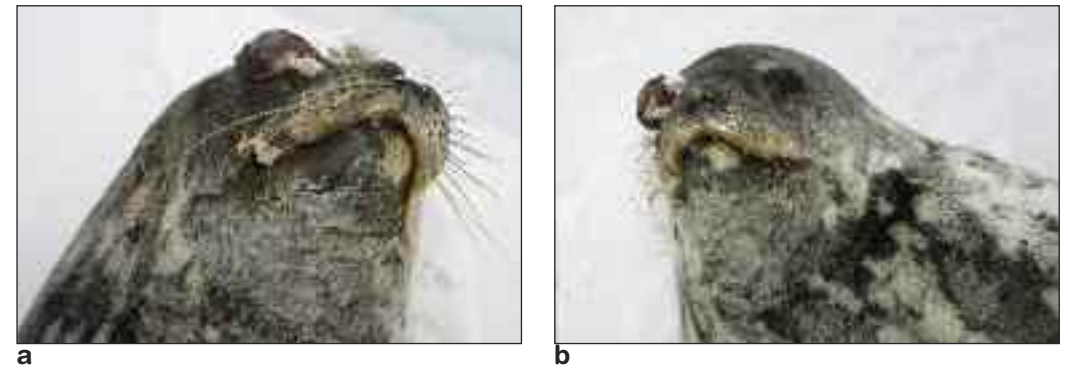


Fig. 7: In spite of its disablement, this bull with the right gouged out eye was one of the dominant males at Hutton Cliffs during the breeding season 2007-08; it defended its territories by attacking every other males fiercely, both underwater and on the ice.



Fig. 8: An almost completely moulted pup still suckling (late lactation).

During the very long term research program in the Erebus Bay (the longest, on-going program on a marine mammal of all times), started in the 1960s (Cameron and Siniff, 2004), many aspects of the Weddell seal biology and ecology were deeply investigated; between them, the works on diving behav-

our and diet composition (dietary behaviour) are amongst the most thorough studies carried out on marine mammals by far.

But there is a specific and well delimited period on the reproductive ecology of the Weddell seal that received very little attention in the perspective of the above-quoted two points of view: lactation and early weaning (Figure 8).

Notably, the associate diving behaviour of mums and their own pups during the first weeks after parturition were almost completely unknown and undetected.

This situation generated the substantial impossibility to give an ultimate answer to the questions on the mum feeding strategies and the ontogeny of foraging in the pups during this crucial period: the most important for the newborn survival and, after all, for the continuation of the species.

Even if “mum fasting” is not a very common maternal care strategy in the mammalian group (Boness and Bowen, 1996), in the prevailing literature, the lactating females of the family *Phocidae* are generally indicated as “capital breeders”, that means they should rely only on their metabolic resources (in form of blubber acquired and stored until just before parturition to sustain both milk production and energy needs) all along the pre-weaning period (Jönsson, 1997); and their pups should be fed exclusively on maternal milk by a fast transfer of high fat milk from mother to pup until the weaning, which in phocid seal, occurs in a drastic and abrupt manner.

On the contrary “eared seals” (Family *Otariidae*) are “income breeders” with longer lactation (from some months up to three years) during which both lactating females and breeding pups forage in the sea (Oftedal, 1993; Boness and Bowen, 1996; Atkinson, 1997; Sato *et al.*, 2003).

Nevertheless the maternal feeding strategies of phocid seals can not precisely be determined and categorized (at the intra-specific - if not intra-population - level as well) since the capital strategy (fasting) is not absolute in all species, with mothers and un-weaned pups that are known, or suspected, to feed to some extent (limited food intake fed more or less opportunistically) at least in the last part of lactation (Bonner, 1984; Gentry and Kooyman, 1986; Oftedal *et al.*, 1987; Bowen, 1991; Costa, 1991; Oftedal, 1993; Bowen *et al.*, 1992; Boness and Bowen, 1996; Atkinson, 1997; Lydersen and Kovacs, 1999;

Krafft *et al.*, 2000; Bowen *et al.* 2001; Eisert *et al.* 2005; Sato *et al.*, 2003; Wheatley *et al.*, 2008b).

Specifically, the scientific debate on the maternal strategy adopted by the Weddell seal lactating females passed through an evolution that moved from an older starting point considering them “extreme capital breeders” (Oftedal *et al.* 1987; Tedman and Green, 1987), to a more recent position for which Weddell seal shows a “swinging” between a pure capital breeding strategy to a more complex one that presents some characteristics of the income breeders (Testa *et al.*, 1989; Hindell *et al.*, 1999 and 2002).

Reasons supporting the first hypothesis (fasting during lactation) are based essentially on the indisputable evidence that every lactating female undergoes a considerable loss of weight during lactation (up to more than 40% of the initial body mass) (Figure 9a-b) and on few papers reporting absence of food in the stomachs of post-partum females and of prey remains in scats (Mansfield, 1958; Tedman and Bryden, 1979; Thomas and De Master, 1983; Reijnders *et al.*, 1990).

On the contrary diving data and biochemical analyses (biomarkers, fatty acids), as well as sporadic direct observations of seals resurfacing with a prey, showed a feeding activity by mothers in late lactation (Testa *et al.*, 1989; Hindell *et al.*, 1999 and 2002; Sato *et al.*, 2002; Eisert *et al.*, 2005; Wheatley *et al.*, 2008a and 2008b).

But, even if the occurrence of these feeding events was demonstrated (mostly indirectly), their extent, their adaptive/ecological value (are they just occasional predations or expression of a specific maternal strategy?) and their possible categorization/generalization are still unknown and very little detected.

Besides, with regards to the pups, there is only one scientific report concerning the presence of prey items (crustaceans) together with milk in the stomach of a single un-weaned pup (Lindsey, 1937), while Sato indicated there is not evidence of feeding activity during the synchronous shallow dives by mother-pup pairs in late lactation (Sato *et al.*, 2003).

Therefore the aim of this work is to try to shed light on the diving skill development in pups during lactation and early weaning (from birth until about



a



b

Fig. 9: A Weddell seal mum-pup pair at two days after parturition (a - early lactation) and at about a month (b - late lactation): the loss of maternal body mass is easily detectable (b - the pelvic girdle became evident) as well as the pup growth.

10 weeks of life) along with the associate behaviour of lactating females.

And, in particular, it wants to offer a complementary and deep sight of mother diving behaviour along with that of her own pup (mum/pup pair diving behaviour) during lactation from birth until 35 days post partum.

Three different analytical approaches (a “traditional” dive data analysis; a more innovative dive data analysis; the stable isotopes analysis of both prey and predator tissues) were applied to study the Weddell seal at the Hutton Cliffs colony (Ross Islands, Erebus Bay) during lactation and early weaning; and the produced results were interpreted in a inter-disciplinary and comparative perspective to provide new and thorough information on the ontogeny of foraging in pups and the dietary behaviour (maternal strategy) in lactating females.

2. MATERIALS AND METHODS



2.1 - Study site and field work



Fig. 1: The Weddell seal colony and study site (Hutton Cliffs) in the Ross Island.



Fig. 2

Hutton Cliffs (Ross Sea, McMurdo Sound, 77° 44' South - 160° 30' East) are rocky sheer spurs on the multi-year-fast-ice, few kilometres far from the US "McMurdo Station", that are easily reachable by snow-mobiles and tracked vehicles (Figure 2).

Along the main crack in the ice, that runs parallel to the shoreline beneath the cliffs, Weddell seal adult females (several dozens) gather every year in October to start the breeding season; at the peak of presences, with newborns and males, the colony can comprise up to about 200 individuals (Figure 3).

This study was carried out at Hutton Cliffs during the Antarctic summer seasons 2006/07 and 2007/08 as a part of a larger research project on Weddell seal on behalf of the Smithsonian National Zoological Park (Smithsonian Institution, Washington D.C.), funded by National Science Foundation (Project number B-024-M).

During the two field seasons a remote camp was set up from mid October till mid December close to the pressure ridge perpendicular to the shoreline, about half kilometre far from the main crack. It was also very close to Turtle Rock, a tiny island that traditionally hosts another smaller colony.

The remote camp included a laboratory-hut to process biological samples and store them frozen (Figure 4).

The bathymetry in the McMurdo Sound (a southern extension of the Ross Sea with a maximum depth of about 700 - 800 metres) follows a steep trend



Fig. 3 and 4: The main crack along the Hutton Cliffs shoreline: the difference in height of the two edges shows the intensity of the tidal action.

and along the south-western coasts of the Ross Islands the sea bottom reaches the depth of 300 meters, 2 to 5 kilometres of shore (but overcoming the depth of 200 meters within 1 km) and not exceeding the depth of about 500 meters near the breeding colonies (Testa *et al.*, 1999; Castellini *et al.*, 1991).

Nevertheless, Hindell reported depths up to 850 meters just south of Turtle Rock (Hindell *et al.*, 2002).

2.2 - Animal handling and samplings.

Post-parturient adult females were captured using a head-bag as described by Stirling (Stirling, 1966) and restrained manually, with no need of sedation by an intravenous injection of 5 - 7 ml diazepam ("Valium", 5 mg.ml-1) most of the times.

Normally, after few attempts to free themselves from the head-bag by rolling on their back and trying to bite the handler, the seals calmed down and did not try to slip away until the end of biological samplings and device deployment (Figure 5a-b-c-d).

The choice of not sedating post-parturient females were adopted to reduce the risk of the newborn to be abandoned by their mothers, events that are not so rare and represent a significant cause of mortality, above all, among pups born from primiparous females (Kaufman *et al.*, 1975; Hastings and Testa, 1998).



a



b



c



d

Fig. 5: The seal capture: the operator put the head-bag on the seal (a - b); then he has to control the animal reaction and avoid it frees itself (c); when the seal calms down, biological samplings can be carried out (d).



a



c

Fig. 6: When weighing a seal, it is important that the animal do not get out of the head-bag: figure 6c shows the consequences if it happens.



b

After biological samplings seals were weighted: they were positioned in a pole net continuing to maintain the head-bag on (to avoid injuries on the animal's face and mouth due to the mesh net rubbing) and lifted up by a portable tripod and a winch. The weight was read on a digital scale (Figure 6a-b-c).

Samplings on the newborn pups followed the same procedure with the advantage that the few day old pups were simply captured by hands and weighted using a canvas bag or a much smaller pole net (Figure 7). In late lactation, when pups were bigger and heavier, a wooden bit was used to handle them.

When a pup was captured, one of the researchers had to prevent the mother from reaching it or escaping and leaving it alone; the adult female was controlled by a board. (Figure 8)

At the end of treatments we always verified that the pup went back to its mother and she accepted it positively.

Every seal was marked on the dorsal fur with commercial hair bleach (Kaufmann *et al.*, 1975) so as to be easily recognizable for the following re-captures.



Fig. 7



Fig. 8

Samplings on the mum-pup pairs (blood, milk, weight) began at circa 2-3 days postpartum and they were serially repeated every 3-4 days.

The history of the adult females was known because Weddell seals in Erebus Bay have been tagged since 1960s in a long-term marking studies (Siniff *et al.*, 1977; Testa and Siniff, 1987; Hastings and Testa, 1998) and the dates of birth of the newborns were recorded by daily surveys in the colony.

Unfortunately, data on mum-pup weights during lactation and early weaning were not made available for this study.

2.3 - Stable Isotopes

Analysis of naturally occurring carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) stable isotopes in animal tissue is an ecological research tool that became very popular and widely applied in the last 30 years (e.g. DeNiro and Epstein, 1978 and 1981; Minagawa and Wada, 1984; Owens, 1987; Rau *et al.* 1991; Lajtha and Michener, 1994; Hobson *et al.*, 1996 and 1997; Burns *et al.*, 1998; Kelly, 2000; Lesage *et al.*, 2002; Kurle, 2002; Post, 2002; Dehn *et al.*, 2007; Crawford *et al.*, 2008).

Its applications in ecology are manifold and stable carbon and nitrogen isotope analyses were used in mammalian trophic ecology to individualize the level in the food chain at which an animal forages, the foraging locations in

terrestrial and marine food webs, the prey-predator relationship and the nutritional status (Ambrose and DeNiro, 1986; Rau *et al.*, 1992; Burns *et al.*, 1998; Kelly, 2000; Lesage *et al.*, 2002; Kurle, 2002; Vanderklift and Ponsard, 2003; Cherel *et al.*, 2007; Crawford *et al.*, 2008).

The rationale of this method is based on the essential requirement that the natural and relative abundance of carbon and nitrogen isotopes in the consumer's biological tissues is directly linked to their diet.

When food is ingested, dietary nutrients obtained by digestion are synthesized to build up animal tissues; but due to the differences in the atomic mass, heavier isotopes (generally less common) are metabolized or excreted at different rates in the physiological processes compared to the respective lighter isotopes. The latter are more likely excreted respect to the first ones, and that leads to a slight selective retention of the heavier isotopes (Kelly, 2000; Kurle, 2002; Robbins *et al.*, 2005; Miron *et al.*, 2006; Crawford *et al.*, 2008; Stegall *et al.*, 2008).

Consequently, raising from a step to another in the food chain, stable isotope relative abundance will undergo a natural variation whose magnitude can be described by predictable changes in the isotopic ratios.

This process of differential excretion and metabolism (chemically due to the different amount of energy needed to break the atomic bonds, even if not completely understood) is called isotopic fractionation and is element specific.

The isotopic ratios (relative abundances of the stable isotopes) are commonly expressed in delta notation (δ) as parts per thousand or per mil (‰) and show the deviation from accepted international standards:

$$\delta^{\text{H}}\text{X} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000$$

where:

- δ is the isotope ratio of the sample relative to the international standard
- X is the element
- ^H is the mass number of the heavy isotope
- R_{sample} is the isotopic ratio between the heavy to light isotopes of X
- R_{standard} is the isotopic ratio of the international standard

1 is subtracted from the R_{sample}/R_{standard} ratio for obtaining δ values that are immediately recognizable as “enriched” or “depleted” compared to the international standards.; positive and negative values of $\delta^{\text{H}}\text{X}$ respectively indicate a higher or a lower isotopic ratio than the standard.

The expression as parts per thousand or per mil (‰) of the equation result is given by multiplying X 1000 the number obtained before.

The international standards are Pee Dee Belemnite for carbon and atmospheric nitrogen for nitrogen (DeNiro and Epstein, 1978 and 1981)

The isotopic enrichment is therefore progressively driven by the trophic steps (every animal will be isotopically more enriched than its preys and less than its predators) but is also thought being element, tissue and species specific (Kelly, 2000; Kurle, 2002; Crawford, 2008).

$\delta^{15}\text{N}$ increases in a predictable manner between 2‰ and 4‰ per trophic level while the $\delta^{13}\text{C}$ enrichment is only about 0.8‰. For this, nitrogen stable isotope ratios are usually used to make inferences on the trophic position of a consumer within the food web and to reconstruct its diet (dietary tracer), while carbon isotopic signature is exploited by ecologists as a reliable tracer of the source of carbon throughout the food web (Hobson and Welch, 1992; Kelly, 2000; Post, 2002; Vanderklift and Ponsard, 2003; Crawford *et al.*, 2008).

One of the main problems for a correct isotopic data interpretation is that the integration of stable isotopes in the consumer's body reflects the biochemical turnover rate of every single tissue, so that any single tissue will provide dietary information at different temporal scales (its “own” temporal scale): tissues with high rates of turnover (Blood - Liver) will show dietary data relative to more recent feeding events while tissues with low rates of turnover (Bones - Keratinized tissue) integrate isotopic record over a much longer period and more remote feeding bouts (Hobson, 1993; Hobson *et al.*, 1996; Dalerum and Angerbjorn, 2005).

2.3.1 - Seals' plasma and milk

Stable isotope analyses were carried out on seals' plasma (both from mothers and pups), on milk and on prey items, to assess the presence and the extent of foraging activities during lactation and, possibly, the diet composition.

Blood samples of 10 to 20 ml volume (to be used not only for stable isotope analyses) were collected by venipuncture from extradural veins (Cline *et al.*, 1969; Geraci, 1971). Plasma was separated by centrifugation directly in the field in the laboratory hut not later than 2 hours after the collection.

Milk samples of 30 to 50 ml volume (to be used not only for stable isotope analyses) were collected by suction with a modified 50-ml plastic syringe about 20-30 minutes after an intramuscular injection of oxytocin (30 IU) to stimulate a muscular contraction of the lactiferous ducts and facilitate milk ejection (oxytocin is naturally released in response to the suckling stimulus).

Both plasma and milk were stored frozen until analysis.

Blood and milk samples were collected from 11 mum/pup pairs during the first field season (2006 - 2007) for about 5 (for mums) and 10 (for pups) weeks, while no biological samples from the second field season (2007 - 2008) were available for stable isotope analysis.

Each plasma sample was prepared in order to obtain 3 sub-aliquots to be analyzed at the mass spectrometer: one whole plasma sample and two lipid-extracted plasma samples (aqueous and protein phases).

Of each milk samples only the two lipid-extracted sub-aliquots (aqueous and protein phases) were analyzed.

Lipid extraction is now a consistent methodology because $\delta^{13}\text{C}$ values are expected to be lower for samples with high lipid content since lipids are depleted in ^{13}C ; thus high contents of lipids in tissues could lead to a bias (too low $\delta^{13}\text{C}$ values) and isotopic data misinterpretation (Parker, 1964; De Niro and Epstein, 1977; Tieszen *et al.*, 1983; Ramsay and Hobson, 1991; Kelly, 2000; Thompson *et al.*, 2000; Lesage *et al.*, 2002; Kurle, 2002; Kurle and Worthy, 2002).

• Whole plasma

100 μl of plasma were dried for 40 hours at 60 $^{\circ}\text{C}$ in the oven and ground before to be put in 3.5 X 5 mm tin capsules (≈ 0.5 mg).

• Lipid-extracted plasma (by chloroform and methanol).

1. to 150 μl of plasma
2. add 600 μl of MeOH
3. vortex well
4. add 150 μl of CHCl_3
5. vortex well
6. add 450 μl of H_2O
7. vortex well
8. spin at 10,000 RPM for 3 minutes (4 $^{\circ}\text{C}$)

At the end of this procedure I got 3 separated fractions in the vial containing:

- i) CHCl_3 and lipids - bottom;
- ii) proteins - intermediate;
- iii) and MeOH/ H_2O with electrolytes and free amino acids - supernatant.

The two upper layers (aqueous and protein phases) were saved and carefully put in different vials using Pasteur's pipettes (Figure 9a-b).

The bottom layer (lipids) was discarded.

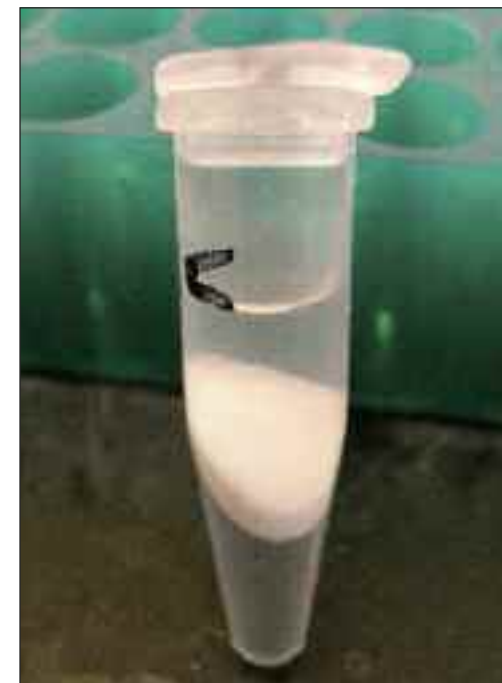


Fig. 9a-b: After the precipitation reaction the three fractions are clearly stratified (a): upper layer, aqueous phase; intermediate layer, protein phase (like a pat); lower layer, lipid phase. The three fractions were separated using a Pasteur's pipette (b).

As for the whole plasma, aqueous and protein phase samples were dried for 40 hours at 60 °C but in a heating block under the fume hood to allow methanol to evaporate with no danger. Since of the aqueous phase remained only a very thin film on the vial wall, it was scraped off to be put in 3.5 X 5 mm tin capsules (≈ 0.5 mg) while protein were ground (≈ 0.5 mg).

- Lipid extracted milk (by chloroform and methanol).

First lipid extraction.

1. to 200 μ l of milk
2. add 300 μ l of MeOH
3. vortex well
4. add 300 μ l of CHCl_3
5. vortex well
6. add 300 μ l of H_2O
7. vortex well
8. spin at 10,000 RPM for 3 minutes (4 °C)
9. put the upper two layers (aqueous and protein fractions) in a single vial and the third (lipid fraction) in another one

Second lipid extraction (from the two sub-samples)

1. to the two sub-samples add 300 μ l of MeOH
2. vortex well
3. add 200 μ l of CHCl_3
4. vortex well
5. add 100 μ l of H_2O
6. spin at 10,000 RPM for 3 minutes (4 °C)

At the end of the second lipid extraction I put the aqueous fractions of the two subsamples in a single vial and the protein fraction of the first sub-samples in another one.

As expected there was not a protein layer in the second sub-samples.

Aqueous and protein phase samples were dried for 40 hours at 60 °C in the heating block under the fume hood to allow methanol to evaporate with no

danger. Since of the aqueous phase remained only a very thin film on the vial wall, it was scraped off to be put in 3.5 X 5 mm tin capsules (≈ 0.5 mg) while proteins were ground (≈ 0.5 mg).

Stable isotope analyses on Weddell seals' plasma and milk were performed in a continuous flow isotope ratio spectrometer at the Geophysical laboratory of Carnegie Institution, Washington DC, USA.

2.3.2 - Prey items

Prey items (provided by Programma Nazionale di Ricerche in Antartide - PNRA) were collected during various PNRA oceanographic campaigns in different locations within the Ross sea but especially in the Terra Nova Bay area.

Whole prey muscle samples and lipid-extracted prey muscle samples (by a modified technique from Bligh and Dyer, 1959) were dried for 40 hours at 60 °C in the oven and ground before being put in 3.5 X 5 mm tin capsules (≈ 1.0 mg). Stable isotope analysis of prey samples were performed in a continuous flow isotope ratio spectrometer (Thermo Electron Flash EA 1112 coupled to Thermo Electron Delta Plus XP) at the "Istituto Ambiente Marino Costiero - Consiglio Nazionale delle Ricerche" (IAMC-CNR) in Naples, Italy.

2.4 - TDRs

TDRs ("Mk 9", Wildlife Computers, Redman, Washington) and radio transmitters (164-165 MHz, Model MM150 backmount transmitters, Advanced Telemetry Systems, Inc., Isanti, MN) were attached on the seals' mid-back by gluing



a
Fig. 10



b

them with a 5-minute setting epoxy resin directly to their dorsal fur (Boness *et al.*, 1994; Bornemann *et al.*, 1998); during the second field season TDRs and radio transmitters at first were attached to 8-finger webbing patches and then the single fingers were glued to the fur (Figure 10a-b).

Although the pristine plan counted to obtain dive data from 20 mum/pup pairs (10 experimental, 10 control) in both field season 1 and 2, due to malfunctioning, loss of devices and anticipated closure of the scientific activities during field season 2, complete dive data sets were recorded from 16 lactating females (field season 1: 10 - field season 2: 6) and 8 newborns (field season 1).

TDRs began to record when the sensor was wet with a sampling frequency of 10 seconds and a depth resolution of 1 meter. Maximum operative depth was 500 meter.

About lactating females, data capture periods ranged from 31 until 54 days from parturition (overall mean 40 DD; 38 DD in field season 1 - 42 DD in field season 2) while for pups ranged from 42 until 81 days (mean 67.8 DD).

2.4.1 - Dive data analysis

Dive profiles are a simplify 2D representation of the three-dimensional spatial use of the underwater environment based on depth and duration variables that allow to plot geometric shapes of the depth-time profile.

These geometric shapes were widely used to classify and assign ecological functions to the dives (Le Boeuf *et al.*, 1988 and 1993; Hindell *et al.*, 1991; Asaga *et al.*, 1994; Schreer and Testa, 1995; Schreer and Testa, 1996; Crocker *et al.*, 1997; Lesage *et al.*, 1999; Schreer *et al.*, 2001; Baechler *et al.*, 2002; Davis *et al.*, 2003; Halsey *et al.*, 2007).

Dive Data were downloaded and decoded using software provided by the manufacturer (Wildlife Computer Inc.).

Dive data analyses were performed by means of MultiTrace-Dive software (MT-Dive, Jensen Software Systems), Self-Organising Maps (SOMs - software packages by Michele Scardi) and a custom built software called "Associator" (Roberto Palozzi - Francesco Alvisini).

2.4.2 - Dive definition

For the aims of the dive classification, primarily a dive was defined as any descent below water surface deeper than 4 meter (≥ 4 m) and lasting at least 100 second (≥ 100 s), even if, in some specific cases, dives were further filtered (see afterwards) and considered for the analytical purposes only if they were able to match both the new defining parameters and the temporal and spatial restraints.

The depth threshold of 4 metres were set following the specific literature which indicates to consider a depth that was at least twice the TDR resolution (Bowen *et al.*, 1999; Burns, 1999; Hooker and Baird, 1999; Krafft *et al.*, 2000; Arnould and Hindell, 2001; Jorgensen *et al.*, 2001; Schreer *et al.*, 2001; Baechler *et al.*, 2002; Sato *et al.*, 2002) and taking into account the thickness of the ice that, in Hutton Cliffs, can be up to several meter thick.

It is important to underline that, how highlighted by Hooker and Baird (2001), the "common-rule" of applying two time the TDR resolution is not founded on any rigorous scientific definition.

The minimum duration (100 s) was chosen so that the TDR temporal sampling frequency (10 s) corresponded to 10% of the shortest dives; graphically it means that every dive was plotted by 10 points at least (Wilson *et al.* 1995; Schreer and Testa, 1995).

Instead the "Associator" is not a tool for dive classification but to associate mum's diving activity (time spent in/out the water and in shallow/deep water) with that of her own pup; so it was necessary to define the depth discriminating between shallow and deep water (see afterward).

2.4.3 - MT-Dive (Traditional approach)

MT-Dive is a software that scans raw data from TDRs and provides a graphic output of the dive profiles generated on the basis of the parameters pre-emptively set by the user (Figure 11).

The Dive Parameters window requires several values to be inserted to specify the software what is a dive, a dive stop, a wiggle and a bout by combining the different parameters.

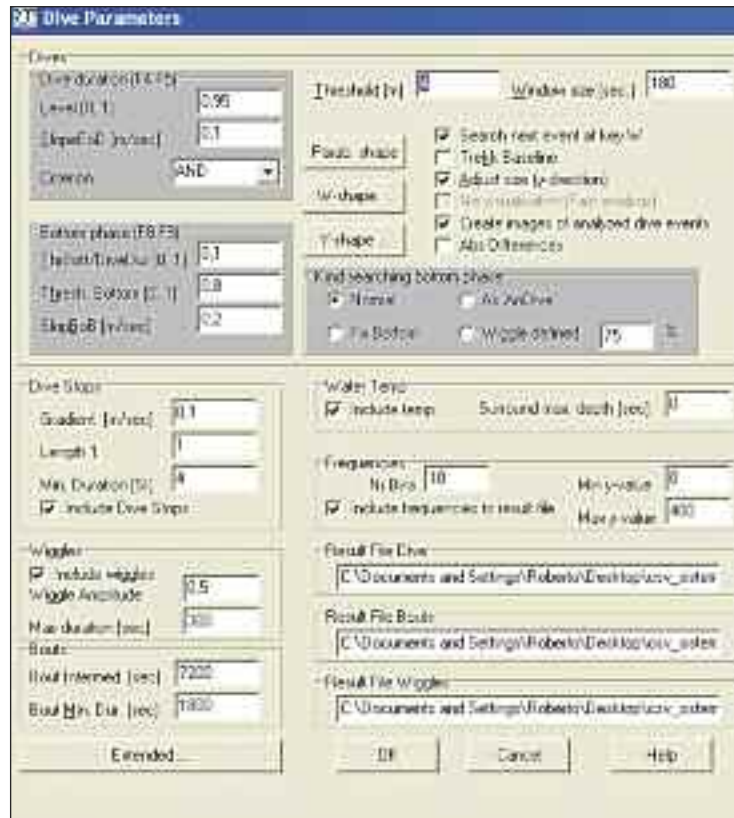


Fig. 11

A dive event in MT-Dive is defined by:

- Dive threshold T: the minimum depth to consider a dive. I set 4 meter.
- Level L Criterion: percentage of the maximum recorded depth to detect the end of diving. When the animal comes back over this percentage the software will consider the first successive data as the last one of the dive. I set 0.95 (95%).
- Slope EoD S: criterion to detect the beginning and the end of diving. The smallest gradient (vertical velocity, m/s) underneath which the dive is considered ended. I set 0.1.
- Threshold bottom H: percentage of the maximum recorded depth underneath which a bottom phase is considered. I set 0.8 (80%).
- Slope BoB B: criterion to detect the beginning and the end of a bottom phase.

Gradient (vertical velocity, m/s) underneath of which a bottom phase exists. I set 0.2.

- Relation Bottom-Surface R: this value affects the classification of dive profiles since when

$$\text{Bottom Phase Duration/Total Dive Duration} > R$$

U-shaped dives exist. I set 0.1.

The baseline (0, the water surface) was set by “zero offset correction” and checked by visual inspection so that every diving event found by the software was “biologically” consistent (i.e., if dives lasting more than 50 hours were found, the baseline was modified).

By default MT-Dive classifies the dives within 5 preordained profiles: U, V, Y, W and parabola(u)-shaped (Figure 12a-b-c-d-e).

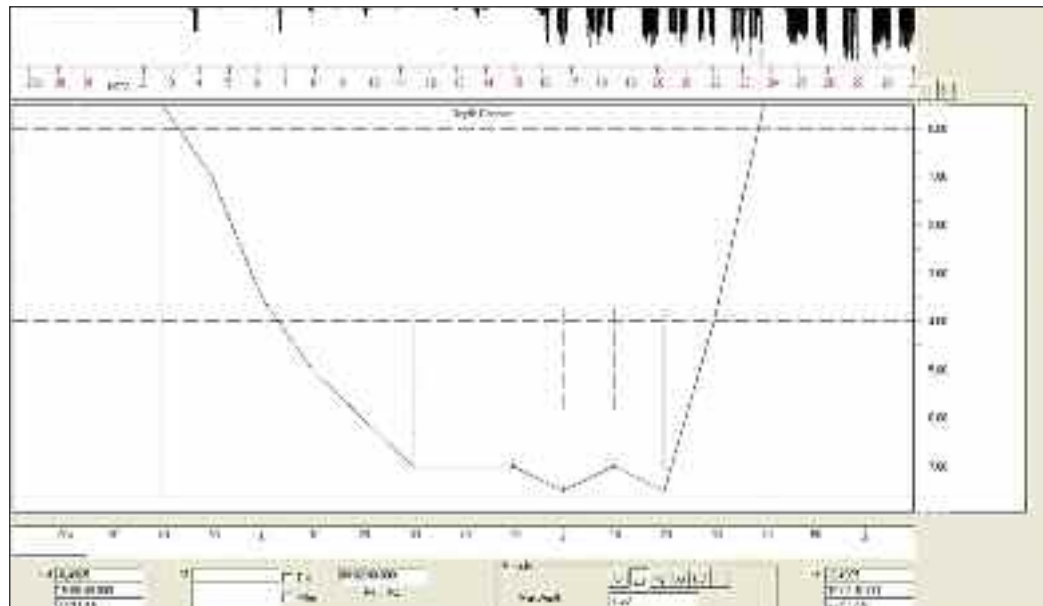
Researcher-user’s arbitrariness in the value assignment is very high since parameters are specie-specific and often they represent the subject of the research.

For example, the detection of the bottom time (the amount of time spent at more than a certain percentage of the dive maximum depth) is evidently depending on the bottom threshold assigned to the software: in the literature concerning the pinniped diving behaviour it ranges from 70% to 85% with no any rigorous scientific justification (Schreer and Testa, 1996; Bowen *et al.*, 1999; Burns, 1999; Lesage *et al.*, 1999; Jorgensen *et al.*, 2001; Baechler *et al.*, 2002; Baylis *et al.*, 2005).

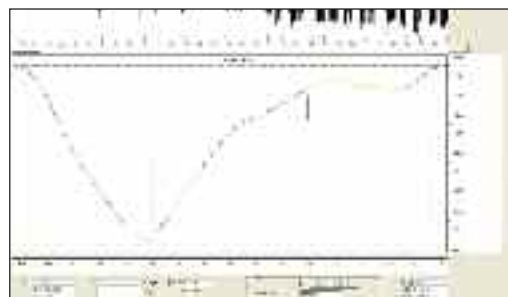
All data relative to the dive events of a seal found by the software were successively expressed in a Excel sheet and pooled together in categories (mothers - pups) and in intermediate periods (early lactation - 1 to 13 DPP; mid lactation - 14 to 26 DPP; 27 to 33 DPP; 34 to 42 DPP; 42 to 63 DPP; 63 to the end of recording period) for comparison.

2.4.4 - Self Organizing Maps

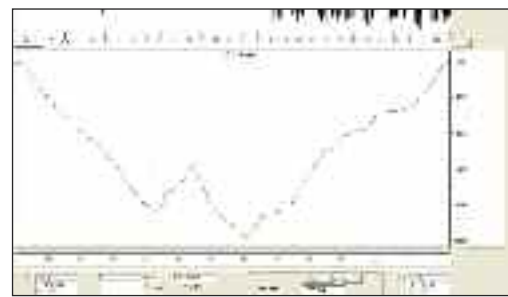
Artificial Neural Networks (ANNs) are powerful data analysis tools that are arising as an alternative mean to traditional statistical methods, in ecology as well; they are based on interconnected computational units, typically organized



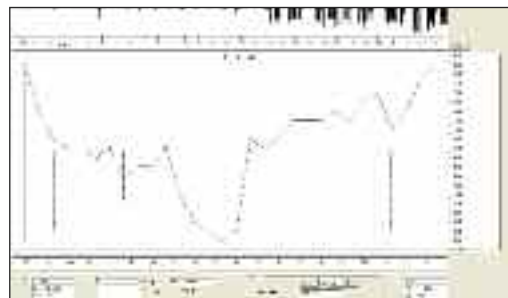
a



b



c



d



e

Fig. 12: The five clusters used by MT-Dive to categorize the dives: U- (generally associated with the foraging activity, a), V- (in this case with a right-skewed “tail”, b), W- (c), Y- (d) and parabola- (e) shaped profiles.

into layers (Input - Hidden - Output layers), that work as a biological brain (Lek and Guegan, 1999).

ANNs learn through training which can be “supervised” or “unsupervised”.

I used a Self-Organizing Map - SOM (Kohonen, 1982 and 2001), a type of artificial neural network that is trained using unsupervised learning and that provides an output layer made of a network of neurons arranged on a hexagonal lattice (Park *et al.*, 2006), to classify seal’s dive profiles (dive shapes).

The reason for this choice is in the intrinsic ability of the SOMs to give effective answers to two of the main problems the dive data analysis presents: the management of enormous amounts of raw data (more than a million of records in the specific case of this work) and the need for objectivity in the dive classification (Schreer *et al.*, 1998; Lek and Guegan, 1999; Giraudel and Lek, 2001).

In fact, SOM algorithm produces a two-dimensional discretized representation of the data space making possible to display a high-dimensional input dataset in a lower dimensional output space (clusters). In this process the map preserves the topology by sorting the hidden nodes in order that each one will be connected to those representing similar cluster vectors (nearest neighbours on the grid); and the second consequence is that since data are clustered on the basis of their perceived closeness (unsupervised learning) the classification is not known and set by the users a priori.

This makes SOM useful for reducing multivariate data seeking clusters within them and to visualize low-dimensional views of high-dimensional data.

The SOM limit is that the user must decide the number of clusters since the algorithm requires the number of rows and columns (Giraudel and Lek, 2001).

2.4.5 - Dive Data (SOM)

Baselines were adjusted by a software package (“Baseline” by Michele Scardi) and data filtered to skip dives less deep than 4 meters and shorter than 100 seconds (“Filtra Dive” by Michele Scardi).

Then dives were normalized for the duration and the depth.

Every dive was cut in 50 “slices” and inserted in a defined rectangle regardless of its duration and depth: this standardization allowed all the dives to be rep-

resented by 50 depths and durations and to be compared for the shape.

The learning processes of the SOMs were carried out using a software package developed by Michele Scardi (2004).

SOMs were run applying both the Euclidean and the Hellinger distance (the latter was used because it is well-suited to be applied with frequency distributions) and in two different map sizes: 3X4 (12 clusters) and 9X12 (108 clusters) grids.

Smaller SOMs act as non hierarchical classifiers (very similar to a K-means cluster analysis) while larger SOMs, which can be regarded as non linear ordinations, provide a much more faded transition between clusters.

The graphic outputs (the Maps) were then displayed in Excel sheets along the relative data managed by pivot tables.

2.4.6 - The Associator

The Associator is a simple software package developed with the specific aim to associate (align) mum's dive data with those of her own pup (Figure 13).

It searches the first and the last sampling in common between the two individuals (same date and time both for the mum and pup) and provides the amount of time (number of records) and when seals are:

- both mum and pup OUT of the water - AA
- mum OUT, pup IN the water - AB
- mum IN, pup OUT of the water - BA
- mum and pup IN the water - BB

The Associator also provides the same info referred to three different animal conditions: i) OUT of the water, ii) IN Shallow water and iii) IN Deep water. The 9 combinations are:

- both mum and pup OUT of the water - AA
- mum OUT, pup IN Shallow water - AS
- mum OUT, pup IN Deep water - AD
- mum IN Deep water, pup OUT - DA
- mum IN Deep water, pup IN Shallow water - DS

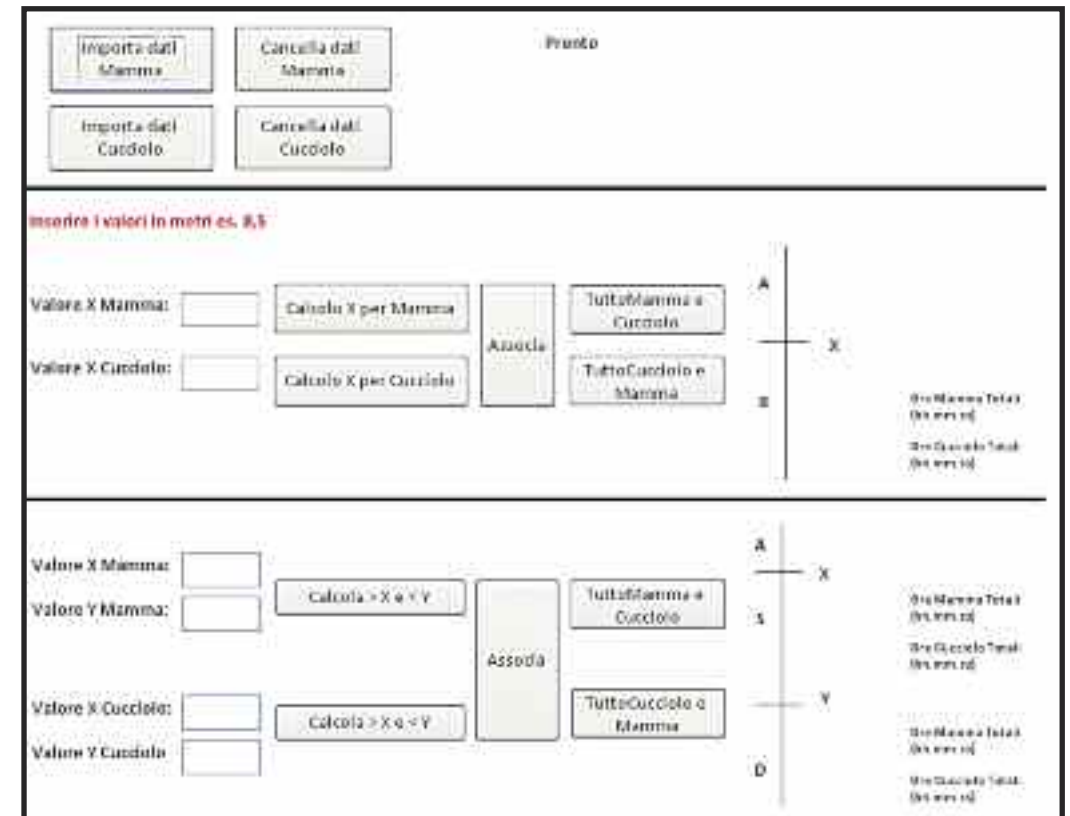


Fig. 13: The Associator allows to load dive data of two individuals (a lactating female and its own pup for the purpose of this work) and associate them as regards date and time. The user has to insert the baseline value for each individual and/or the shallow/deep water value.

- mum IN Deep water, pup IN Deep Water - DD
- mum IN shallow water, pup OUT - SA
- mum IN shallow water, pup IN Shallow water - SS
- mum IN shallow water, pup IN Deep water - SD

For each animal, the software requires the entry of the value corresponding to the water surface (0 meter) and that of the beginning of the deep water.

Water surface (0 meter) was set at the same value of the baselines obtained by MT-Dive “Zero Offset Correction”.

The value for deep water (14.5 meter) was selected in correspondence of

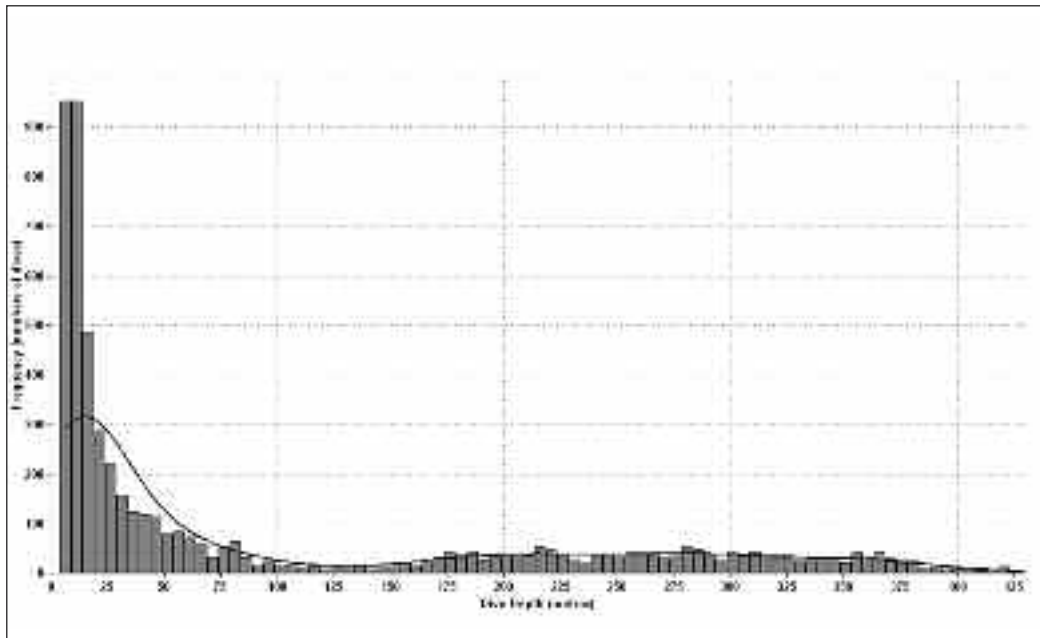


Fig. 14: Kernell density line on the dive-depth frequency distribution of both mums and pups; the peak (14.5 m.) was used to discriminate between shallow and deep water.

the Kernel Density peak after pooling together all the dives performed by mums and pups during lactation. (Figures 14 and 15a-b).

Data in the 2X2 contingency table were statistically analyzed using X^2 .

A relevant problem with the statistical analysis was that every record (sampling) in a dive is completely depending on the previous one and to allow a correct application of the (independent data), only a record per hour was randomly taken.

Since all the dives were shorter than one hour, in this way there was not ant possibility to withdraw 2 data from the same dive.

All statistical analyses (Paired Wilcoxon tests, Mann-Whitney test, X^2 test) were performed using Past software.

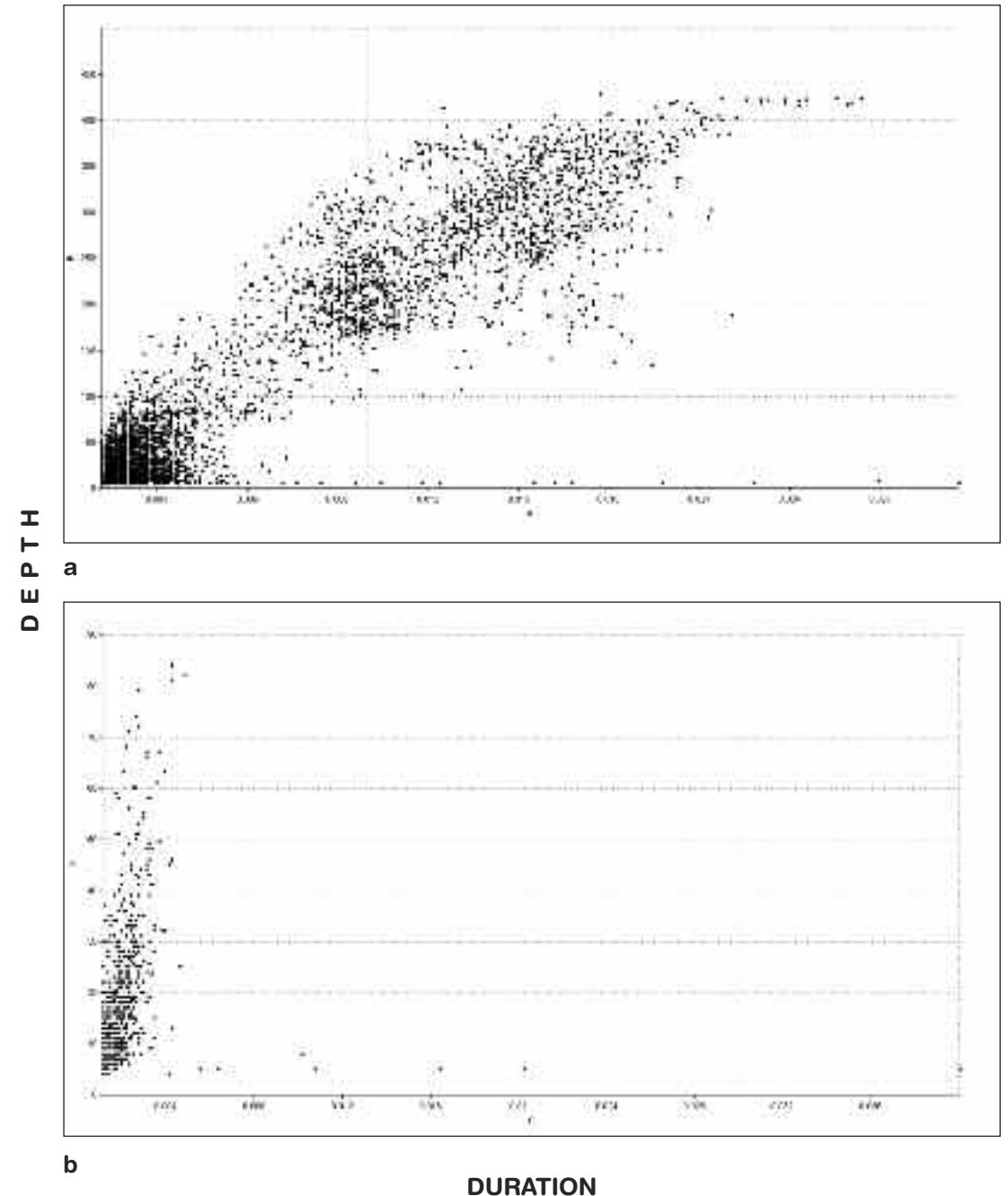


Fig. 15: Durations and maximum depths of mums' (a) and pups' (b) dives throughout lactation (0-35 Days Post Partum). Mums showed a bi-modal distribution (two different clouds of points).

In the X-axe: 0.0015 = 2'10"; 0.0031 = 4'20"; 0.006 = 8'40"; 0.009 = 13'00"; 0.01505 = 21'40"; 0.021065 = 30'20"; 0.027 = 42'40".

3. RESULTS



3.1 - Stable Isotopes

3.1.1 - Seals

Table 1a-b-c shows stable isotope and the C/N ratios for all of the seal sampled in 7 sampling events repeated during lactation, as regards mother's plasma (3 fractions), pup's plasma (3 fractions) and milk (2 fractions) .

Sampling events occurred at (Days Post Partum):

1. 2/3 DPP
2. 6/7 DPP
3. 12/13 DPP
4. 18/19 DPP
5. 24/26 DPP
6. 31/33 DPP
7. 37/38 DPP

The 8th sampling event for pups was not considered for statistical analysis because of its too wide temporal range (samples collected along 3 weeks).

Aqueous fraction, protein fraction and whole plasma curves of lactating females, pups and milk are shown in figure 1a-b-c, 2a-b-c and 3a-b-c.

Since the scientific design of this work was based on longitudinal data and, consequently, the seal sample groups could not be independent, the statistical significance between sampling events was tested by paired Wilcoxon tests with Bonferroni correction to address the multiple comparisons.

Throughout lactation (7 sampling events - same animals at each sampling event) the differences were not significant (paired Wilcoxon tests, p(same median)>0.007) in all the comparisons between fractions (aqueous vs. protein, aqueous vs. whole sample and protein vs. whole sample); within fractions, between isotopes ($\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$); between mothers and pup; and between animals and milk. But in almost all cases p(same median) was remarkably lower than the p-value 0.05 (p(same median) \approx 0.02).

Sampling events	1st	2nd	3rd	4th	5th	6th	7th	MEAN	SE
MUMs $\delta^{13}\text{C}$ TREND									
AQ. MUMs	-24.64	-24.45	-24.37	-24.21	-24.55	-24.95	-24.60	-24.54	0.09
PR. MUMs	-24.01	-23.98	-23.93	-23.68	-23.78	-23.72	-23.90	-23.85	0.05
WH. MUMs	-25.28	-25.23	-25.13	-25.10	-25.13	-25.17	-25.03	-25.15	0.03
MUMs $\delta^{15}\text{N}$ TREND									
AQ. MUMs	12.54	12.21	11.93	11.75	11.91	11.65	11.97	11.99	0.11
PR. MUMs	13.46	13.40	13.31	13.34	13.29	13.25	13.23	13.34	0.04
WH. MUMs	13.33	13.38	13.30	13.23	13.23	13.13	13.07	13.24	0.04
MUMs C/N TREND									
AQ. MUMs	4.63	3.82	3.95	4.12	4.65	5.20	4.77	4.49	0.18
PR. MUMs	3.45	3.46	3.45	3.39	3.40	3.41	3.49	3.43	0.01
WH. MUMs	3.99	3.95	3.95	3.97	4.04	4.08	4.11	4.01	0.02

a

Sampling events	1st	2nd	3rd	4th	5th	6th	7th	MEAN	SE	8th
PUPs $\delta^{13}\text{C}$ TREND										
AQ. PUPs	-25.93	-25.50	-25.14	-25.13	-25.02	-25.08	-25.10	-25.27	0.12	-25.06
PR. PUPs	-24.62	-24.61	-24.49	-24.49	-24.53	-24.27	-24.34	-24.48	0.05	-24.43
WH. PUPs	-25.85	-25.98	-26.00	-25.88	-25.93	-26.05	-26.03	-25.97	0.03	-26.28
PUPs $\delta^{15}\text{N}$ TREND										
AQ. PUPs	13.89	13.33	13.28	13.33	13.59	13.64	13.43	13.49	0.07	13.41
PR. PUPs	14.87	14.77	14.60	14.64	14.63	14.70	14.73	14.70	0.04	14.69
WH. PUPs	15.00	14.73	14.60	14.83	14.70	14.75	14.77	14.77	0.05	14.63
PUPs C/N TREND										
AQ. PUPs	4.14	4.33	4.60	4.76	4.43	4.55	5.28	4.58	0.14	5.16
PR. PUPs	3.46	3.50	3.49	3.50	3.49	3.54	3.59	3.51	0.02	3.55
WH. PUPs	4.07	4.24	4.47	4.36	4.49	4.55	4.56	4.39	0.07	4.29

b

Sampling events	1st	2nd	3rd	4th	5th	6th	7th	MEAN	SE
MILK $\delta^{13}\text{C}$ TREND									
AQ. MILK	-29.46	-29.23	-29.06	-29.16	-29.03	-28.90	-28.90	-29.11	0.07
PR. MILK	-26.32	-25.97	-25.77	-25.97	-25.93	-25.95	-25.80	-25.96	0.07
MILK $\delta^{15}\text{N}$ TREND									
AQ. MILK	6.79	6.33	5.71	5.44	5.93	6.18	5.85	6.03	0.17
PR. MILK	14.97	14.88	14.85	14.70	14.52	14.75	14.55	14.75	0.06
MILK C/N TREND									
AQ. MILK	0.49	7.79	7.44	7.72	7.75	7.27	7.98	7.78	0.15
PR. MILK	4.42	4.09	4.09	4.36	4.24	4.20	4.15	4.19	0.04

c

Table 1: Summary of the isotopic (carbon and nitrogen) and C/N ratios relative to lipid extracted aqueous fraction and protein fraction of plasma and milk and to whole plasma, recorded during lactation: (a) mums; (b) pups; (c) milk.

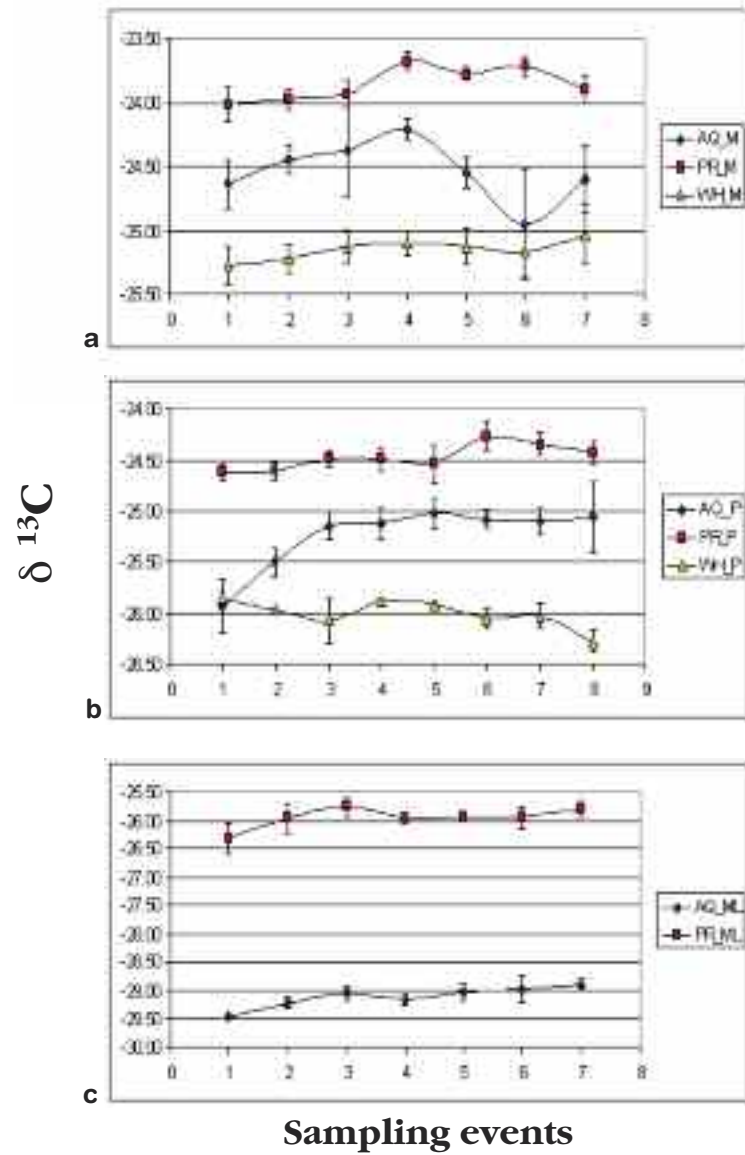


Fig.1: $\delta^{13}\text{C}$ curves of (a) mums' plasma, (b) pups' plasma and (c) milk concerning the aqueous fraction, protein fraction and whole plasma during lactation and early weaning (pups). Mean value of each sampling event \pm Standard Error.

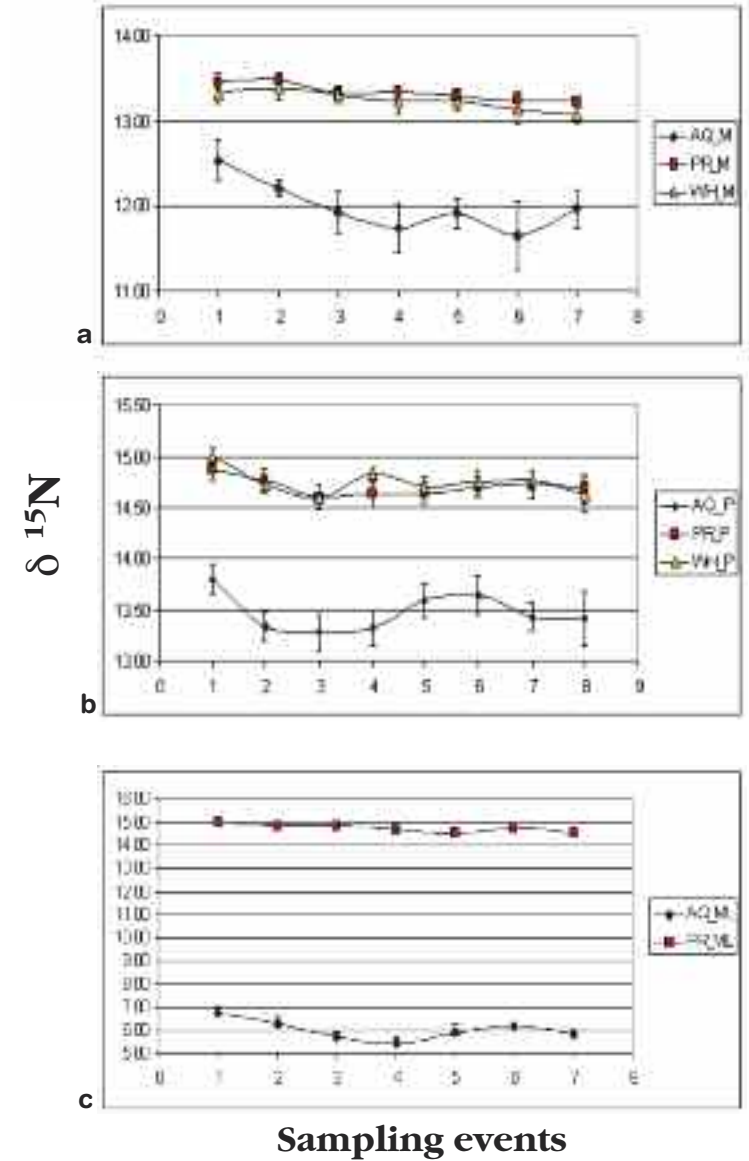


Fig.2: $\delta^{15}\text{N}$ curves of (a) mums' plasma, (b) pups' plasma and (c) milk concerning the aqueous fraction, protein fraction and whole plasma during lactation and early weaning (pups). Mean value of each sampling event \pm Standard Error.

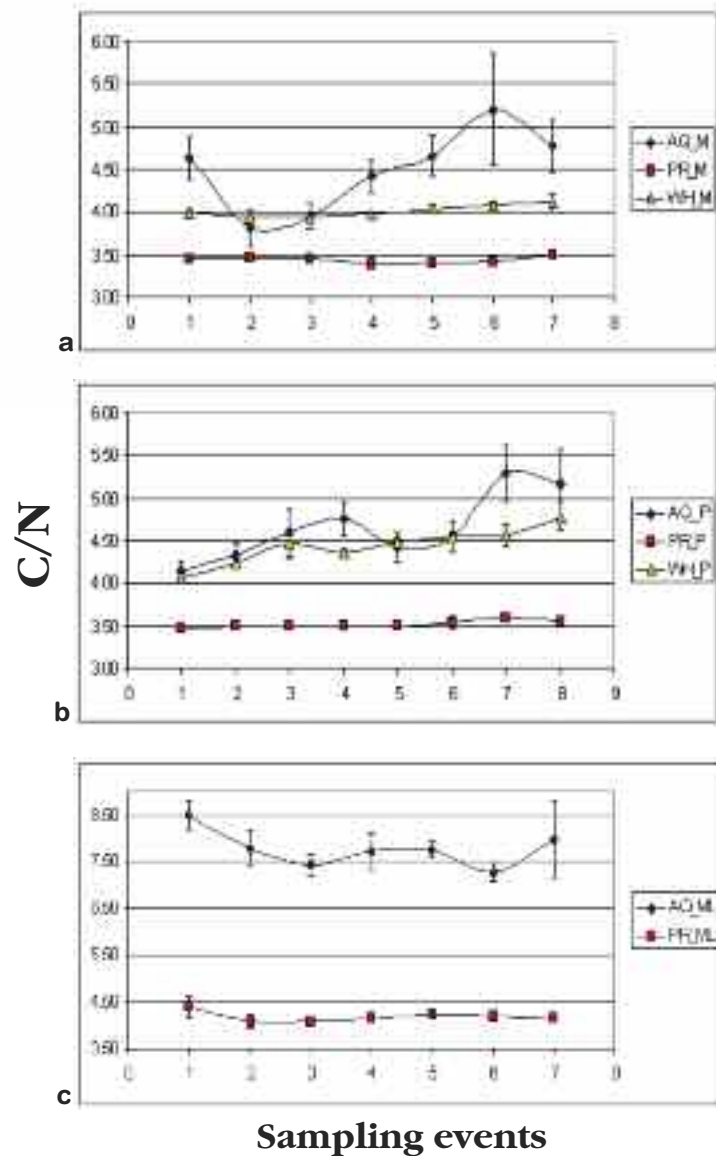


Fig.3: C/N curves of (a) mums' plasma, (b) pups' plasma and (c) milk concerning the aqueous fraction, protein fraction and whole plasma during lactation and early weaning (pups). Mean value of each sampling event \pm Standard Error.

At the first (F) and last (L) sampling event, considering data from all sampled animals, Mann-Whitney tests showed very strong statistical significance between:

- different fractions within same groups

mum aqueous $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$, (F) $n = 8$, $p\text{-same} = 0.00092$, (L) $n = 7$, $p\text{-same} = 0.00217$;
 mum protein $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$, (F) $n = 8$, $p\text{-same} = 0.0009$, (L) $n = 7$, $p\text{-same} = 0.00207$;
 pup aqueous $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$, (F) $n = 9$, $p\text{-same} = 0.0004$, (L) $n = 7$, $p\text{-same} = 0.0021$;
 pup protein $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$, (F) $n = 9$, $p\text{-same} = 0.00039$, (L) $n = 7$, $p\text{-same} = 0.002$;
 milk aqueous $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$, (F) $n = 7$, $p\text{-same} = 0.0021$, (L) $n = 4$, $p\text{-same} = 0.02652$;
 milk protein $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$, (F) $n = 6$, $p\text{-same} = 0.0049$, (L) $n = 4$, $p\text{-same} = 0.0304$;

- same fractions within different groups

mum-pup aqueous $\delta^{13}\text{C}$, (F) $n = 8/9$, $p\text{-same} = 0.00061$, (L) $n = 7$, $p\text{-same} = 0.1792$;
 mum-pup protein $\delta^{13}\text{C}$, (F) $n = 8/9$, $p\text{-same} = 0.00441$, (L) $n = 7$, $p\text{-same} = 0.01733$;
 mum-pup aqueous $\delta^{15}\text{N}$, (F) $n = 8/9$, $p\text{-same} = 0.0017$, (L) $n = 7$, $p\text{-same} = 0.004$;
 mum-pup protein $\delta^{15}\text{N}$, (F) $n = 8/9$, $p\text{-same} = 0.0006$, (L) $n = 7$, $p\text{-same} = 0.0021$;
 mum-milk aqueous $\delta^{13}\text{C}$, (F) $n = 8/7$, $p\text{-same} = 0.0014$, (L) $n = 7/4$, $p\text{-same} = 0.0107$;
 mum-milk protein $\delta^{13}\text{C}$, (F) $n = 8/6$, $p\text{-same} = 0.0024$, (L) $n = 7/4$, $p\text{-same} = 0.0106$;
 mum-milk aqueous $\delta^{15}\text{N}$, (F) $n = 8/7$, $p\text{-same} = 0.0014$, (L) $n = 7/4$, $p\text{-same} = 0.01$;
 mum-milk protein $\delta^{15}\text{N}$, (F) $n = 8/6$, $p\text{-same} = 0.0023$, (L) $n = 7/4$, $p\text{-same} = 0.01$;
 pup-milk aqueous $\delta^{13}\text{C}$, (F) $n = 9/7$, $p\text{-same} = 0.0001$, (L) $n = 7/4$, $p\text{-same} = 0.0107$;
 pup-milk protein $\delta^{13}\text{C}$, (F) $n = 9/6$, $p\text{-same} = 0.0017$, (L) $n = 7/4$, $p\text{-same} = 0.0103$;
 pup-milk aqueous $\delta^{15}\text{N}$, (F) $n = 9/7$, $p\text{-same} = 0.001$, (L) $n = 7/4$, $p\text{-same} = 0.0099$;
 pup-milk protein $\delta^{15}\text{N}$, (F) $n = 9/6$, $p\text{-same} = 1$, (L) $n = 7/4$, $p\text{-same} = 0.338$.

The only p-values bigger than 0.05 were those obtained when compared aqueous $\delta^{13}\text{C}$ ratios of mums and pups during last sampling event and protein $\delta^{15}\text{N}$ ratios of mums and pups both in the first and last sampling event.

C/N ratio can not be considered significantly correlated both with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ within and between groups (Paired Wilcoxon test, $p(\text{same median}) \approx 0.018$) because of Bonferroni correction ($p\text{-value} = 0.0125$ and $p\text{-value} = 0.017$ for 4 and 3 comparison respectively), even if paired Wilcoxon test values were very close to Bonferroni corrected p-values; values of mum aqueous vs.

mum whole ($p(\text{same median}) = 0.0747$), pup aqueous vs. pup whole ($p(\text{same median}) = 0.0747$) and mum aqueous vs. pup aqueous ($p(\text{same median}) = 0.6121$) showed no statistical differences.

As regards the carbon isotopic signature, mums and pups showed a considerable difference between lipid-extracted protein fraction and whole plasma (mum protein $\delta^{13}\text{C} = -23.86 \pm 0.05$, whole $\delta^{13}\text{C} = -25.15 \pm 0.03$, mean difference of 1.29‰; pup protein $\delta^{13}\text{C} = -24.48 \pm 0.04$, whole $\delta^{13}\text{C} = -25.07 \pm 0.05$, mean difference of 1.49‰. Mean \pm SE) with the aqueous isotopic carbon ratios both intermediate between the protein fraction and the whole plasma (mum aqueous $\delta^{13}\text{C} = -24.54 \pm 0.09$; pup aqueous $\delta^{13}\text{C} = -25.27 \pm 0.12$. Mean \pm SE) (Figure 4).

This pattern with protein fraction $\delta^{13}\text{C}$ values higher than aqueous fraction and whole plasma respectively was the same both in mums and pups but the latter were translated downer so that pup protein carbon isotopic signature was very similar to mum aqueous $\delta^{13}\text{C}$ and pup aqueous carbon isotopic sig-

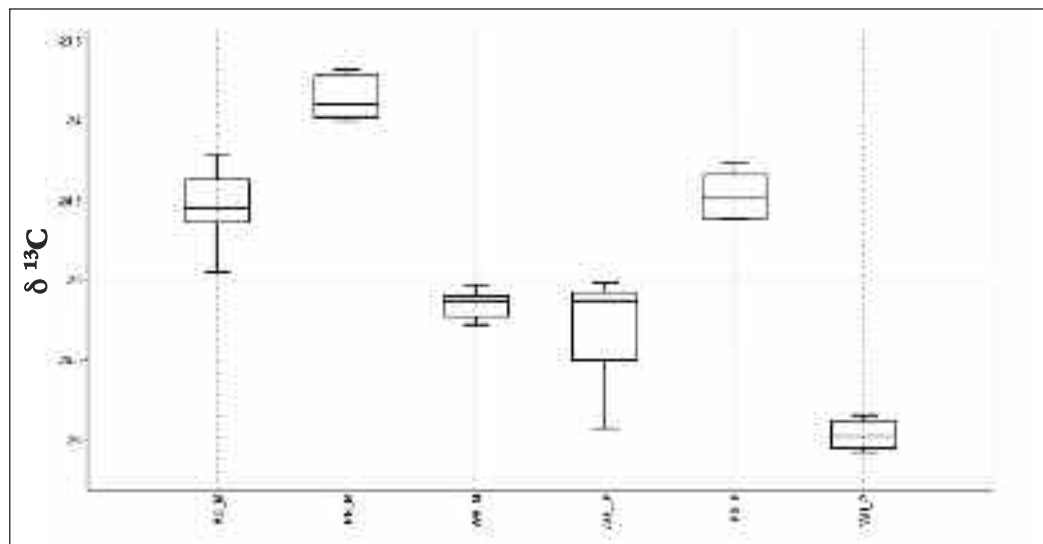


Fig.4: Box Plot of $\delta^{13}\text{C}$ plasma aqueous fraction, plasma protein fraction and whole plasma of mums and pups. The two categories presented the same pattern but pups' protein values were very similar to those of mums' aqueous and pups' aqueous values were very similar to those of mum's whole plasma. Boxes represent the second and third quartile and the medians; whiskers show the first and fourth quartile, with the extreme values.

nature to mum whole plasma $\delta^{13}\text{C}$ (see Figure 1).

Mum aqueous $\delta^{13}\text{C}$ curve showed a sinusoidal trend throughout lactation while pup aqueous $\delta^{13}\text{C}$ curve indicated an increase in the first part (two first weeks) and then a substantial stabilization (Figure 5).

At sampling event 1 pup aqueous mean carbon ratio was lower than that of whole plasma (-25.93 ± 0.26 SE, -25.85 ± 0.06 SE).

The $\delta^{15}\text{N}$ patterns are different: in both mums and pups protein $\delta^{15}\text{N}$ and whole plasma $\delta^{15}\text{N}$ values were almost coinciding (mum protein $\delta^{15}\text{N} = 13.34 \pm 0.04$, mum whole plasma $\delta^{15}\text{N} = 13.24 \pm 0.04$, mean difference of 0.1‰; pup protein $\delta^{15}\text{N} = 14.71 \pm 0.04$, pup whole plasma $\delta^{15}\text{N} = 14.77 \pm 0.05$, mean difference of -0.06‰. Mean \pm SE) and they were greater than aqueous fraction (mum aqueous $\delta^{15}\text{N} = 11.99 \pm 0.11$; pup aqueous $\delta^{15}\text{N} = 13.49 \pm 0.07$. Mean \pm SE).

As for isotopic carbon signature the pattern amongst fractions was corresponding between mum and pup groups but, on the contrary, the latter was

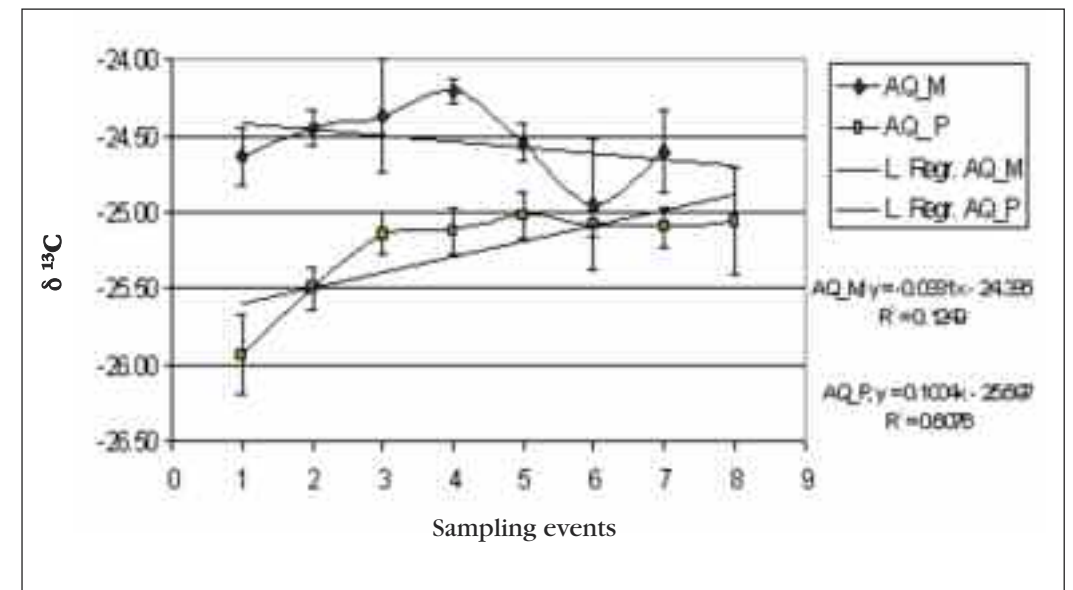


Fig.5: $\delta^{13}\text{C}$ aqueous curves of mums and pups throughout lactation and early weaning with linear regressions (that are converging). Mean value of each sampling event \pm Standard Error.

translated higher (Figure 6).

Therefore, as expected, pups showed higher plasma isotopic nitrogen signature ratios than mothers throughout the course of lactation in all samples of about $\approx 1.5\text{‰}$ (pup - mum's mean difference: aqueous fractions, 1.5‰ ; protein fraction, 1.37‰ ; whole plasma, 1.53‰), that were very remarkable even if these differences do not integrate a trophic step (Jenkins *et al.*, 2001; Sare *et al.*, 2005) (Figure 7).

Moreover pup's plasma stable nitrogen isotope ratios were almost coinciding with those of milk, the actual food of the newborns (Figure 8a-b).

Despite lipid extraction, milk carbon signature was depleted relative to pups and above all, maternal plasma both in aqueous and protein fractions (milk aqueous $\delta^{13}\text{C} = -29.12 \pm 0.07$; milk protein $\delta^{13}\text{C} = -25.96 \pm 0.07$).

Considering the milk protein fraction in comparison to the pup's plasma protein fraction and pup's whole plasma, $\delta^{15}\text{N}$ did not show fractionation between milk and pup's plasma since their isotopic signatures were practically coincident (milk protein $\delta^{15}\text{N} = 14.75 \pm 0.06$, mean differences of 0.04‰ and -0.02 relative to pup's plasma protein fraction and pup's whole plasma respectively. Mean \pm SE). But the difference between aqueous fractions was very important (milk aqueous $\delta^{15}\text{N} = 6.03 \pm 0.17$, mean differences of -7.46‰ relative to pup's plasma aqueous. Mean \pm SE).

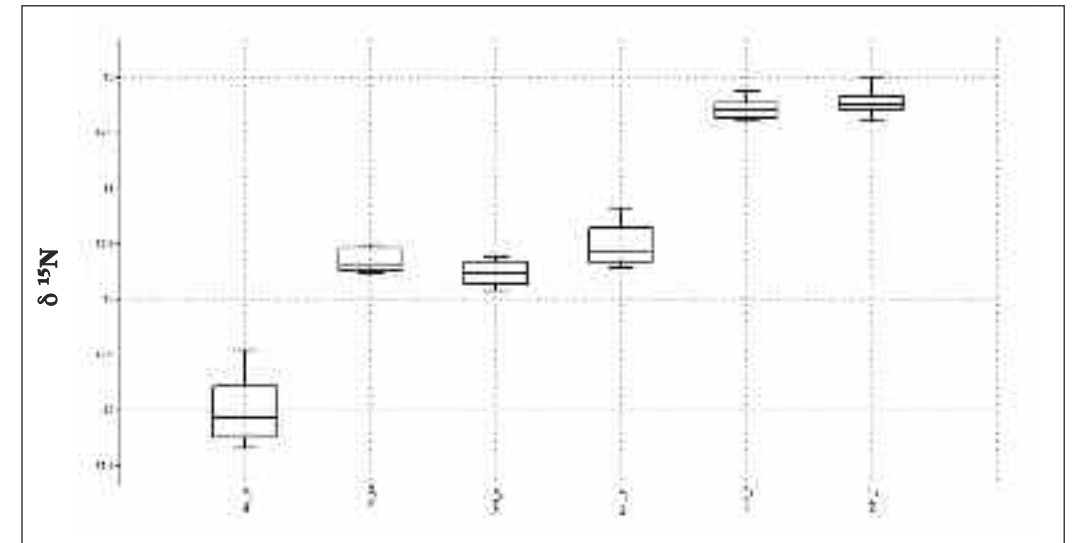


Fig.6: Box Plot of $\delta^{15}\text{N}$ plasma aqueous fraction, plasma protein fraction and whole plasma of mums and pups. The two categories presented the same pattern but pups' aqueous values were very similar to those of mums' protein and whole plasma values. Boxes represent the second and third quartile and the medians; whiskers show the first and fourth quartile, with the extreme values.

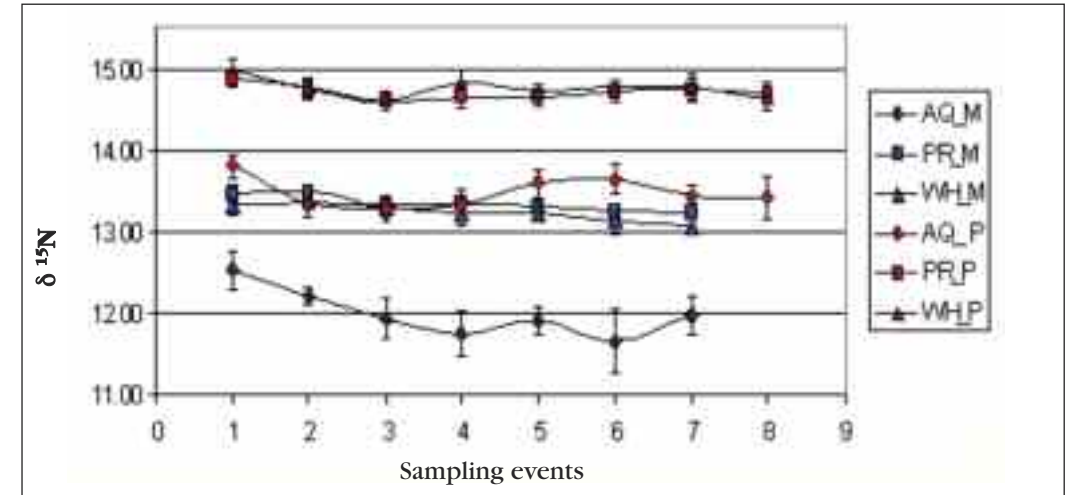
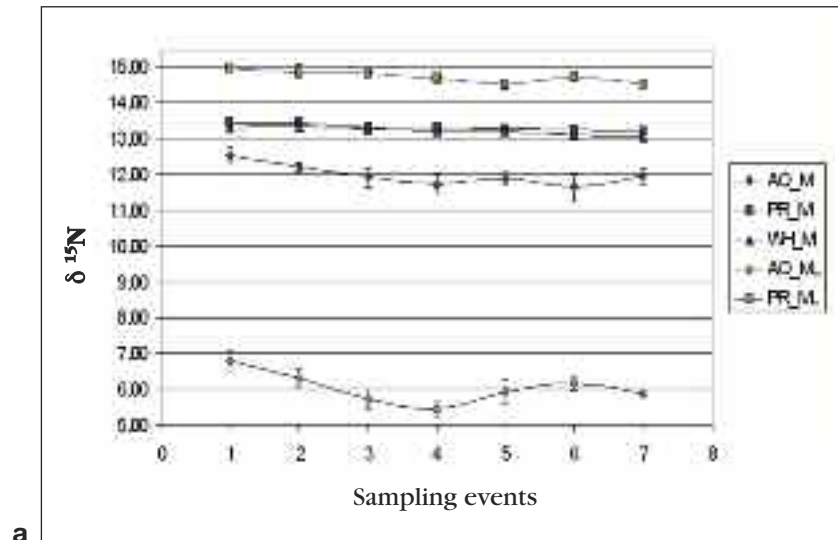
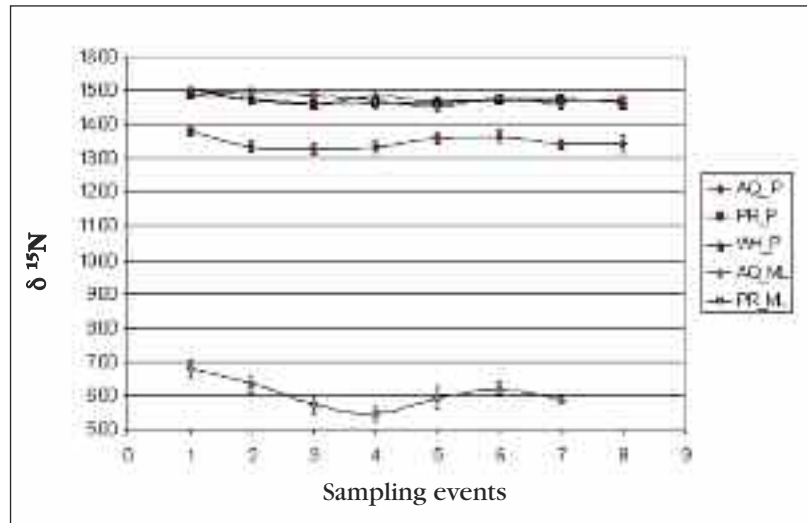


Fig. 7: $\delta^{15}\text{N}$ aqueous, protein and whole plasma curves of mums and pups. Mean value of each sampling event \pm Standard Error.



a



b

Fig. 8: $\delta^{15}\text{N}$ aqueous, protein milk curves plotted with ^{15}N aqueous, protein and whole plasma curves of (a) mums and (b) pups respectively. Mean value of each sampling event \pm Standard Error.

3.1.2 - Prey items

The carbon and nitrogen stable isotope ratios of Weddell seal prey items are reported in table 2a (whole samples) and Table 2b (lipid-extracted samples), along with the isotopic values of mums' and pups' whole plasma and mums', pups' and milk's lipid extracted aqueous and protein fractions.

SPECIES	n (x2rep0)	$\delta^{13}\text{C}$	\pm SE	$\delta^{15}\text{N}$	\pm SE
<i>Chionodraco hamatus</i>	6	-25.48	0.62	12.86	0.30
<i>Dissostichus mawsoni</i>	4	-29.93	2.89	14.96	0.33
<i>Trematomus hansonii</i>	11	-23.50	0.85	13.52	0.29
<i>Trematomus bernacchii</i>	9	-21.45	0.35	10.94	0.15
<i>Trematomus eulepidotus</i>	3	-24.33	0.81	12.52	0.57
<i>Trematomus pennelli</i>	1	-23.60	0.00	14.55	0.00
<i>Pagothenia borchgrevinki</i>	5	-30.01	0.43	11.52	0.52
<i>Megaledone seboi</i> (?)	1	-22.68	0.00	12.47	0.00
<i>Trematomus newnesi</i>	1	-25.46	0.00	14.41	0.00
<i>Pleuragramma antarcticum</i>	12	-28.56	0.35	10.62	0.34
WS MUM parturition	4	-25.28	0.15	13.33	0.13
WS MUM late lactation	3	-25.03	0.23	13.07	0.09
WS PUP parturition	4	-25.85	0.06	15.00	0.07
WS PUP weaning	4	-26.28	0.11	14.63	0.17

a

SPECIES	n (x2rep0)	$\delta^{13}\text{C}$	\pm SE	$\delta^{15}\text{N}$	\pm SE
<i>Chionodraco hamatus</i>	6	-26.77	0.30	13.32	0.47
<i>Dissostichus mawsoni</i>	4	-25.46	0.75	15.66	0.18
<i>Trematomus hansonii</i>	6	-23.32	0.21	14.68	0.19
<i>Trematomus bernacchii</i>	9	-18.97	0.25	12.21	0.17
<i>Trematomus eulepidotus</i>	3	-21.37	0.36	11.40	0.22
<i>Trematomus pennelli</i>	1	-22.59	0.00	11.30	0.00
<i>Pagothenia borchgrevinki</i>	5	-22.91	0.60	11.38	0.30
<i>Megaledone seboi</i> (?)	1	-23.72	0.00	11.67	0.00
<i>Trematomus newnesi</i>	1	-23.88	0.00	10.84	0.00
<i>Pleuragramma antarcticum</i>	12	-24.25	0.16	9.85	0.16
WS MUM parturition AQ	8	-24.64	0.19	12.54	0.24
WS MUM parturition PR	8	-24.01	0.13	13.46	0.10
WS MUM late lactation AQ	7	-24.60	0.26	11.97	0.22
WS MUM late lactation PR	7	-23.90	0.11	13.23	0.04
WS PUP parturition AQ	9	-25.93	0.26	13.80	0.15
WS PUP parturition PR	9	-24.62	0.08	14.87	0.11
WS PUP weaning AQ	7	-25.06	0.34	13.41	0.26
WS PUP weaning PR	7	-24.43	0.11	14.69	0.14
WS Milk parturition AQ	7	-29.46	0.10	6.79	0.27
WS Milk parturition PR	6	-26.32	0.27	14.97	0.10
WS Milk late Lactation AQ	4	-28.90	0.16	5.85	0.05
WS Milk late Lactation PR	4	-25.80	0.15	14.55	0.13

b

Table 2: Summary of the isotopic (carbon and nitrogen) ratios relative to Weddell seal prey items and of the mums (parturition and late lactation), pups (parturition and early weaning) and milk (parturition and late lactation – only lipid extracted samples). Non lipid extracted samples (a) and lipid extracted samples (b). Mean value \pm Standard Error.

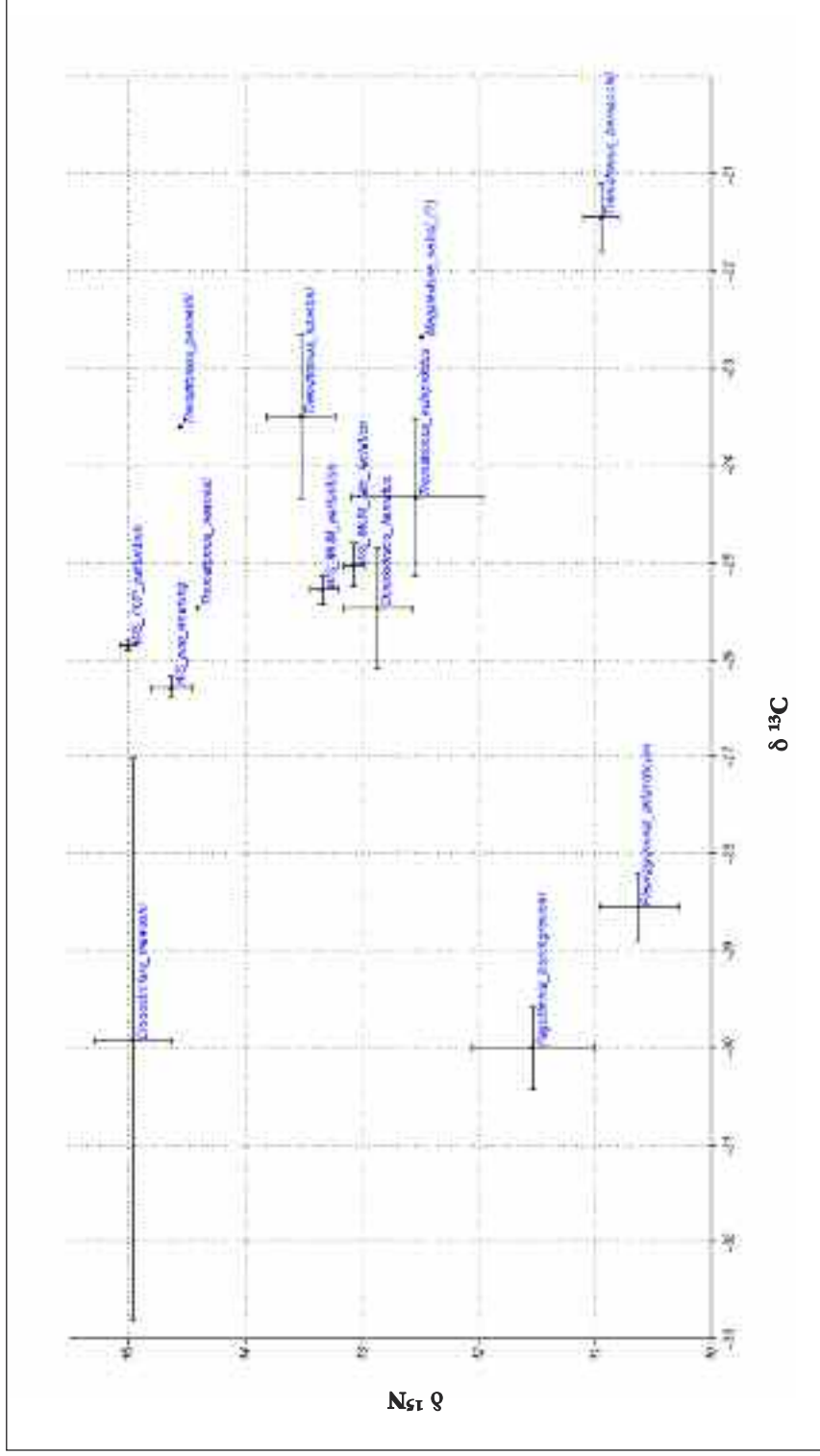


Fig. 9a: The Ross sea trophic web reconstructed by stable carbon and nitrogen isotopes analysis carried out on whole samples. Mean value ± Standard Error.

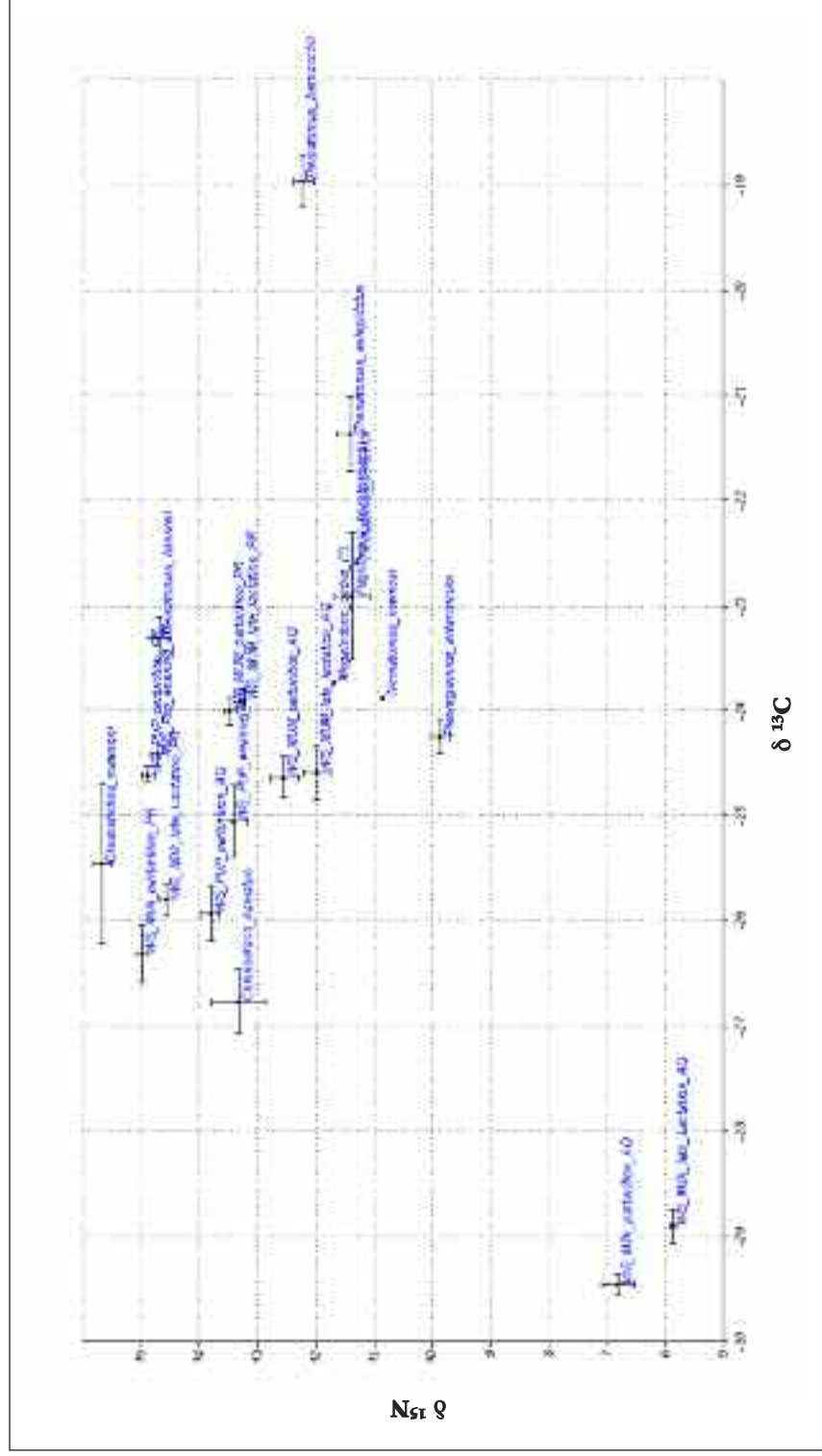


Fig. 9b: The Ross sea trophic web reconstructed by stable carbon and nitrogen isotopes analysis carried out on lipid-extracted samples. Mean value ± Standard Error.

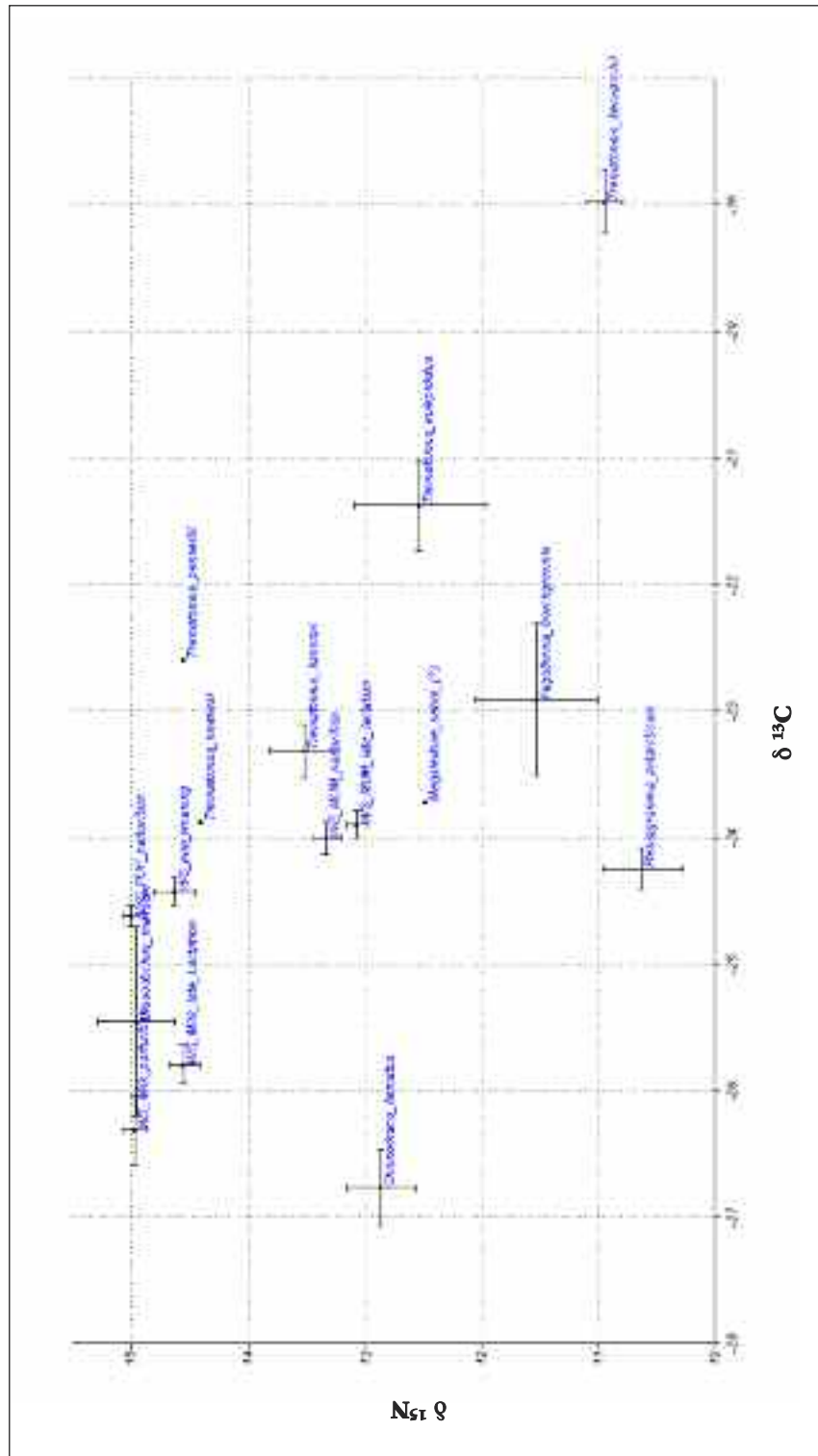


Fig. 9c: The Ross sea trophic web reconstructed by stable carbon and nitrogen isotopes analysis carried out on lipid extracted samples (for carbon isotopic ratios) and whole samples (for nitrogen isotopic ratios). Mean value \pm Standard Error.

Among non lipid-extracted samples the most carbon enriched fish was the emerald rockcod, *Trematomus bernacchii* ($\delta^{13}\text{C} = -21.45 \pm 0.35$. Mean \pm SE), while the most carbon depleted one was the bald notothen, *Pagothenia borchgrevinkii* ($\delta^{13}\text{C} = -30.01 \pm 0.43$. Mean \pm SE); after lipid removal *Trematomus bernacchii* ($\delta^{13}\text{C} = -18.97 \pm 0.25$. Mean \pm SE) was still the most carbon enriched while the most depleted one was *Chionadraco hamatus* ($\delta^{13}\text{C} = -26.77 \pm 0.30$. Mean \pm SE).

It is very important to highlight that *Chionadraco hamatus* and *Megalopterus bonapartei* showed a slight decrease in the $\delta^{13}\text{C}$ values from lipid-extracted samples compared to whole samples.

For nitrogen, the highest value was that of the large Antarctic cod, *Dissostichus mawsoni* (Whole samples: $\delta^{15}\text{N} = 14.96 \pm 0.33$. Lipid-extracted samples: $\delta^{15}\text{N} = 15.66 \pm 0.18$. Mean \pm SE), while the Antarctic silverfish, *Pleuragramma antarcticum*, had the lowest one (Whole samples: $\delta^{15}\text{N} = 10.62 \pm 0.34$. Lipid-extracted samples: $\delta^{15}\text{N} = 9.85 \pm 0.18$. Mean \pm SE).

Figure 9a-b shows the Ross sea trophic web reconstructed using the results of the stable isotope analyses on whole samples and lipid extracted samples, respectively; but since lipid extraction affects $\delta^{15}\text{N}$ values as well - as this work seemed to confirm (Sotiropulos et al., 2004; Sweeting et al., 2006; Zhao et al., 2006; Mintenbeck et al., 2008), figure 9c shows the Ross sea trophic web based on $\delta^{13}\text{C}$ values of lipid-extracted samples and $\delta^{15}\text{N}$ values of untreated samples.

This last trophic web was used for data interpretation and conclusion.

3.2 - DIVE DATA

3.2.1 - MT-Dive

A total of 25275 dives were analysed by MT-Dive:

- 4518 lactating female dives, season 1; 10 individuals
- 4951 lactating female dives, season 2; 6 individuals
- 15806 pup dives, season 1; 8 individuals

The recording periods ranged from 31 to 54 days after parturition (mean 39.7, SD 5.1344) amongst adult females and from 42 to 81 days after parturition (mean 67.8, SD 12.6124) amongst pups.

For comparisons, data were subdivided in 4 (mums) and 5 (pups) pools corresponding to:

- Adult females

1. Early Lactation - 1 to 13 DPP
2. Mid Lactation - 14 to 26 DPP
3. Late Lactation - 27 to 33 DPP
4. Final - 34 to the end of the recording period

- Pups

1. Early Lactation - 1 to 13 DPP
2. Mid Lactation - 14 to 26 DPP
3. Late Lactation - 27 to 33 DPP
4. End Lactation - 34 to 42 DPP
5. Early Weaning - 43 to the end of the recording period

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All these dive data are shown in Table 3a-b.

Mum's data of pool 1, 2 and 3 from the two field seasons were statistically compared showing no significant differences in number of dives, means of maximum depths, means of mean depths and mean durations (Mann-Whitney test, $p > 0.05$ in almost all cases except during comparison of number of dives in pool 2, $p = 0.045$).

Mothers and pups were statistically compared for the same above mentioned categories but with regard only to the seals of the six related pairs (mum with her own pup; M40-P6651, M105-P6695, M265-P6645, M515-P6698, M819-6568 and M1043-P6787); the paired - Wilcoxon tests showed no relevant statistical differences between mum and pup's number of dives ($p > 0.05$), but significant for all the other categories ($p < 0.05$).

The deepest and longest dives of lactating females and pups throughout lactation and for each pool were:

- Mums, lactation (33 days):

1. field season 1: 422.0 m. (M265), 1990 sec. (M946)
2. field season 2: 423.0 m. (M114), 2300 sec. (M3013)

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- Pups, lactation (42 days): 113 m. (P6787), 910 sec. (P6651)
- Mums, pool 1:
 1. field season 1: 378.0 m. (M946, Figure 10), 1770 sec. (M946, Figure 11)
 2. field season 2: 199.0 m. (M578), 890 sec. (M578)
- Pups, pool 1: 0 m., 0 sec.
- Mums, pool 2:
 1. field season 1: 418.0 m. (M946, Figure 12), 1990 sec. (M946 Figure 12)
 2. field season 2: 394.0 m. (M1343), 1610 sec. (M114)
- Pups, pool 2: 35.0 m. (P6568, Figure 13), 470 sec. (P6568, Figure 14)
- Mums, pool 3:
 1. field season 1: 422.0 m. (M265), 1920 sec. (M946)
 2. field season 2: 423.0 m. (M114, Figure 15), 2300 sec. (M3013, Figure 16)
- Pups, pool 3: 80.5 m. (P6787), 570 sec. (P6651)
- Mums, final:
 1. field season 1: 433.5 m. (M479), 1900 sec. (M265)
 2. field season 2: 468.5 m. (M935, Figure 17), 2150 sec. (M3013)
- Pups, pool 4: 113.0 m. (P6787, Figure 18), 910 sec. (P6651, Figure 19)
- Pups, early weaning: 366.0 m. (P6698, Figure 20), 1280 sec. (P6645, Figure 21a-b)

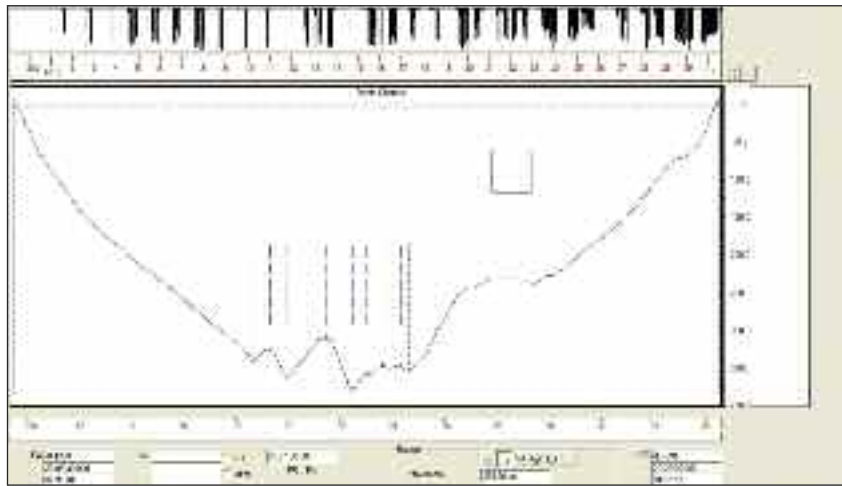


Fig. 10

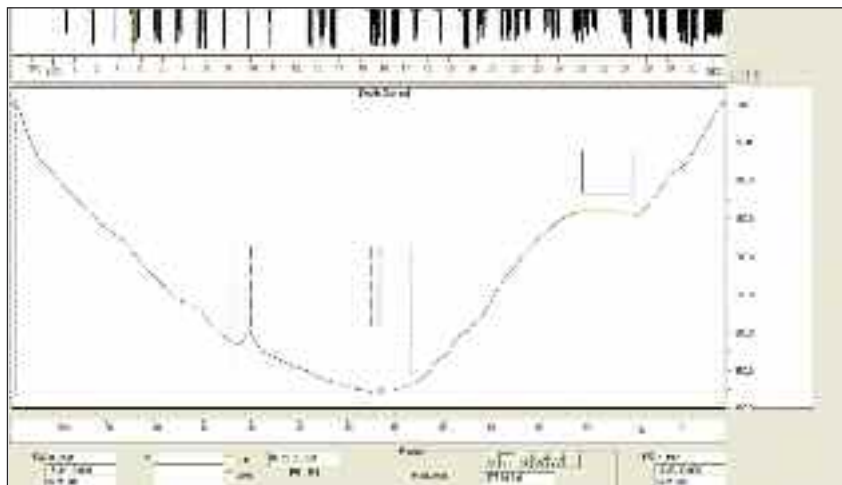


Fig. 11

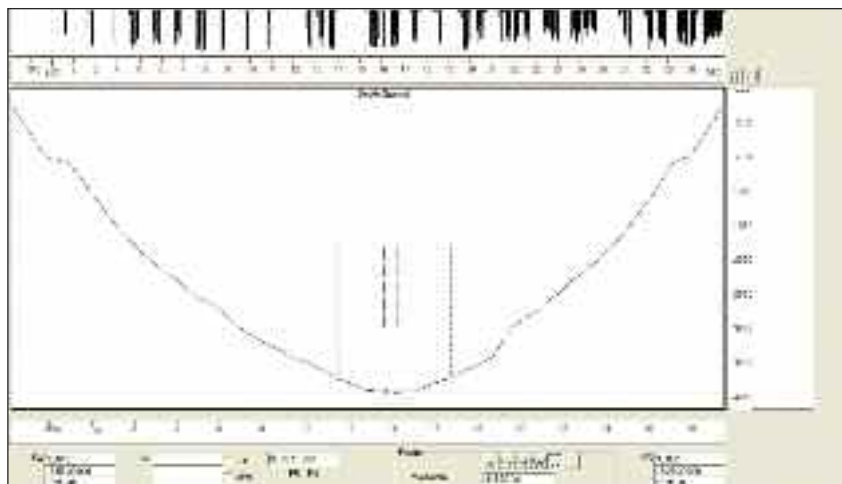


Fig. 12

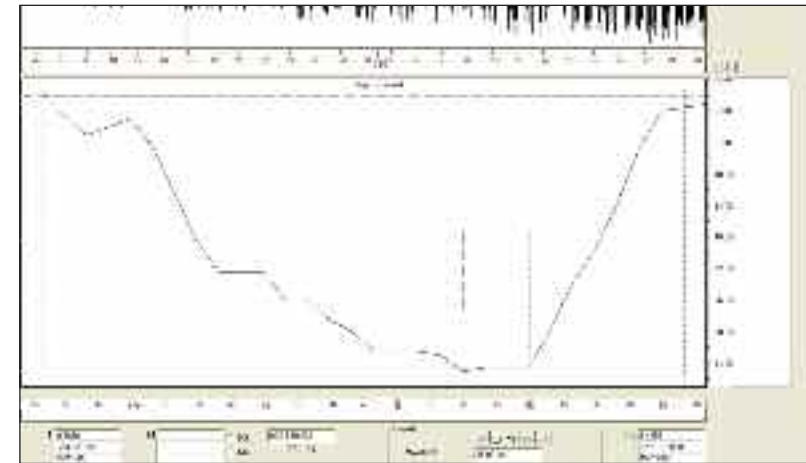


Fig. 13

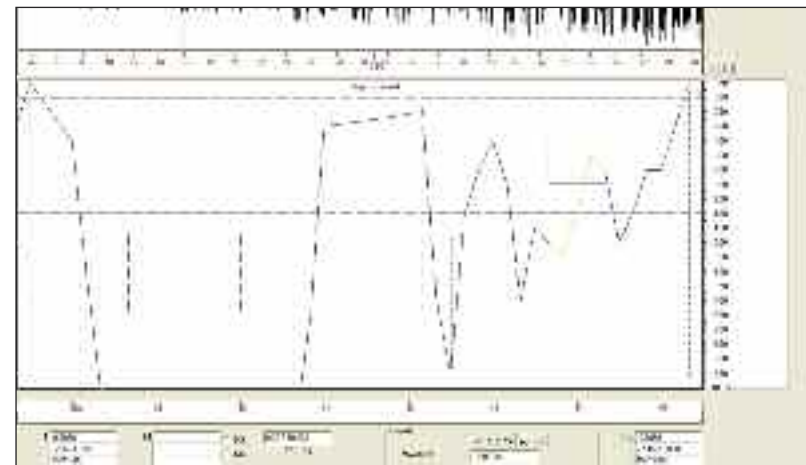


Fig. 14

Fig. 14: There is a certain degree of doubt whether considering this profile a unique dive or two immersions since the animal spends more than a minute very close to the water surface (too close?) and it is very likely the seal breathed.

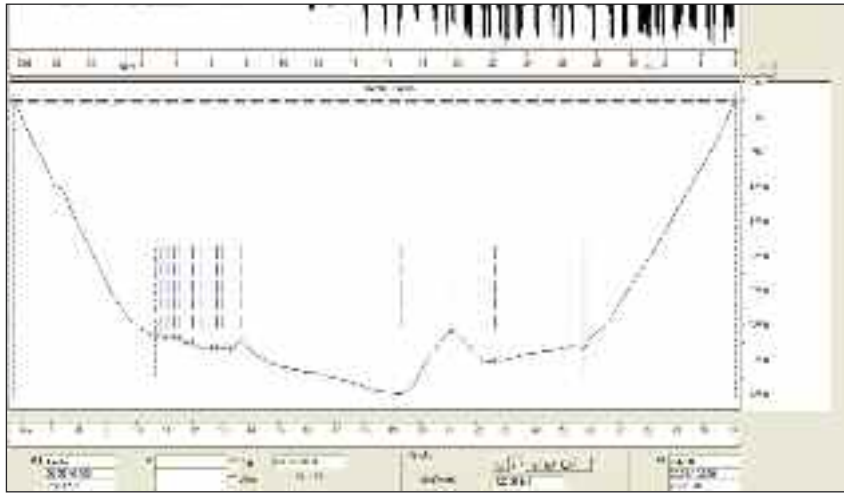


Fig. 15

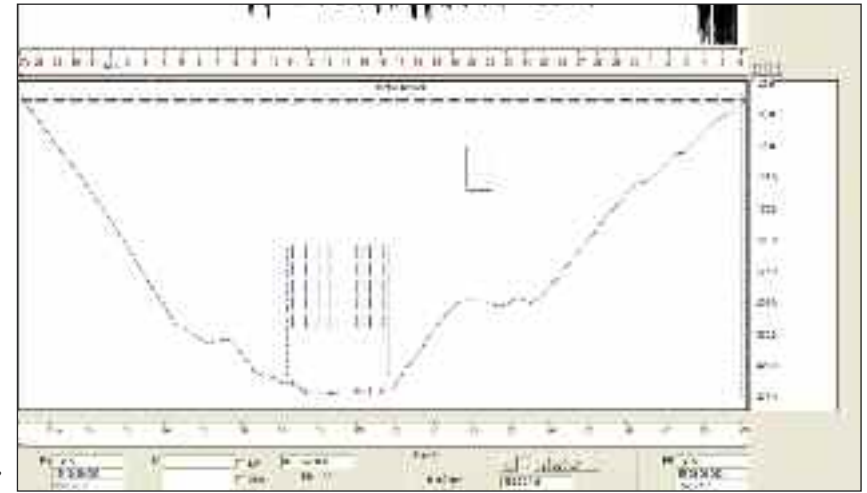


Fig. 17

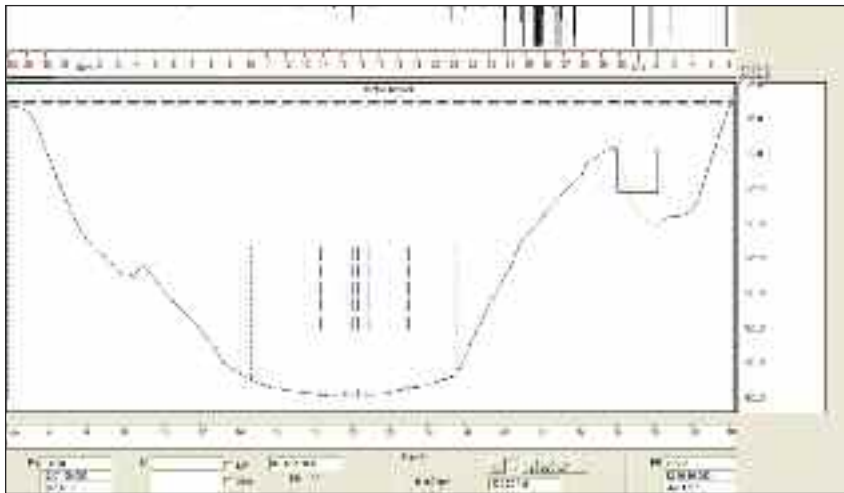


Fig. 16

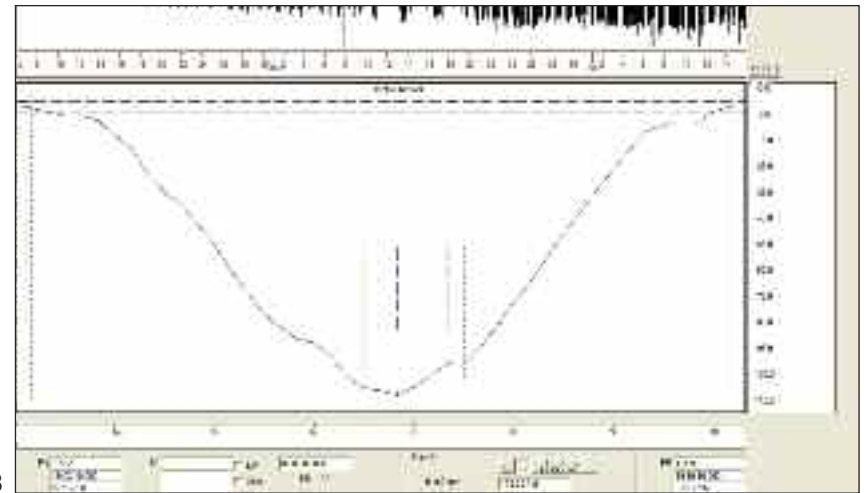


Fig. 18

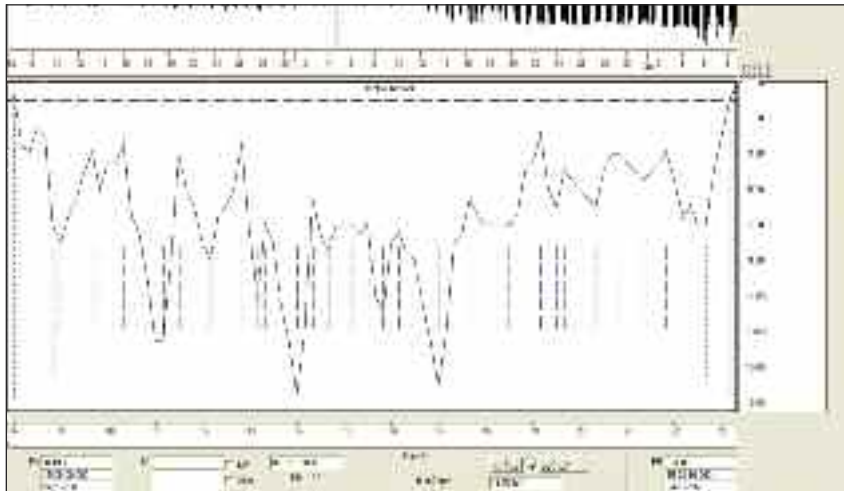
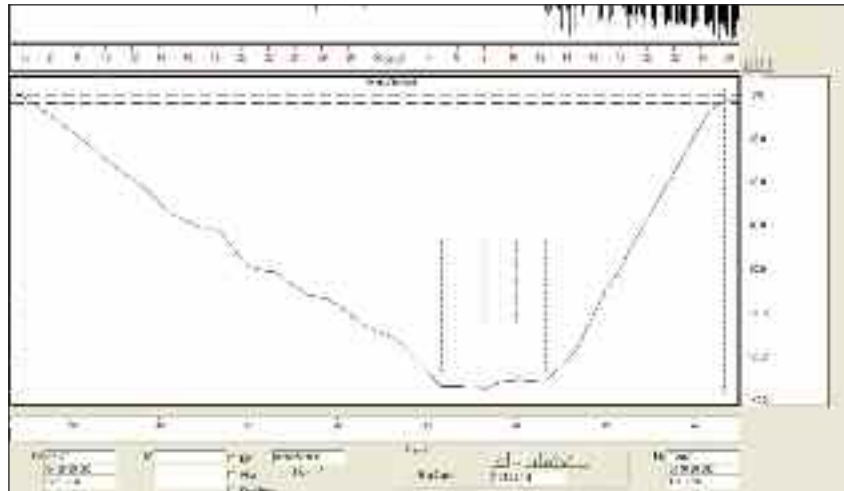


Fig. 19

Fig. 19: It is relevant to notice that the pups' longest dives (see also Fig. 14), during lactation, were those performed in shallow water, with one or more peaks a little bit deeper.



a

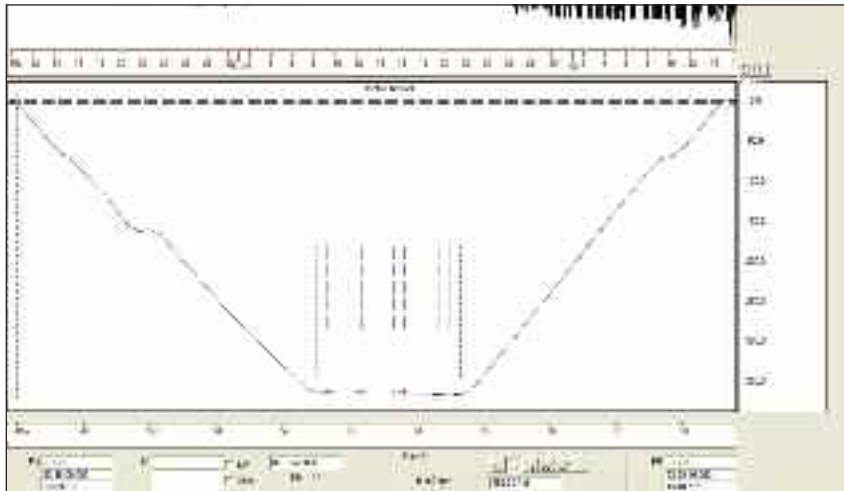
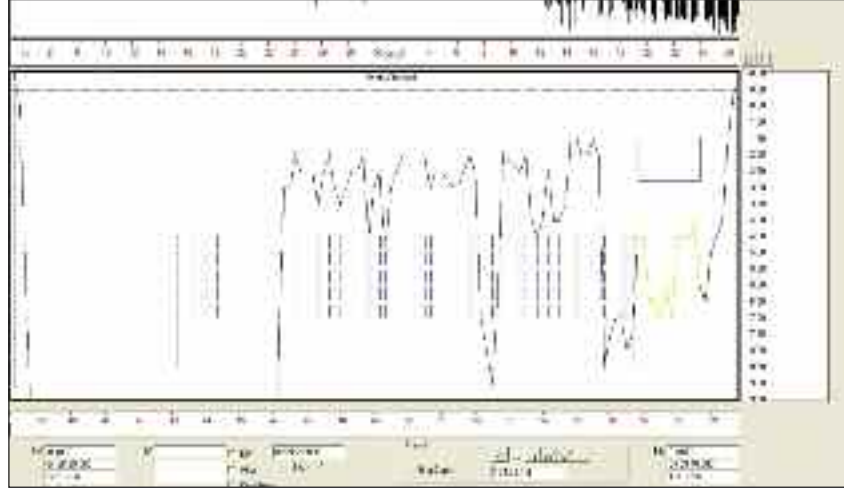


Fig. 20



b

Fig. 21: These two profiles show an important problem of the software in singling out the dive: the first profile (a) is recognized as a single dive and as the first part of the following dive (b).

Both lactating females and pups showed increasing number of dives, means of maximum depths, means of mean depths and mean dive durations.

Time per day spent underwater increased throughout lactation and early weaning as well (Figure 22).

No statistical analyses were applied to compare the proportion of U, u, V, W and Y shaped profiles since these info were considered not completely representative (misleading) of the real underwater activity.

It is relevant pointing out that U-shapes were the great majority in all cases, both for mums and pups (see Table 3a-b).

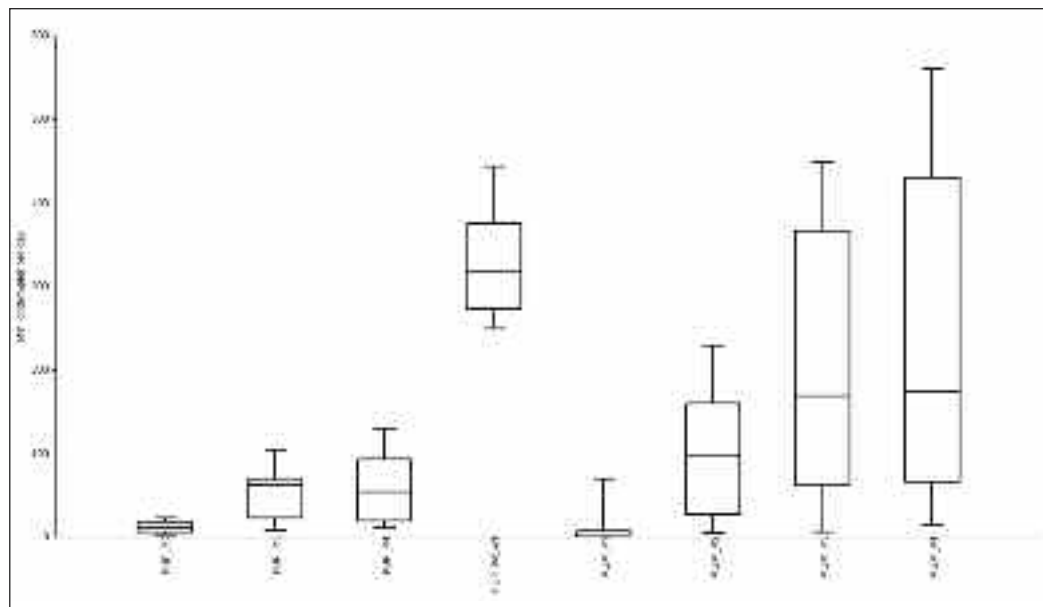


Fig. 22: Box Plot of mums and pups' maximum depths in pool 1, 2, 3, 4 and weaning. Pups did not dive in pool 1. Boxes represent the second and third quartile and the medians; whiskers show the first and fourth quartile, with the extreme values.

3.2.2 - SOMs

Data for SOMs were further filtered to avoid trivial results “contaminated” by the numerous dives recorded in very shallow water, the profiles of which could not be clearly categorized and interpreted.

Mothers and pups dives (singularly and pooled together) performed during the first 5 weeks of lactation were discriminated by individualizing the peak of the Kernel density in the distribution of the depths and durations.

As result, the dives analyzed using SOMs were:

- Mums (35 DPP) - all dives deeper than 15.0 meter and longer than 02 min. 40 sec. 2781 dives
- Pups (35 DPP) - all dives deeper than 10.0 meter and longer than 02 min. 00 sec. 375 dives
- Mums and Pups (35 DPP) - all dives deeper than 14.5 meter and longer than 02 min. 40 sec. 3014 dives
- Pups (early weaning) - all dives deeper than 17.0 meter and longer than 05 min. 00 sec. 5172 dives

Data and graphic outputs are shown in Tables 4 to 10 and Figures 23 to 29.

Due to their homogeneity, only the results of the SOMs, that were run using the Hellinger distance, are reported:

- Mums (35 DPP): Hellinger distance.
 1. SOM 3X4

the most represented cluster ($y/x = 3/4$, Figure 23a-b, Table 4) was the one more assimilable to a V-profile (26.1%) which also showed the highest mean depth (263.7 meter; minimum 28.0, maximum 422.5) and mean duration (17 min. 59 sec.; minimum 02 min. 40 sec., maximum 34 min. 20 sec.). The deepest dive (428 meter) was clustered within a parabola-profile cluster ($y/x = 1/3$) and the longest (38 min. 00 sec.) within a V/parabola and a V/skewed right profile clusters ($y/x = 1/1$ and $1/2$).

No U-shaped profile clusters were clearly represented.
 2. SOM 9X12

the most represented cluster ($y/x = 6/3$, Figure 24a-b, Table 5) was the

one more assimilable to a parabola-profile (2.4%); the highest mean depth (322.8 meter; minimum 113.5, maximum 423.0) was that of a U/parabola-profile cluster ($y/x = 7/12$) and the highest mean duration (23 min. 13 sec.; minimum 05 min. 30 sec., maximum 27 min. 40 sec.) that of a parabola-profile cluster ($y/x = 8/5$). The deepest dive (428 meter) was clustered within a U-profile cluster ($y/x = 4/1$) and the longest (2 x 38 min. 00 sec.) within a skewed right and a U profile clusters ($y/x = 6/12$ and $9/5$).

U-shaped and V-shaped profile clusters were clearly represented in the map accounting for 8.3% and 14.9% of the total respectively; clusters with bi and tri-modal dive profiles also appeared .

- Pups (35 DPP): Hellinger distance.

1. SOM 3X4

the most represented cluster ($y/x = 1/2$, Figure 25a-b, Table 6) was the one more assimilable to a slightly skewed-left V-profile (11.7%) which also showed the highest mean duration (03 min. 55 sec.; minimum 02 min. 00 sec., maximum 06 min. 10 sec.). The highest mean depth (44.6 meter; minimum 20.0, maximum 81.0) was within a V-profile cluster ($y/x = 1/1$).

The deepest dive (84 meter) was clustered within the same slightly skewed-left V-profile cluster ($y/x = 1/2$) and the longest (07 min. 00 sec.) within a skewed right profile cluster ($y/x = 3/1$).

No U-shaped profile clusters were represented at all.

2. SOM 9X12

Due the limited number of dives (375) the SOM 9X12 was not performed.

- Mums and Pups (35 DPP): Hellinger distance.

1. SOM 3X4

the most represented cluster ($y/x = 3/4$, Figure 26a-b, Table 7) was the one more assimilable to a V-profile (24.6%) which also showed the highest mean depth (264.3 meter; minimum 28.0, maximum 422.5) and mean duration (18 min. 05 sec.; minimum 02 min. 40 sec., maximum

37 min. 30 sec.). The deepest dive (428 meter) was clustered within a parabola-profile cluster ($y/x = 3x3$) and the longest (2 x 38 min. 00 sec.) within a V/skewed right profile and a V/parabola clusters ($y/x = 3x1$ and $3x2$).

No U-shaped profile clusters were clearly represented.

2. SOM 9X12

the most represented cluster ($y/x = 6x3$, Figure 27a-b, Table 8) was the one more assimilable to a parabola/V-profile (2.6%) which also showed the highest mean depth (328.0 meter; minimum 175.0, maximum 419.0) and mean duration (22 min. 26 sec.; minimum 09 min. 50 sec., maximum 33 min. 10 sec.). The deepest dive (428 meter) was clustered within a U-profile cluster ($y/x = 3/2$) and the longest (2 x 38 min. 00 sec.) within a U and a skewed right profile clusters ($y/x = 9x9$ and $7x12$).

U-shaped and V-shaped profile clusters were clearly represented in the map accounting for 10.6% and 16.7% of the total respectively; clusters with bi and tri-modal dive profiles were less evident.

As expected, due to the low percentage “weight” of the pups compared with their mums, the results of these maps tightly followed those of Mums (35 DPP), giving an indirect confirmation of the SOM reliability.

- Pups (early weaning): Hellinger distance.

1. SOM 3X4

the most represented cluster ($y/x = 2/3$, Figure 28a-b, Table 9) was the one more assimilable to a U-profile (22.4%; V-profile 8.0%); the highest mean depth (118.7 meter; minimum 19.5, maximum 366.0) was that of the V-profile cluster ($y/x = 1/4$) and the highest mean duration (07 min. 11 sec.; minimum 05 min. 10 sec., maximum 17 min. 10 sec.) that of a skewed right-profile cluster ($y/x = 3/1$). The deepest dive (366.0 meter) was clustered within the V-profile cluster ($y/x = 1/4$) and the longest (31 min. 40 sec.) within a parabola/V-profile - slightly skewed left - cluster ($y/x = 2/4$).

U-shaped profile clusters accounted for 51.7% of the total.

2. SOM 9X12

the most represented cluster ($y/x = 1/4$, Figure 29a-b, Table 10) was the one more assimilable to a U-profile (2.9%); the highest mean depth (149.7 meter; minimum 51.0, maximum 363.0) was that of a parabola-profile cluster ($y/x = 2/1$) and the highest mean duration (08 min. 11 sec.; minimum 05 min. 10 sec., maximum 16 min. 00 sec.) that of a skewed right-profile cluster ($y/x = 9/6$). The deepest dive (366.0 meter) was clustered within a parabola-profile cluster and the longest (31 min. 40 sec.) within an another Y-profile cluster.

U-shaped and V-shaped dives accounted for 24.6% and 1.1% (only 1 cluster clearly detectable) of the total respectively; multi-modal dive profiles were very evident.

All SOMs showed a substantive importance of the skewed right-shaped profiles while skewed left-shaped profiles were much less represented both from a quantitative point of view and as regards to the depth range. In fact, skewed left shapes were recorded only in dives that were performed in shallow water.

ID Count		x				
y		1	2	3	4	Grand Total
1		211	314	517	725	1767
2		140	168	218	159	685
3		104	66	80	79	329
Grand Total		455	548	815	963	2781

Depth Mean		x				
y		1	2	3	4	Grand Total
1		237.6	221.0	220.6	263.7	240.4
2		167.7	52.5	46.6	88.1	82.4
3		204.9	82.1	21.9	25.1	92.6
Grand Total		208.6	152.6	154.6	215.1	184.0

Mean Durat.		x				
y		1	2	3	4	Grand Total
1		0.16.29	0.14.11	0.14.35	0.17.59	0.16.08
2		0.14.43	0.04.36	0.04.27	0.06.17	0.07.01
3		0.16.16	0.10.14	0.04.13	0.03.48	0.09.08
Grand Total		0.15.54	0.10.46	0.10.51	0.14.53	0.13.04

Table 4: Summary of mums (35 DPP) 3X4 SOM: number of dives, mean depth and mean duration.

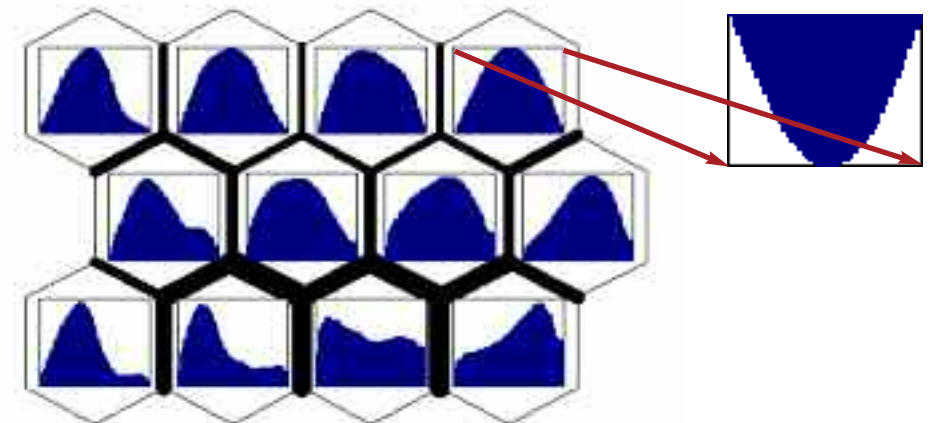


Fig. 23: Mums (35 DPP) 3X4 SOM graphic output; the red arrows indicate the most represented shape. The thickness of the lines among hexagons is proportional to the distance between two neighbour-clusters.

ID Count		x												Grand Total
y		1	2	3	4	5	6	7	8	9	10	11	12	
1	26	18	33	15	13	15	12	5	15	16	20	22	205	
2	15	28	17	21	13	11	10	9	12	9	10	24	185	
3	25	25	30	13	20	20	18	15	12	18	19	25	254	
4	4	5	21	25	18	20	10	9	17	21	17	25	297	
5	6	8	28	15	13	11	17	24	15	15	23	26	313	
6	40	50	66	50	13	28	14	20	25	28	12	29	382	
7	24	50	38	56	15	27	18	38	27	10	16	41	365	
8	24	07	70	44	54	31	34	26	25	16	20	23	412	
9	20	16	26	33	24	26	22	18	25	10	26	24	300	
Grand Total		302	371	312	273	200	191	183	154	105	152	180	2781	

Mean Depth		x												Grand Total
y		1	2	3	4	5	6	7	8	9	10	11	12	
1	31.3	18.3	51.7	35.8	30.2	34.6	19.8	17.2	22.5	21.0	29.0	36.2	377.0	
2	172.5	154.6	36.5	52.0	38.1	26.1	39.2	23.9	25.7	22.2	43.6	202.7	98.0	
3	252.0	185.6	159.6	137.0	77.5	37.6	31.1	18.0	20.4	19.7	57.3	135.4	119.4	
4	257.5	200.6	236.6	210.3	72.7	67.7	67.0	44.7	62.1	214.7	21.8	250.0	105.1	
5	745.1	224.6	227.3	122.8	105.5	26.6	26.8	25.8	43.6	129.6	211.5	288.6	189.0	
6	181.1	218.6	305.8	242.3	52.3	65.9	54.9	42.9	58.5	56.5	28.0	285.8	173.8	
7	233.2	31.1	257.9	212.7	150.7	70.6	51.0	57.0	61.2	42.5	45.5	222.0	201.8	
8	332.2	282.0	272.6	219.4	277.2	230.4	200.1	159.5	104.0	66.0	188.7	250.7	237.8	
9	222.8	26.1	227.3	275.0	322.5	304.7	227.1	261.5	265.0	282.4	289.6	221.7	281.1	
Grand Total		225.4	245.3	228.3	237.2	173.1	114.7	127.1	83.5	98.0	101.1	128.3	259.0	184.0

Mean Durat.		x												Grand Total
y		1	2	3	4	5	6	7	8	9	10	11	12	
1	0.16	0.10	0.16	0.14	0.12	0.11	0.10	0.09	0.08	0.07	0.06	0.05	0.04	0.03
2	0.14	0.13	0.08	0.08	0.05	0.03	0.03	0.04	0.02	0.02	0.01	0.01	0.01	0.01
3	0.13	0.14	0.13	0.09	0.05	0.02	0.03	0.04	0.03	0.03	0.02	0.02	0.01	0.01
4	0.14	0.19	0.16	0.13	0.11	0.09	0.08	0.06	0.05	0.04	0.03	0.02	0.01	0.01
5	0.14	0.13	0.11	0.08	0.07	0.04	0.03	0.03	0.02	0.02	0.01	0.01	0.01	0.01
6	0.13	0.15	0.20	0.14	0.09	0.05	0.03	0.03	0.02	0.02	0.01	0.01	0.01	0.01
7	0.17	0.22	0.17	0.12	0.08	0.05	0.04	0.03	0.02	0.02	0.01	0.01	0.01	0.01
8	0.17	0.18	0.17	0.12	0.09	0.07	0.06	0.05	0.04	0.03	0.02	0.01	0.01	0.01
9	0.17	0.19	0.18	0.13	0.10	0.08	0.07	0.06	0.05	0.04	0.03	0.02	0.01	0.01
Grand Total		0.15	0.17	0.15	0.12	0.09	0.07	0.06	0.05	0.04	0.03	0.02	0.01	0.01

Table 5: Summary of mums (35 DPP) 9X12 SOM: number of dives, mean depth and mean duration

ID Count		x				Grand Total
y		1	2	3	4	
1	29	44	38	37	148	
2	37	41	41	16	135	
3	29	29	16	18	92	
Grand Total		95	114	95	71	375

Mean Depth		x				Grand Total
y		1	2	3	4	
1	44.6	38.7	25.6	29.1	34.1	
2	28.5	17.4	16.9	20.2	20.6	
3	20.2	14.6	12.7	15.9	16.3	
Grand Total		30.8	24.9	19.7	23.8	24.9

Mean Durat.		x				Grand Total
y		1	2	3	4	
1	0.03	0.03	0.03	0.03	0.03	
2	0.03	0.02	0.03	0.02	0.02	
3	0.02	0.02	0.03	0.03	0.02	
Grand Total		0.03	0.03	0.03	0.03	0.03

Table 6: Summary of pups (35 DPP) 3X4 SOM: number of dives, mean depth and mean duration.

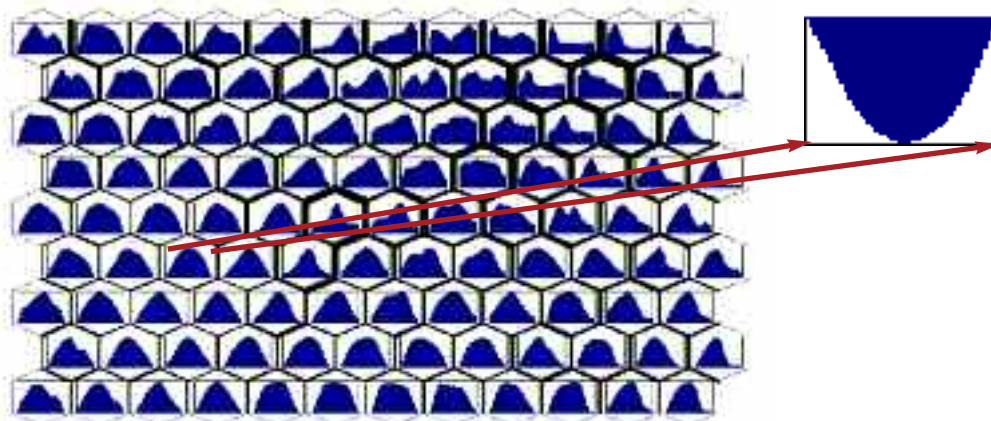


Fig. 24: Mums (35 DPP) 9X12 SOM graphic output; the red arrows indicate the most represented shape. The thickness of the lines among hexagons is proportional to the distance between two neighbour-clusters.

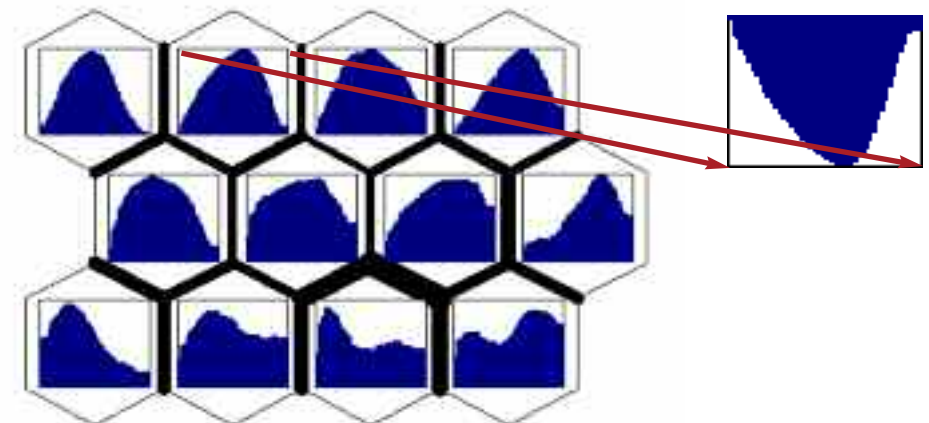


Fig. 25: Pups (35 DPP) 3X4 SOM graphic output; the red arrows indicate the most represented shape. The thickness of the lines among hexagons is proportional to the distance between two neighbour-clusters.

ID Count		x				
y		1	2	3	4	Grand Total
1		133	78	78	95	384
2		187	247	178	206	818
3		206	316	550	740	1812
Grand Total		526	641	806	1041	3014

Mean Depth		x				
y		1	2	3	4	Grand Total
1		201.2	63.5	20.4	23.6	92.6
2		78.3	48.9	37.9	72.5	59.2
3		244.5	210.9	222.0	264.3	239.9
Grand Total		174.5	130.5	161.8	204.4	172.1

Mean Durat.		x				
y		1	2	3	4	Grand Total
1		0.16.31	0.08.34	0.04.14	0.03.42	0.09.14
2		0.07.32	0.04.10	0.04.25	0.05.31	0.05.20
3		0.17.06	0.13.35	0.14.40	0.18.05	0.16.09
Grand Total		0.13.33	0.09.21	0.11.24	0.14.17	0.12.20

Table 7: Summary of mums and pups (35 DPP) 3X4 SOM: number of dives, mean depth and mean duration.

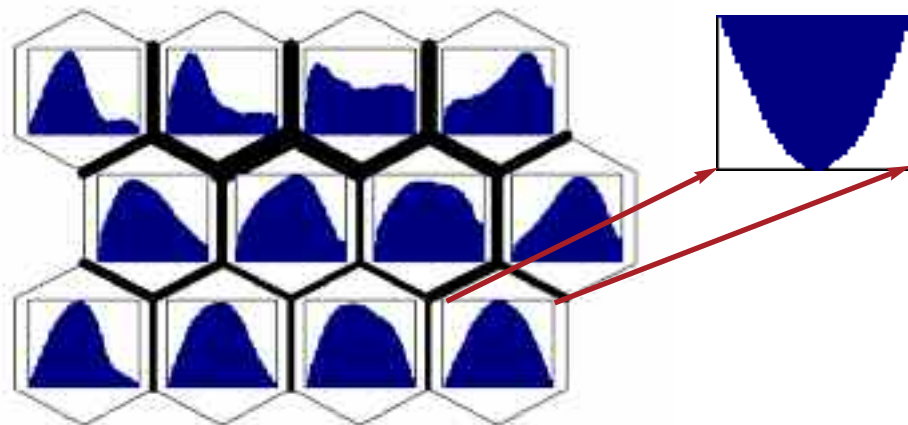


Fig. 26: Mums and pups (35 DPP) 3X4 SOM graphic output; the red arrows indicate the most represented shape. The thickness of the lines among hexagons is proportional to the distance between two neighbour-clusters.

ID Count		x												
y		1	2	3	4	5	6	7	8	9	10	11	12	Grand Total
1		32	17	20	17	26	21	15	10	15	20	17	12	215
2		21	26	21	34	23	23	14	15	15	12	10	31	243
3		31	40	33	27	24	18	22	28	18	18	20	21	318
4		43	21	35	16	15	11	31	22	14	16	16	44	306
5		67	65	54	15	14	10	8	30	17	24	26	26	390
6		45	35	77	29	18	13	34	31	23	30	24	28	384
7		15	71	22	55	14	24	20	27	23	19	22	45	362
8		43	48	37	45	43	41	36	30	18	37	28	37	430
9		41	74	49	19	40	36	37	21	44	24	41	36	418
Grand Total		362	367	348	257	202	183	209	218	194	192	206	282	3014

Mean Depth		x												
y		1	2	3	4	5	6	7	8	9	10	11	12	Grand Total
1		73.7	115.7	96.8	59.7	25.3	29.8	22.6	29.1	21.3	30.1	34.8	52.5	66.8
2		160.5	170.9	52.4	41.3	59.0	19.8	24.4	19.2	21.3	19.9	63.8	178.8	77.7
3		264.2	220.0	179.0	59.7	67.0	24.1	25.3	29.1	19.7	19.7	47.4	115.2	120.9
4		263.7	190.2	70.0	236.2	42.0	30.1	53.3	34.8	26.5	42.3	77.0	232.9	189.8
5		232.2	295.7	229.3	100.8	105.2	26.8	26.0	29.5	37.5	37.5	200.7	231.7	191.2
6		234.6	209.4	328.0	323.4	51.4	26.5	45.4	54.5	41.0	56.0	196.8	249.4	175.0
7		127.5	307.2	79.0	296.5	75.2	69.5	48.4	44.2	59.2	49.8	43.4	328.3	176.5
8		251.2	297.1	289.6	226.2	269.4	299.6	70.6	137.0	55.2	83.8	148.9	284.9	321.8
9		221.4	283.8	271.4	134.5	233.0	343.7	261.2	48.8	305.2	263.8	271.6	253.4	280.6
Grand Total		228.8	250.9	231.9	134.3	143.8	130.8	81.6	88.3	111.7	75.9	139.8	233.6	172.3

Mean Durat.		x												
y		1	2	3	4	5	6	7	8	9	10	11	12	Grand Total
1		0.18.00	0.09.26	0.05.39	0.05.32	0.07.00	0.08.00	0.03.53	0.12.27	0.04.21	0.04.00	0.25.16	0.08.28	0.07.03
2		0.13.30	0.13.53	0.04.06	0.04.03	0.03.53	0.03.24	0.03.47	0.03.57	0.03.49	0.03.23	0.09.52	0.13.06	0.07.56
3		0.13.45	0.14.16	0.15.12	0.07.10	0.05.59	0.03.19	0.03.27	0.04.17	0.03.29	0.03.59	0.04.49	0.10.25	0.09.50
4		0.14.47	0.13.24	0.12.50	0.14.10	0.07.19	0.04.00	0.04.18	0.03.36	0.03.10	0.04.00	0.20.39	0.17.42	0.10.36
5		0.14.38	0.18.14	0.12.33	0.12.18	0.05.24	0.03.48	0.03.51	0.03.22	0.03.14	0.03.38	0.22.00	0.18.48	0.13.46
6		0.16.02	0.15.00	0.22.26	0.12.41	0.05.24	0.03.31	0.03.31	0.03.35	0.03.37	0.03.25	0.14.19	0.18.54	0.12.17
7		0.10.04	0.21.48	0.04.10	0.13.47	0.04.04	0.04.49	0.04.40	0.03.55	0.03.45	0.04.24	0.04.41	0.21.55	0.12.28
8		0.10.58	0.19.24	0.12.43	0.13.46	0.21.45	0.22.26	0.06.00	0.05.52	0.04.03	0.04.30	0.25.02	0.20.50	0.10.30
9		0.12.03	0.18.36	0.19.54	0.05.36	0.10.04	0.20.38	0.14.07	0.14.56	0.15.00	0.04.16	0.19.50	0.18.44	0.11.08
Grand Total		0.15.03	0.17.11	0.15.09	0.12.33	0.10.47	0.10.33	0.08.58	0.05.24	0.07.27	0.06.37	0.11.46	0.18.03	0.12.29

Table 8: Summary of mums and pups (35 DPP) 9X12 SOM: number of dives, mean depth and mean duration.

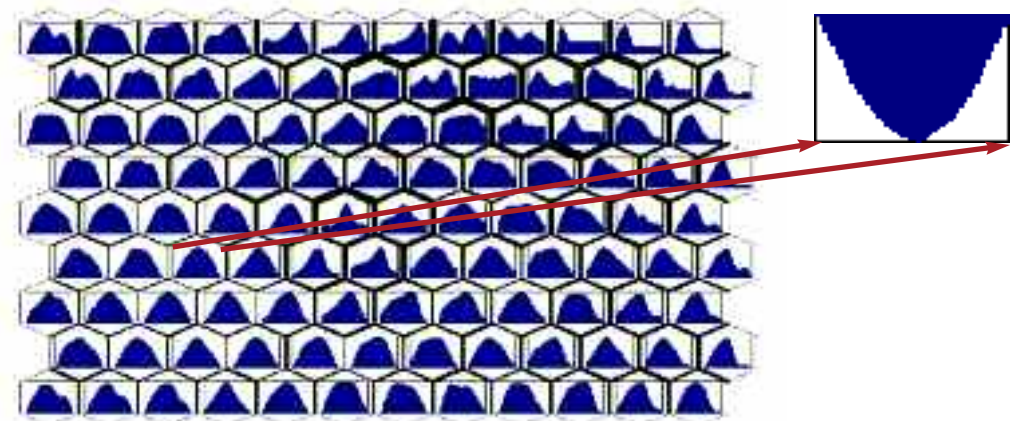


Fig. 27: Mums and pups (35 DPP) 9X12 SOM graphic output; the red arrows indicate the most represented shape. The thickness of the lines among hexagons is proportional to the distance between two neighbour-clusters.

ID Count		x				
y		1	2	3	4	Grand Total
1		207	360	638	412	1617
2		331	877	1160	450	2818
3		156	187	262	132	737
Grand Total		694	1424	2060	994	5172

Mean Depth		x				
y		1	2	3	4	Grand Total
1		80.8	86.6	100.7	118.7	99.6
2		61.2	97.6	116.6	110.1	103.2
3		51.6	29.3	43.1	72.1	46.6
Grand Total		64.9	85.9	102.3	106.6	94.0

Mean Durat.		x				
y		1	2	3	4	Grand Total
1		0.07.04	0.06.55	0.06.27	0.06.42	0.06.42
2		0.06.26	0.06.35	0.06.32	0.06.38	0.06.33
3		0.07.11	0.07.03	0.06.33	0.06.36	0.06.49
Grand Total		0.06.48	0.06.44	0.06.30	0.06.39	0.06.38

Table 9: Summary of pups (early weaning) 3X4 SOM: number of dives, mean depth and mean duration.

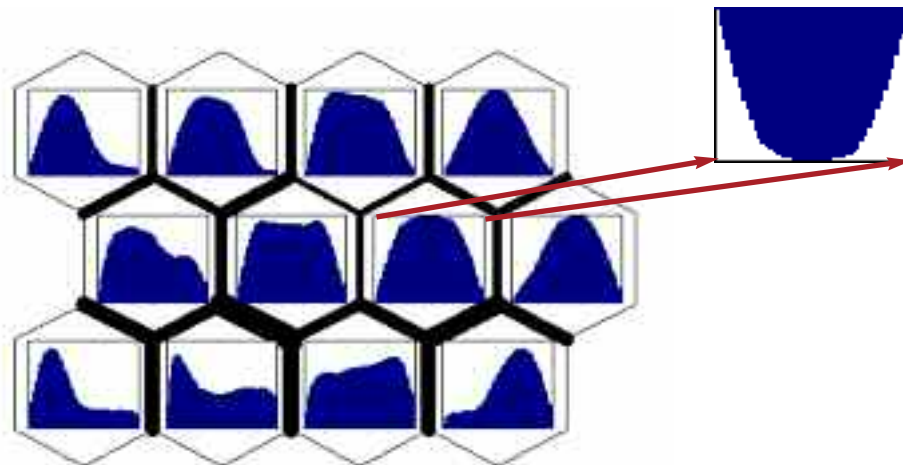


Fig. 28: Pups (early weaning) 3X4 SOM graphic output; the red arrows indicate the most represented shape. The thickness of the lines among hexagons is proportional to the distance between two neighbour-clusters.

ID Count		x												
y		1	2	3	4	5	6	7	8	9	10	11	12	Grand Total
1		45	98	110	149	55	36	23	30	51	34	88	51	727
2		65	112	79	103	48	48	45	85	88	60	96	45	808
3		62	75	79	54	42	85	96	91	131	90	105	54	926
4		175	14	30	47	37	40	37	37	65	31	24	29	507
5		54	86	80	27	45	19	49	19	37	36	63	21	481
6		45	89	25	25	35	24	29	30	52	29	28	23	409
7		45	39	44	39	19	13	26	25	42	30	32	34	384
8		45	78	40	33	37	44	43	50	34	21	19	47	400
9		33	81	59	57	34	26	24	36	49	27	20	35	480
Grand Total		484	590	507	629	395	330	362	357	611	362	430	331	5172

Mean Depth		x												
y		1	2	3	4	5	6	7	8	9	10	11	12	Grand Total
1		105.1	116.9	126.9	129.2	119.2	100.1	108.6	121.4	153.1	104.3	104.6	128.8	113.8
2		142.7	128.1	117.9	113.5	101.7	100.4	81.2	103.9	117.3	122.3	125.9	122.5	117.1
3		125.5	113.0	107.0	100.7	100.5	108.9	106.2	112.9	112.7	116.7	100.5	131.6	115.8
4		116.7	86.6	84.7	84.3	82.8	86.0	86.1	85.4	106.1	123.9	119.0	89.1	89.1
5		105.4	107.1	89.3	45.1	59.3	81.7	79.7	85.7	95.3	86.1	89.5	47.6	83.0
6		103.0	70.9	45.1	41.5	68.2	72.4	77.3	61.8	72.8	37.5	80.7	55.9	68.6
7		118.9	91.7	63.7	58.9	30.9	35.3	63.1	39.1	40.2	24.3	44.5	79.1	62.5
8		67.5	87.1	76.7	59.9	60.5	40.3	43.0	24.2	20.5	25.4	39.0	70.3	60.0
9		88.3	108.4	86.7	76.5	57.7	93.8	95.8	40.9	28.2	25.4	43.1	72.8	70.6
Grand Total		111.9	105.3	96.9	95.8	75.4	88.7	79.8	83.8	67.3	68.8	101.3	93.7	94.0

Mean Durat.		x												
y		1	2	3	4	5	6	7	8	9	10	11	12	Grand Total
1		0.06.21	0.06.17	0.06.21	0.05.30	0.06.15	0.06.07	0.06.16	0.07.10	0.06.21	0.07.10	0.06.25	0.06.06	0.06.20
2		0.06.33	0.06.10	0.06.13	0.05.15	0.05.57	0.06.21	0.06.13	0.05.38	0.07.02	0.06.31	0.06.31	0.06.40	0.06.24
3		0.06.55	0.06.31	0.06.25	0.05.58	0.07.32	0.06.50	0.06.50	0.07.14	0.06.44	0.06.48	0.06.27	0.06.28	0.06.44
4		0.06.12	0.06.25	0.06.36	0.06.06	0.06.29	0.06.16	0.06.20	0.07.06	0.06.40	0.06.30	0.06.30	0.06.21	0.06.20
5		0.06.55	0.06.10	0.06.15	0.05.51	0.06.25	0.06.48	0.06.20	0.05.20	0.06.10	0.06.30	0.06.20	0.07.09	0.06.25
6		0.06.45	0.06.18	0.06.46	0.05.04	0.06.22	0.06.37	0.06.25	0.05.30	0.06.31	0.06.03	0.06.39	0.07.42	0.06.30
7		0.06.45	0.07.01	0.06.31	0.07.18	0.07.07	0.06.37	0.06.48	0.05.42	0.06.15	0.06.55	0.06.29	0.05.28	0.06.44
8		0.07.05	0.06.40	0.06.20	0.07.23	0.06.47	0.06.98	0.06.43	0.07.22	0.07.07	0.06.44	0.07.33	0.08.21	0.06.53
9		0.07.21	0.07.21	0.06.56	0.07.17	0.06.40	0.08.11	0.06.57	0.07.16	0.07.10	0.07.10	0.06.30	0.05.20	0.07.11
Grand Total		0.06.47	0.06.30	0.06.27	0.05.31	0.06.34	0.06.44	0.06.36	0.05.58	0.06.43	0.06.43	0.06.35	0.05.38	0.06.38

Table 10: Summary of pups (early weaning) 9X12 SOM: number of dives, mean depth and mean duration.

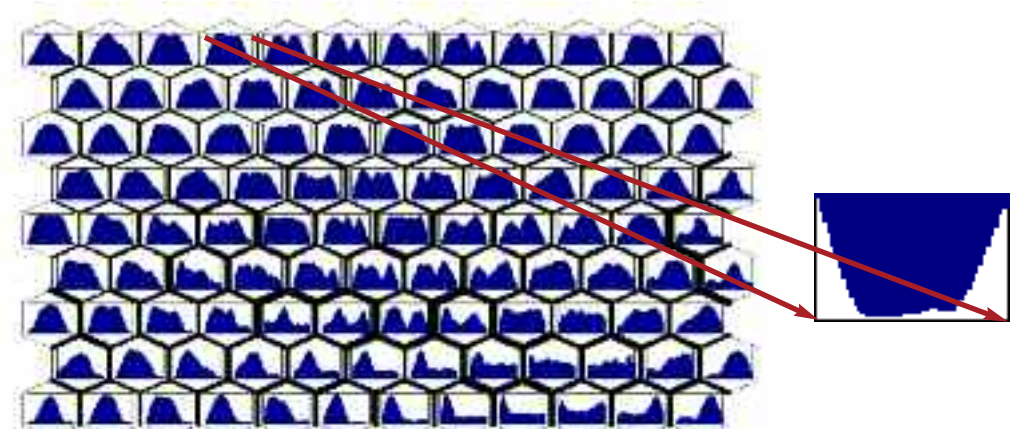


Fig. 29: Pups (early weaning) 9X12 SOM graphic output; the red arrows indicate the most represented shape. The thickness of the lines among hexagons is proportional to the distance between two neighbour-clusters.

3.2.3 - The Associator

Results of the 6 pairs concerning the time spent IN (overall and in shallow or deep water) or OUT of the water during the exactly associate period of data recording of the mums with their “own” pups are presented in Tables 11 and 12.

X² tests performed within each pairs showed a very strong statistical significance between the observed and the expected frequencies in 4 cases ($p << 0.01$); an almost significant difference ($p = 0.056$); and a non relevant difference ($p = 0.075$).

While the time OUT of the mothers was significantly correlated with their

1 rec./hour	Pair 1	Pair 2	Pair 3	Pair 4	Pair 5	Pair 6		
AA	305	556	491	438	311	391		
AB	62	23	19	71	66	67		
BA	181	41	137	22	166	62		
BB	62	80	25	8	88	18		
X ²	6.709	308	27.54	3.663	24.16	3.172		
p	0.01	6E-69	2E-07	0.056	9E-07	0.075		

%	Pair 1	Pair 2	Pair 3	Pair 4	Pair 5	Pair 6	Mean	± SE
AA	50.88	79.35	73.71	80.70	50.56	72.87	68.01	5.60867
AB	9.48	2.93	2.99	13.69	10.19	12.74	8.67	1.91435
BA	30.79	5.64	18.85	3.86	28.38	10.04	15.93	4.56366
BB	8.85	12.08	4.45	1.75	12.86	4.36	7.39	1.85804

%	Pair 1	Pair 2	Pair 3	Pair 4	Pair 5	Pair 6	Mean	± SE
AA	50.88	79.35	73.71	80.7	50.56	72.87	68.01	5.6086
AD	0.02	0	0.17	0	0.05	0.02	0.04	0.0264
AS	9.47	2.93	2.82	13.68	10.14	12.72	8.63	1.9275
DA	11.31	1.11	7.17	0.09	9.45	1.3	5.07	1.9768
DD	0.22	0.18	0.03	0	0.48	0.07	0.16	0.0723
DS	1.11	0.51	1.21	0	3.61	0.48	1.15	0.524
SA	19.49	4.54	11.68	3.78	16.94	8.73	10.86	2.825
SD	0.03	0.09	0.01	0	0.09	0.06	0.05	0.0161
SS	7.48	11.29	3.19	1.75	8.69	3.75	6.03	1.5103

Table 11: X² tests (a) were performed on data that were sampled randomly (1 record per hour); tables b and c show the percentage of the associate time spent in the water by the mums and their dependent pups of the six analyzed pair. Mean ± Standard Error.

A = OUT; B = IN; S = Shallow; D = Deep water. First letter, mum; second letter, pup.

time IN (paired-Wilcoxon test, $p = 0.02771$), there were no significant difference between time OUT of mothers and pups and time IN of mothers and pups (paired-Wilcoxon test, $p = 0.1159$; $p = 0.1159$).

As for mothers, the time OUT and IN of the pups was significantly correlated (paired-Wilcoxon test, $p = 0.02771$).

On average mums and pups spent 68.01% (min. 50.56%, max 80.70

%) of their associate time resting and pupping on the ice; for the 8.67% (min. 2.93, max 13.69) the pups were in the water and the mothers outside; for the 15.93% (min. 3.86%, max 30.79%) the mothers were in the water and the pups outside; and both mums and pups were together in the water for the 7.39% (min. 1.76%, max 12.86%) of the time.

In the pair 4, the pup spent more time in the water than his mother markedly (see Tables 11 and 12) but he remained almost exclusively in shallow water

Time in deep water was always bigger for mums than pups remarkably.

%	Pair 1	Pair 2	Pair 3	Pair 4	Pair 5	Pair 6	Mean	± SE
MUM A	60.36	82.28	76.70	94.38	60.75	85.61	76.68	5.61101
MUM B	39.64	17.72	23.30	5.62	39.25	14.39	23.32	5.61101
PUP A	81.67	85.00	92.56	84.55	76.94	82.91	83.94	2.08967
PUP B	18.33	15.00	7.44	15.44	23.06	17.09	16.06	2.08967

%	Pair 1	Pair 2	Pair 3	Pair 4	Pair 5	Pair 6	Mean	± SE
MUM A	60.37	82.28	76.70	94.38	60.75	85.61	76.68	5.61
MUM S	27.00	15.92	14.88	5.53	25.72	12.54	16.93	3.33
MUM D	12.64	1.80	8.41	0.09	13.54	1.85	6.39	2.42
PUP A	81.68	85.00	92.56	84.57	76.95	82.90	83.94	2.09
PUP S	18.06	14.73	7.22	15.43	22.44	16.95	15.81	2.04
PUP D	0.27	0.27	0.21	0.00	0.62	0.15	0.25	0.08

Table 12: Percentage of time spent OUT or IN the water (a) and OUT, IN Shallow and IN Deep water (b) individually by the mums and their dependent pups of the six analyzed pair. Mean ± Standard Error.

A = OUT; B = IN; S = Shallow; D = Deep water.

4. DISCUSSION



4.1 - Stable Isotopes

Looking with no critical spirit at the trophic web that springs out from the isotopic analyses of seals and fish (see Figure 9, Chapter 3), the only conclusion that could be drawn would be that the top predators in the Ross sea are the large Antarctic cod, *Dissostichus mawsoni*, and the Weddell seal pups.

Obviously, this paradoxical result can not be accepted but it is very important to move from this ambiguous statement of fact to prevent misleading data interpretations; above all because the isotopic results of this work are consistent with those of previous studies (Burns *et al.*, 1998; Zaho *et al.*, 2004; Bury *et al.*, 2008; Ainley and Siniff, 2009).

4.1.1 - Weddell seal isotopic signatures

Firstly, it is topical to highlight that, when $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios of pups and mums' plasma were compared, the $\delta^{15}\text{N}$ enrichment was contradicted by the $\delta^{13}\text{C}$ decrease.

This is fundamental because, in spite of the fact that nitrogen isotope signature is preferentially used to determine the trophic level at which an animal group forages and its diet composition (Kelly, 2000; Vanderklift and Ponsard, 2003; Crawford *et al.*, 2008. Stegall *et al.*, 2008), the carbon isotopic ratio as well should show a $\delta^{13}\text{C}$ enrichment – even if lighter than nitrogen – from a trophic step to the upper one (De niro and Epstein, 1978; Kelly, 2000), since both the carbon and nitrogen isotope composition of the consumer's tissues are directly related to its diet.

So, it appears too simplistic to justify and interpret the nitrogen enrichment found between mothers and pups' plasma (mums Aq./Pr./Wh., 11.99‰/13.34‰/13.24‰; pups Aq./Pr./Wh., 13.49‰/14.70‰/14.77‰. Means) like the expected trophic step shift (or almost) and base this conclusion on the assumption that pups “were effectively foraging on their mothers” or “consuming their mothers” (Hobson *et al.*, 1997 and 2000; Burns *et al.*, 1998; Jenkins *et al.*, 2001; Ducatez *et al.*, 2008).

In fact, not only carbon-isotope signature decreased from lactating females to pups, but also the protein $\delta^{15}\text{N}$ values of pups' plasma were almost perfectly

coincident with those of milk, showing no enrichment from the diet (milk) to the consumers (pups).

Since milk is the actual “prey” of the newborns (pups do not consume their mothers on the whole but they just assume a single tissue from them) the $\delta^{15}\text{N}$ enrichment was expected to happen between milk and pup's plasma. That was not.

About carbon-isotope ratios, previous studies recorded a depletion of ^{13}C in the same tissues (hairs and blood) from lactating females to dependent offspring in various species (Nelson *et al.*, 1998; Polischuk *et al.*, 2001; Sare *et al.*, 2005) and the explanation of this decrease, passing from a lower trophic step to a higher one, was indicated in the high fat content of milk: lipids are depleted in ^{13}C relative to carbohydrates and proteins and tissues that contain a very high percentage of them will present lower $\delta^{13}\text{C}$ than tissues containing little fat (Parker, 1964; De Niro and Epstein, 1977 and 1978; Pinnegar and Polunin, 1999; Jenkins *et al.*, 2001; Sotiropoulos *et al.*, 2004; Sare *et al.*, 2005; Mintenbeck *et al.*, 2008). But these data on Weddell seal showed depleted $\delta^{13}\text{C}$ values in pup's plasma relative to mums in both whole plasma and lipid-extracted aliquots (mums Aq./Pr./Wh., -24.54‰/-23.85‰/-25.15‰; pups Aq./Pr./Wh., -25.27‰/-24.48‰/-25.97‰. Means) which, however, were enriched relative to milk carbon isotope ratios (milk Aq./Pr., -29.11‰/-25.96‰. Means), as expected.

Summarizing, this scenario involved that:

- when, more correctly, consumer's isotopic signatures (pup's plasma) were compared with those of its actual diet (milk), pup carbon isotope signature was enriched, but not the $\delta^{15}\text{N}$;
- mum $\delta^{13}\text{C}$ ratio was the most enriched amongst mums, pups and milk;
- and pup $\delta^{15}\text{N}$ showed the highest value.

Moreover, at least the first sampling event reflected the “foetal” fractionation (pre-suckling isotopic signature due to nutrients assimilated from maternal blood through the placenta) since plasma has a very fast isotopic turnover rate and it is thought to integrate dietary information within the previous 2 – 14 days (Hildebrand *et al.*, 1996; Burns *et al.*, 1998; Kurle, 2002; Crawford *et al.*, 2008; Ainley and Siniff, 2009); but neither whole plasma nor protein fraction

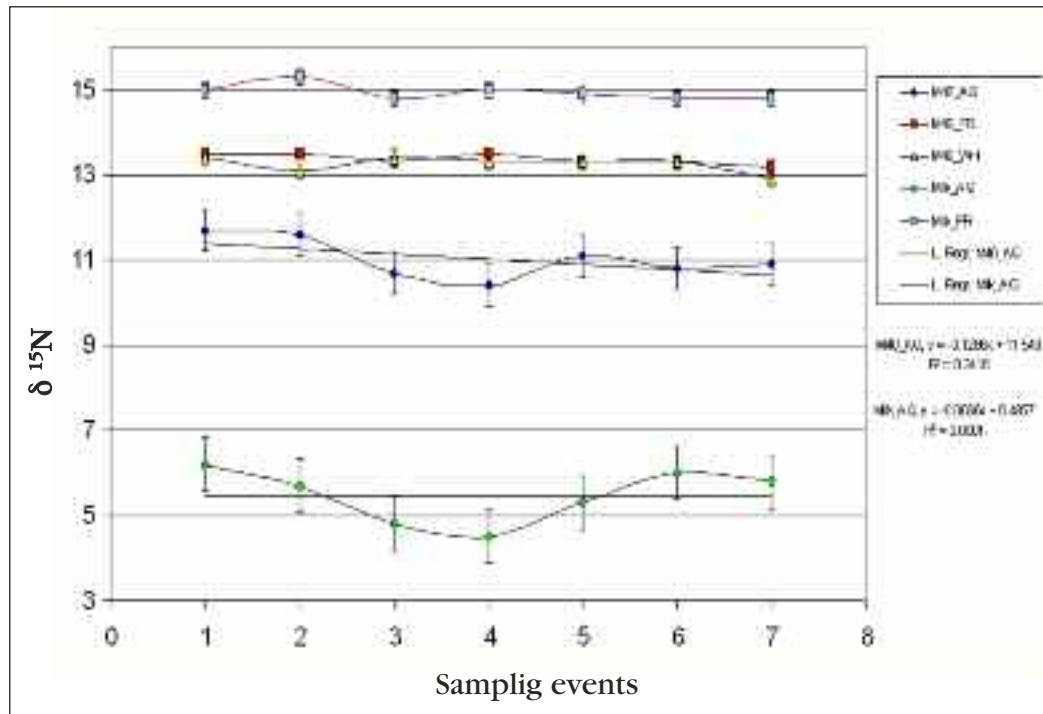


Fig. 1: Mum 40 δ¹⁵N whole plasma and aqueous and protein curves of plasma and milk. Aqueous fractions show an almost perfect parallel trend that seems able to record the effects of the physiological variations not detectable by whole plasma and protein fractions. Mean value of each sampling event ± Standard Error.

presented any evident variation throughout lactation and early weaning that could indicate the passage from foetal nourishment to milk assumption.

Mother's isotopic signatures showed an analogous situation with no oscillations in the curves of whole plasma and protein fraction, even if lactating females (at least most of them, see afterwards) change their nutritional status: feeding until few days prior parturition; fasting during the early and (in some cases) mid lactation; and feeding again from mid and late lactation (Eisert *et al.*, 2005).

Nutritional stress (fasting and energy expenditure to produce milk) should lead to an elevation in δ¹⁵N (Kelly, 2000) but mum's whole plasma and protein fraction trends showed only a constant, slight decrease. On the contrary, aqueous fractions of lactating females, offspring and milk seemed to be able to record

isotopic variations at a higher resolution due, presumably, to the fact that, in aqueous, carbon and nitrogen isotope ratios derive from those compounds that are not metabolized yet (i.e., free amino-acids not yet incorporated to build proteins). A few individuals showed a curve trend that seems to support this hypothesis (Mum 40 was the most paradigmatic case with a perfect parallelism between the aqueous δ¹⁵N mum and aqueous δ¹⁵N milk values) (Figure 1) but the sample size is too small to infer any statistical considerable conclusion.

To complete the scenario, it is necessary bearing in mind that milk composition varies noticeably while lactation progresses and lactating females lose body mass (Stull *et al.*, 1967; Kooyman and Drabek, 1968; Riedman and Ortiz, 1979; Tedman, 1980; Oftedal *et al.*, 1987; Iverson *et al.*, 1993; Oftedal, 1993; Boness and Bowen, 1996; Atkinson, 1997).

4.1.2 - Trophic web by stable isotopes

Some interpretative problems of the isotopic data were also posed by the highest position (relative to nitrogen) of the Antarctic toothfish in the trophic web; this result is consistent with all the previous studies which found in *Dissostichus mawsoni* similar or slightly higher δ¹⁵N values than adult Weddell seals, but it is conflicting with the numerous direct observations of seals resurfacing with large individuals of Antarctic toothfish in their mouths (i.e., Calhaem and Christoffel, 1969; Kim *et al.*, 2005; Ponganis and Stockard, 2007; Ainley and Siniff, 2009).

Besides, it is very interesting to notice that also killer whales (type C) in Antarctica occupy the same trophic level of Weddell seal resulting a little bit depleted in δ¹⁵N relative to the Antarctic toothfish (Krahn *et al.*, 2008); all that seems to confirm that biochemical data on the large Antarctic cod can not be slavishly inserted in a food web because it would involve not considering it as an important prey of marine mammal top predators (Ainley and Siniff, 2009).

One of the possible explanation - not yet considered in literature - of such an elevated δ¹⁵N enrichment in the *Dissostichus mawsoni* tissues, could be in its hypothetic aptitude of acting as scavenger on large animal carcasses (above all seals and cetaceans) that fall over the sea floor and that, under the

ice, can be considered a remarkable source of nitrogen.

In fact, among notothenioids (perciformes, *Notothenioidei*), the predominant group of fishes in the Ross sea to which Antarctic toothfish belongs (Eastman and Hubold, 1999; Vacchi *et al.*, 1999; Eastman, 2000; La Mesa *et al.*, 2004), the particular foraging behaviour known as “rotational feeding” was already described in two species (*Pagothenia borchgrevinki* and *Trematomus bernacchii*) and *Dissostichus mawsoni* could apply this same strategy as well.

Rotational feeding consists in the capability of ripping pieces of flesh from bigger sources of food by rotating their body on the longitudinal axe several times, as moray eels or other anguilliforms do to dismember and eat their preys (Janssen *et al.*, 1992; Vacchi, personal communication, 2009).

The application of the rotational feeding by *Dissostichus mawsoni* could be indirectly confirmed by the discovery of penguin remains in the stomach of four Antarctic toothfish (Fenaughty *et al.* 2003) and by the fact that, in some areas, it is an opportunistic predator that often preys on locally abundant benthic species and competes with penguins and marine mammals (Wohlschlag, 1968; Calhaem and Christoffel, 1969; Yukhov, 1970 and 1971; Testa *et al.*, 1985; Goldsworthy *et al.*, 2001; Fenaughty *et al.* 2003).

By way of summary, *Dissostichus mawsoni* could act as a secondly top-predator, even because it is the only notothenioid fish in the Ross Sea with suitable size and anatomic adaptation to carry out the rotational feeding on large marine mammal carcasses.

Moreover the lack of Antarctic toothfish’s hard part remains in the Weddell seal’s stomach contents and scats (that is considered another evidence to infer that it is not part of the Weddell seal diet) is a misleading info since Weddell seals were documented removing the heads of toothfish before consumption and eating only soft parts because of the large dimension of the fish (Kim *et al.*, 2005; Ainley and Siniff, 2009).

The $\delta^{15}\text{N}$ values of the other prey items showed that at the base of the diet of adult females there is the Antarctic silverfish, *Pleuragramma antarcticum*, above all during late lactation when all mothers resumed a deep diving activity. In early lactation, *Pagothenia borchgrevinki* and *Trematomus bernacchii* as well seemed to be an important integration of their diet, losing

importance throughout lactation. And *Pagothenia borchgrevinki*, above all, could be indicated as the most relevant prey of pups in the early weaning.

These inferences were supported by the carbon isotope signatures that reflected the life style of each single species: $\delta^{13}\text{C}$ values are high in benthic species and low in pelagic species (Burns *et al.*, 1998).

Notothenioids do not possess swim-bladder and mostly they are benthic or demersal but few species that live in the water column use subcutaneous lipid deposits to regulate buoyancy: the pelagic ones have the most extensive deposition of lipids, the epi-benthic and cryopelagic species an intermediate amount, while the benthic Notothenioids – such as *Trematomus bernacchii* – the smallest (Clark *et al.*, 1984; Phleger *et al.*, 1999; Vacchi *et al.*, 1999).

In fact, the lowest $\delta^{13}\text{C}$ values (after *Chionodraco hamatus*) were recorded in *Dissostichus mawsoni* and *Pleuragramma antarcticum* as expected since the toothfish and the silverfish are active, pelagic fishes. The $\delta^{13}\text{C}$ value of *Chionodraco hamatus* (the most carbon depleted) is quite difficult to explain even because it showed a depletion from untreated samples to lipid-extracted samples; but even if *Chionodraco hamatus* is an epibenthic species it spends considerable time in the water column preying heavily on krill (Vacchi *et al.*, 1999) and this could justify such a low carbon isotopic ratio.

Pagothenia borchgrevinki showed an intermediate $\delta^{13}\text{C}$ value that reflects its cryopelagic life style and its carbon isotope signature is likely also the consequence of its diet based on amphipods and copepods (Clarke *et al.*, 1984; Pankhurst, 1990; La Mesa *et al.*, 2004).

4.2 - Diving behaviour

Results of dive data analysis revealed that the lactating females’ diving behaviour was marked by a very outstanding intra-individual variability that conditioned the ontogeny of diving in their pups.

The simple results (See Table 3a-b, Chapter 3) on number of dives, deepest and longest dive, max depth mean, mean depth mean and time underwater per day, referred to the different stages of lactation, were already sufficient to show big differences in the maternal strategies adopted by the Weddell seals at Hutton Cliffs; but it is fundamental to point out that, notwithstanding the

many variations in these parameters, the most traditional approach to dive profile classification, based on five pre-established shapes, provided a predominant presence of U-shaped profiles in both mums and pups and independently from the lactation stages. Moreover, especially the pups showed a negative trend in the percentage of U-shaped profiles from mid lactation to the end (P2, $65.09\% \pm 12.09$; P3, $58.24\% \pm 19.45$; P4, $55.94\% \pm 15.18$. Mean \pm SD), followed by an increase in early weaning (P5, $65.51\% \pm 9.91$. Mean \pm SD).

These results were contrary to the expected ones since, in literature, U-shaped profiles (also called square or rectangular dives) are associated with foraging activity and prey pursuit, while V-shaped profiles (also called triangular dives) are thought to represent exploratory and transit excursions or predator avoidance dives (Kooyman, 1968; Williams and Kooyman, 1985; Le Boeuf *et al.*, 1988 and 1992; Testa *et al.*, 1989; Hindell *et al.*, 1991; Thompson *et al.*, 1991; Bengtson and Stewart, 1992; Slip *et al.*, 1994; Wilson, 1990 and 1995; Wilson *et al.*, 1995; Schreer and Testa, 1996; Burns *et al.*, 1997; Kirkwood and Robertson, 1997; Schreer and Kovacs, 1997; Lesage *et al.*, 1999; Krafft *et al.*, 2000; Ropert-Coudert *et al.*, 2000; Arnould and Hindell, 2000; Schreer *et al.*, 2001; Krafft *et al.*, 2002; Putz and Cherel, 2005; Halsey *et al.*, 2007).

U-shaped (fast descent and ascent with long bottom time characterised by a vertical speed close to 0) and V-shaped (lack of bottom time with instantaneous inversion at the max reached depth) profiles are the main forms at the base of all the others described by scientists in the last 20 years.

So, a pre-eminence of U-shapes should indicate an intense foraging activity that obviously, however, can not reach its maximum intensity in early lactation and it decreases while pups grow and develop their diving and fishing skills. Moreover data showed that the characteristics of the dives performed by the pups during lactation were not compatible with a foraging activity, not even on benthic and demersal preys.

These results made clear that clustering operations based on pre-ordinate geometrical shapes (into the bargain thought to be used to describe the diving activity of any air breathing vertebrates) can not be very reliable (figure 2a-b-c-d-e-f) and, above all, can entail relevant biases in assigning the ecological functions to the dives.

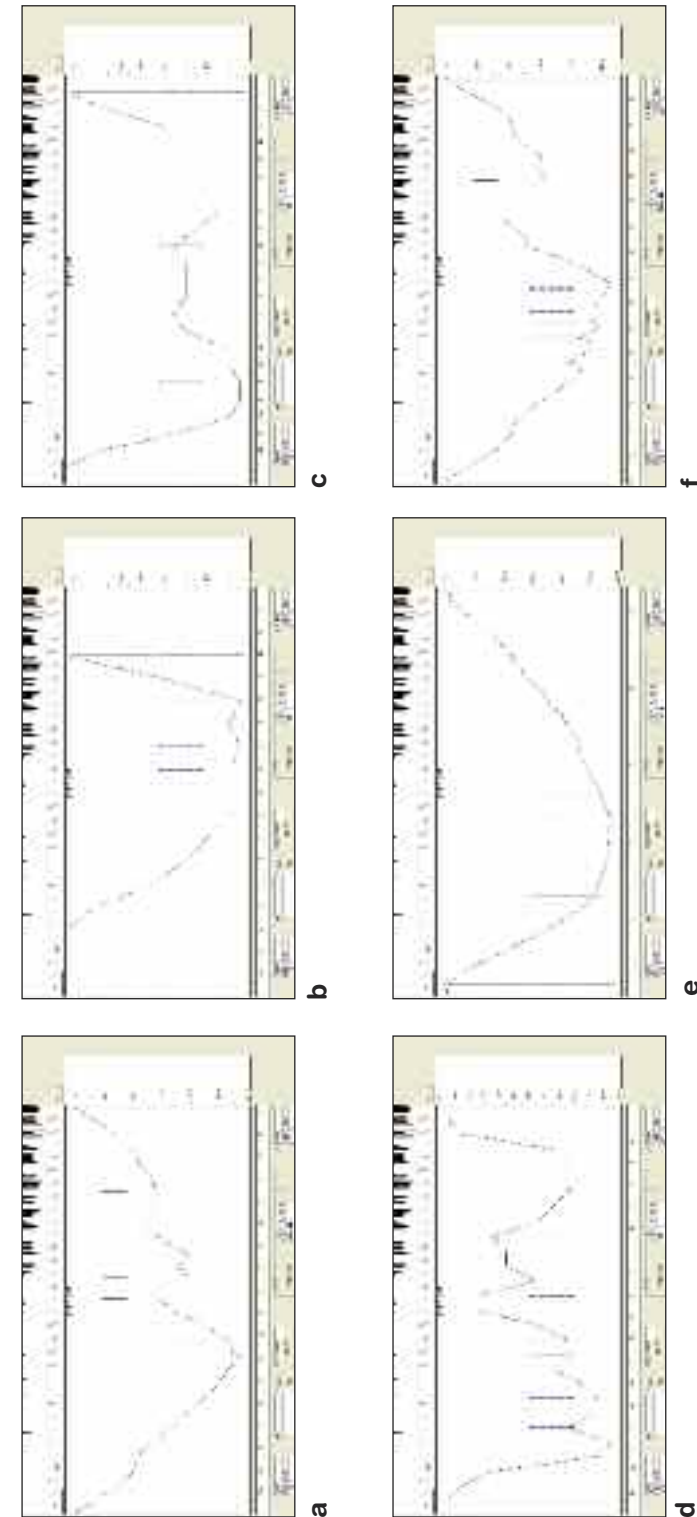


Fig. 2: The six dive profiles showed in the figure were all classified as U-shape despite the fact they are very different one from the other.

So, it was not accidental that SOMs performed on dive data that were recorded in the first five weeks after parturition, showed a complete lack of U-shaped profiles in 3x4 maps, both in mums and pups (which indicated no foraging activity), while only a limited percentage of them was singled out in the 9x12 map performed on lactating females' dive data. And all these clusters of U-shapes presented mean maximum depths (ranging from 253.0 to 322.8 meters) and mean durations (ranging from 13'58" to 21'18") through and through consistent with those of deep diving foraging activity.

On the contrary, the SOMs performed on weaned pups' dive data showed U-shaped profile clusters in both the 3x4 and 9x12 map, strongly indicating foraging activity (or, at least, attempts to forage) from the beginning of the wean, with a very short fasting interval.

Moreover, their mean dive durations were consistent with the calculated Aerobic Dive Limit (ADL: "the maximum breath-hold that is possible without any increase in blood lactate concentration during or after the dive. This limit is dependent upon available O₂ stores, oxygen consumption rate, degree of peripheral vasoconstriction, and rate of lactate production and consumption" – Kooyman, 1989), that seems to widely affect the juvenile diving behaviour and should indicate a diving strategy more aimed at foraging than travelling; even if several dives exceeding the ADL, likely performed by the pups in order to push their physiological limits and increase apnea (Kooyman *et al.*, 1983; Thorson and Le Boeuf, 1994; Burns and Castellini, 1996; Burns *et al.*, 1997; Horning and Trillmich, 1997a and 1997b; Burns, 1999), were recorded in the same period.

It is very interesting to highlight that, among lactating females, the left-skewed shapes were very little represented and formed by shallow-water dives, while right-skewed shapes were more numerous and representative of mid and deep water dives. On the other hand, suckling pups showed no numerical significant differences between left- and right-skewed shapes with mean depth that is very similar to mums' left-skewed shaped profiles, so that the latter seem to be performed with the aim to force pups to develop their swimming skills ("school-dives"). Instead, early weaned pups showed a pattern that closed in on adult females (both left and right skewed shapes were represented but with

a numerical pre-eminence of the second ones) but proportionally shallower and shorter. In what early weaned pups were different was the presence of bi-, tri- and multi-modal profiles that can be interpreted as training dives to improve apnea physiological limits related to foraging activity. V-shaped profiles, which were the deepest dives, were likely those performed to progressively move forward the individual depth range in absolute.

The intra-individual variability about the maternal strategy and mum-pup diving behaviour was stressed by the results of the associated underwater activity analysis as well: not only the six analysed mothers showed a very wide host of facets ranging from "extreme capital breeding" to "income breeding", but also the results of the associated dive data cleared up that the maternal choice affected the ontogeny of diving and foraging in pups.

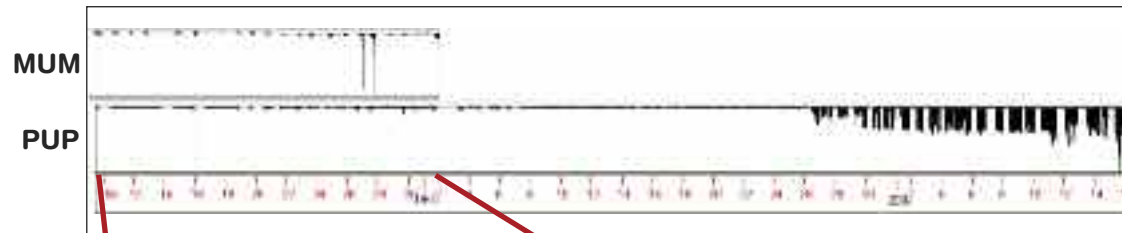
In fact, the newborns' diving activity resulted strictly depending from that of their mothers during lactation by a relation of direct proportion of the time OUT/IN the water between mums and pups.

Certainly, the number of pairs (6) represented a quite small sample, but the diametrically opposite cases of M515-P6698 and M819-P6568, with the first pair being representative of the "extreme capital breeding" maternal strategy and the second one of the "income breeding" (Figures 3a-b-c-d-e and 4a-b-c-d-e), are paradigmatic of the very high intra-individual complexity of the Weddell seals breeding at Hutton Cliffs.

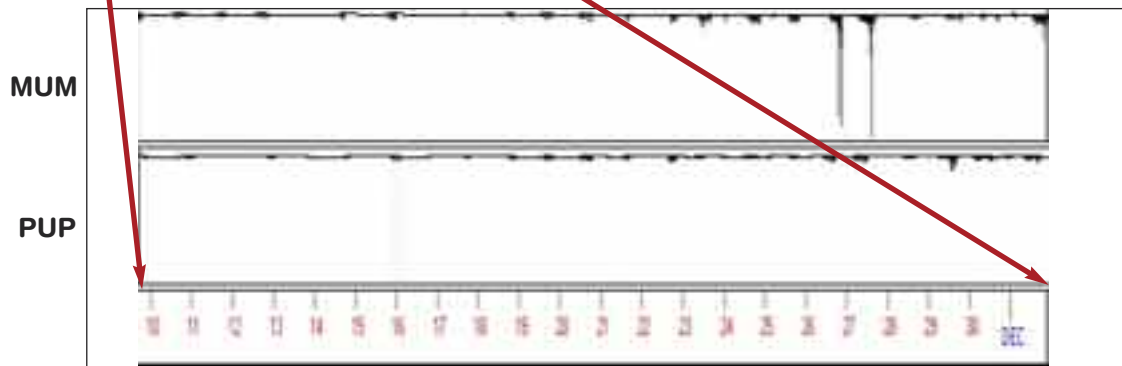
MUM 515				PUP 6698			
Records	Hours	%		Records	Hours	%	
183074	608.54	84.38		164020	465.61	84.96	
10097	30.27	4.63		29548	16.44	30.99	
Record TOT		Hours TOT		Record TOT		Hours TOT	
103971		538.81		193571		532.01	
		% of the water				% of the water	
		0.09				0.00	

Association 1			Association 2		
Records	Hours	%	Records	Hours	%
55529	438	80.70	55529	438	80.70
36545	71	13.69	3	0.00	0.00
7491	71	13.69	2647	13.69	13.69
3406	6	1.76	166	0.09	0.09
			0	0.00	0.00
			3	0.00	0.00
			215	3.79	3.79
			0	0.00	0.00
			3403	1.75	1.75
Chi Square			Chi Square		
3.661122148			0.05523966		
p:			0.05523966		

a

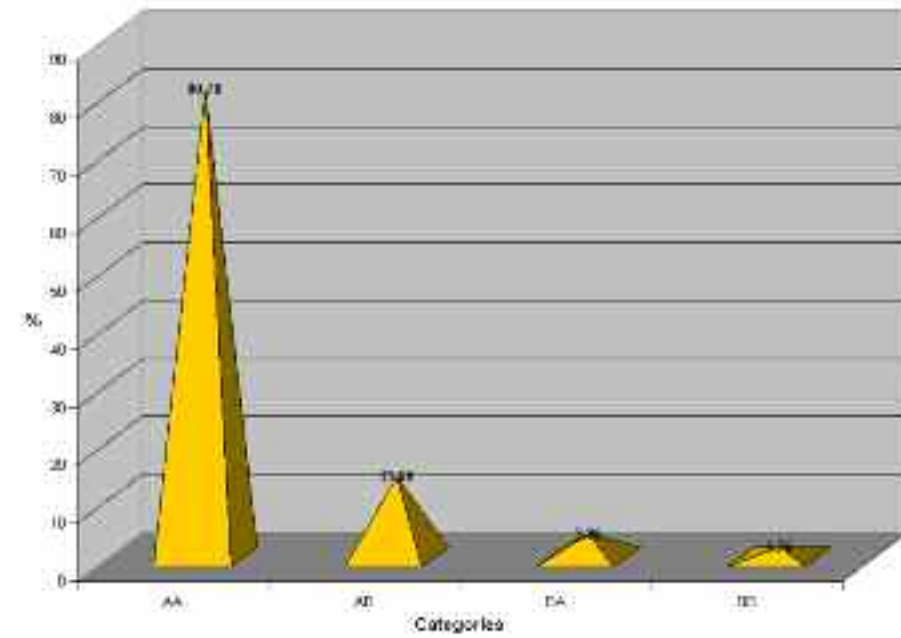


b



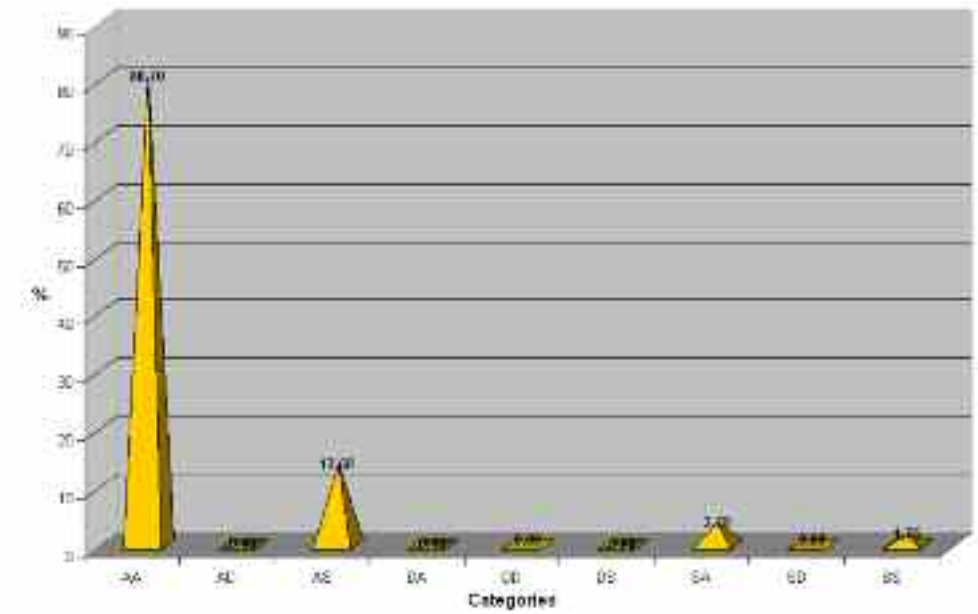
c

Fig. 3: The associate diving behaviour of Mum 515 / Pup 6698 (Pair 4) showed by the Associator and the diving activity.



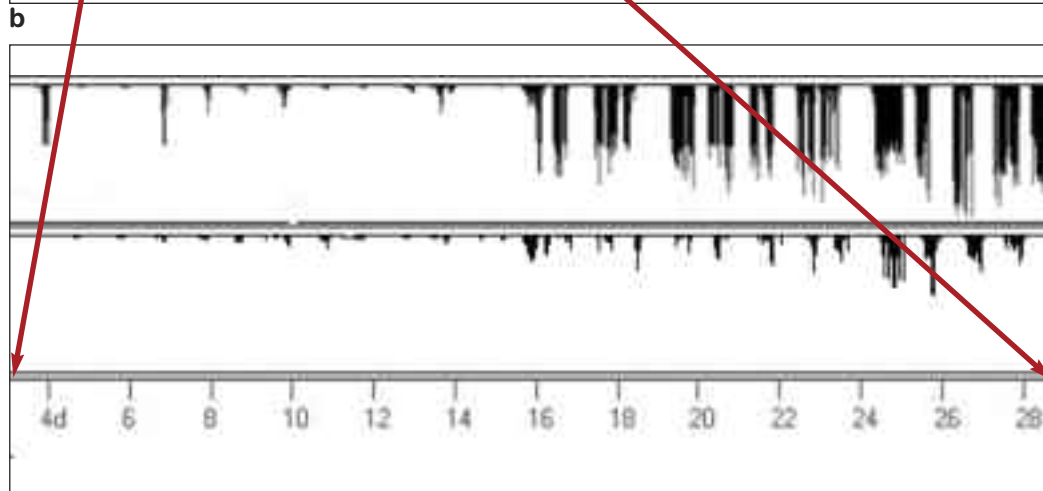
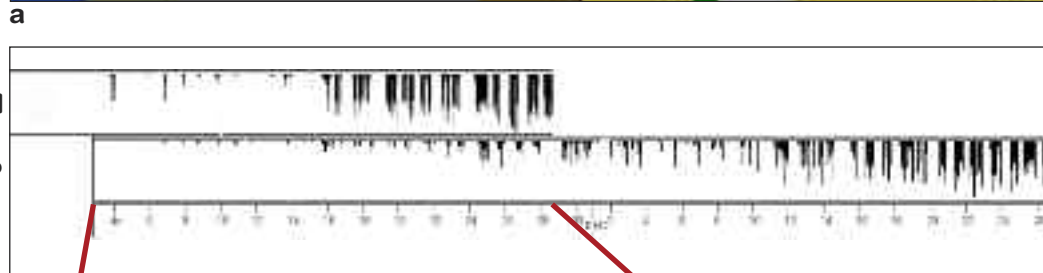
TIME

d



e

MUM 819	Record 126	Rate	%	Associator 1 M	%
A	137984	385.29	60.75	11-835 AA	311 60.55
B	89136	247.60	38.25	231-43 AD	66 10.15
Record TOT		632.89		5867 BA	88 26.94
Rate TOT		632.89		29215 BB	88 12.89
A	137904	60.75	34.45	Associator 2 M	
B	90501	28.71	16.51	11-835 AA	311 60.55
C	30745	13.54	34.45	115 AD	0.05
Record TOT		632.89		2485 AS	10.14
Rate TOT		632.89		21455 DA	9.45
A	174757	465.44	75.54	1095 DD	0.45
B	62903	145.45	23.09	8192 DS	3.61
Record TOT		632.89		8195 SA	16.94
Rate TOT		632.89		194 SD	0.09
A	174757	75.54	34.45	19734 SS	8.69
B	90501	28.44	16.51	Chi Square 24.1522643	
C	1404	0.62	2.69	p 0.0046107	



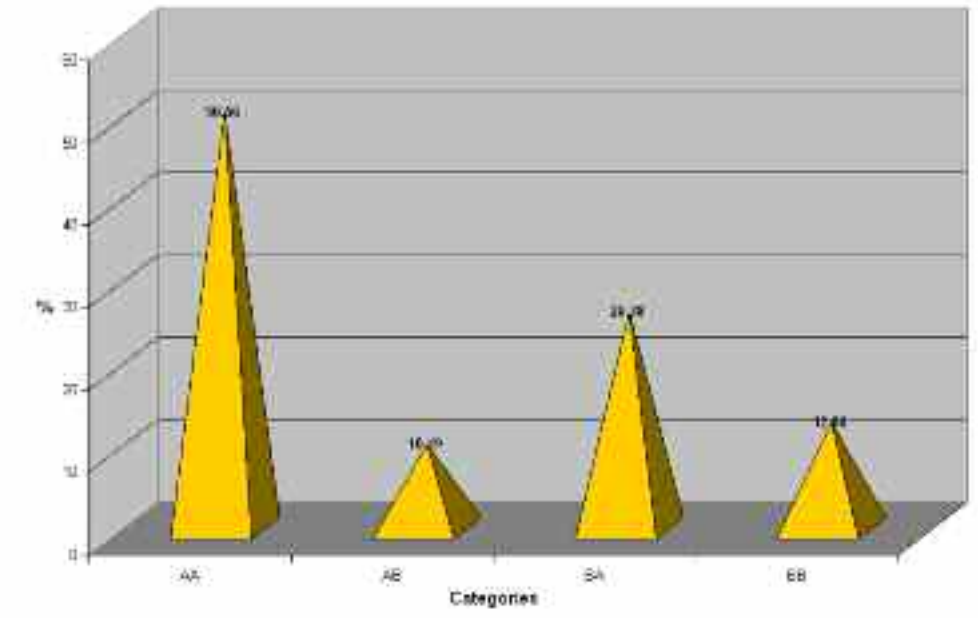
a

b

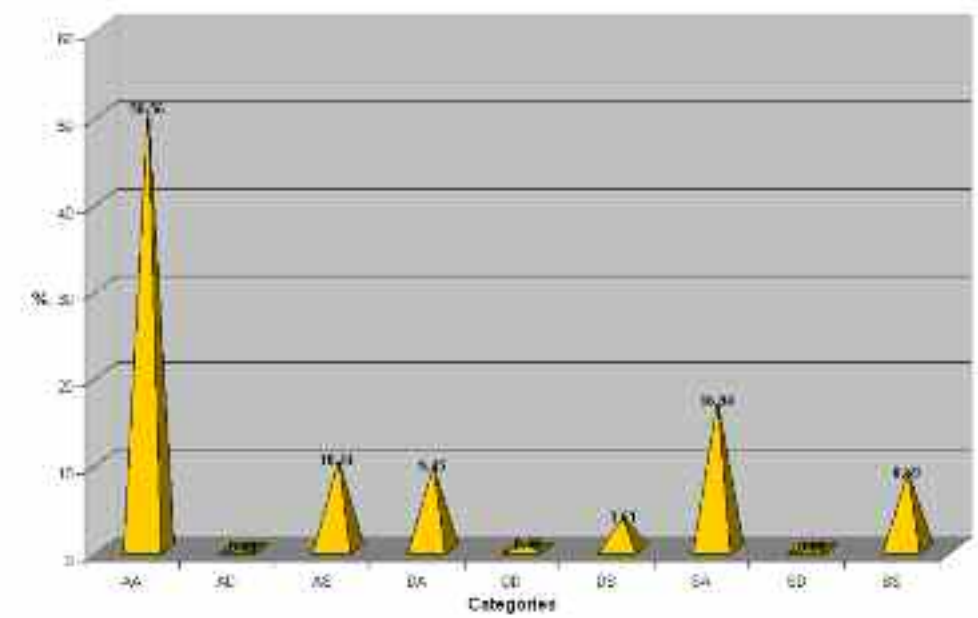
c

Fig. 4: The associate diving behaviour of Mum 819 / Pup 6568 (Pair 5) showed by the Associator and the diving activity.

Mum 819 / Pup 6568



TIME



d

e

5. CONCLUSIONS



5.1 - Conclusions

The innovative approach of this study - based on serial samplings carried out on lactating females and on their own dependent pups from the second day after parturition - allowed to obtain the first associate data (biochemical and behavioural) of mum-pup pairs in the very first weeks of the newborns' life.

These data, processed by analytical methodologies that were thought to assess the scientific question from points of view different from the traditional ones, stressed the importance to tackle the matter of the ontogeny of foraging in Weddell seal pups and the dietary behaviour of lactating females from diverse angle shots, since they can provide new and unexplored answers, both in an evolutionary and methodological perspective.

Specifically the results of this study strongly suggested that:

1. the Weddell seal lactating females of Hutton Cliffs adopt different maternal care strategies, ranging from "extreme capital breeder" (the seal fasts throughout lactation) to "income breeder" (the seal acts like an otariid and after a brief fasting, undertakes foraging cycle) passing through several intermediate gradations of level. It means that their breeding behaviour is based on a very high intra-individual variability. This finding opens a new perspective in the understanding of the evolution of maternal care in pinnipeds since, previously, the foraging cycle strategy was thought to be related to limited dimensions and to be typical of small body sized pinnipeds, such as otariids and smaller phocids weighing less than 100 kilos at parturition (Boness and Bowen, 1996);

2. consequently, the majority of Weddell seal lactating females forages during lactation, with an intensity that is proportional to the level of the adopted maternal strategy;

3. their pups do not forage independently in a relevant and detectable manner during lactation and they completely rely on maternal milk for growth and energetic costs as shown by dive data;

4. the ontogeny of diving in pups is strictly linked to the strategy adopted by their mothers. In an adaptive perspective, the pups association to a capital

or an income breeder mother gets advantages and disadvantages which seem balance each other out: pups that spend more time in water since their first water entries are exposed to a higher risk of pre-weaning mortality but can develop greater swimming and foraging skills, while pups that spend less time in water will probably have an "easier" lactation but a more traumatic approach to the independent foraging after mother's abandoning. This hypothesis could help in explaining the reason why such different maternal strategies are still kept by the evolution;

5. early weaned pups forage on benthic and cryopelagic preys but the onset of the independent foraging could not be detected by stable isotope analysis. Dive data suggest a short or a very short fasting period after lactation (depending on the pup) but it is not possible to be very accurate since the exact end of the maternal cares could not be determined for any single individual.

This study also stressed the importance that more caution is needed when using stable isotope techniques to diet reconstruction research to avoid biases and data misinterpretation.

Stable isotope analysis can be a very useful tool to give response to ecological questions, but its reliability is totally depending on the thorough and whole knowledge of all the physiological and ecological processes taking part in the studied issue.

The study case of the ontogeny of diet in Weddell seal pups is paradigmatic of the numerousness and the complexity of factors that can deeply affect the isotopic fractionation and that can provide values which do not correspond to the "generalized" rule of an enrichment of about 1‰ and 3‰ for carbon and nitrogen respectively per trophic step.

First of all there is still a too wide uncertainty and lack of knowledge on the turnover rates in the different tissues of wild animals (in literature, plasma is thought to integrate dietary information over 48 hours until a couple of weeks); then we still do not know how and how much physiological processes such as nutritional and water stress, pregnancy, production of milk and its changes in composition during lactation, pup growth and intra-specific/population/individual/tissue variability participate to the isotopic fractionation; and, in the case of milk, which is the most suitable tissue of a newborn to be

compared to it?

A further note of caution this study has highlighted is relative to the use of the mean values of dive parameters within a population to homogenously describe the diving behaviour of the group.

As already Burns *et al.* (1997) noticed and this study has confirmed and developed, describing the underwater activity of the Weddell seal at Hutton Cliffs as an average of a more or less big sample of individuals, can result quite misleading and trivialize a scenario that has in the complexity and in the pluralism of the adopted behaviours its real and actual expression.

6. REFERENCES



- Ainley, D. G. and Siniff, D. B. 2009.** Review. The importance of Antarctic foothill fish as prey of Weddell seals in the Ross sea. *Antarctic Science*, **21**(4): 317-327.
- Ambrose, S. H. and DeNiro, M. J. 1986.** The isotopic ecology of East African mammals. *Oecologia* (Berlin), **69**: 395-406.
- Arnould, J. P. Y. & Hindell, M. A. 2001.** Dive behaviour, foraging locations, and maternal-attendance patterns of Australian fur seals (*Arctocephalus pusillus doriferus*). *Can. J. Zool.* **79**: 35-48.
- Asaga, T., Naito, Y., Le Boeuf, B. J. and Sakurai, H. 1994.** Functional analysis of dive types of female northern elephant seals. In: Le Boeuf B. J., Laws R. M. (eds) *Elephant seals*. University of California Press, Berkeley, 310-327.
- Atkinson, S. 1997.** Reproductive biology of seals. *Journals of Reproduction and Fertility. Reviews of Reproduction*, **2**: 175-194.
- Baechler, J., Beck, C. A. & Bowen W. D. 2002.** Dive shapes reveal temporal changes in the foraging behaviour of different age and sex classes of harbour seals (*Phoca vitulina*). *Can. J. Zool.* **80**: 1569-1577.
- Baroni, C. 2001.** Il ghiaccio marino. In *Antartide. Terra di scienza e riserva naturale*. Terra Antartica Publication, pp. 30-36
- Barrett-Hamilton, G. E. H. 1901.** Seals. Res. Voyage S. Y. "Belgica", *Zool.*, 1-20.
- Baylis, A. M. M., Page, B., Peters, K., McIntosh, R., McKenzie, J. & Goldsworthy S. 2005.** The ontogeny of diving behaviour in New Zealand fur seal pups (*Arctocephalus forsteri*). *Can. J. Zool.* **83**: 1149-1161.
- Bengtson, J. L. and Stewart, B. S. 1992.** Diving and haulout behavior of crabeater seals in the Weddell Sea, Antarctica, during March 1986. *Polar Biology*, **12**: 635-644.
- Bertram, G. L. C. 1940.** The biology of the Weddell and crabeater seals. *Sci. Rept. Brit. Graham Land Exp. 1934-37* **1**: 1-139.
- Bligh, E. G. and Dyer, W. J. 1959.** A rapid method of total lipid extraction and purification. *Can. J. of Biochem. And Physiol.*, **37**: 911-917.
- Boness, D. J. and Bowen, W. D. 1996.** The evolution of maternal care in Pinnipeds. *Bioscience* **46**(9): 645-654
- Bonner, W. N. 1984.** Lactation strategies in pinnipeds: problems for a marine mammalian group. *Symposia of the Zoological Society of London*, **51**: 253-272.
- Bowen, W. D. 1991.** Behavioural ecology of pinniped neonates. In *The behaviour of pinnipeds*. Renouf D, ed., London (UK): Chapman and Hall, 66-127.
- Bowen, W. D., Oftedal, O. T. and Boness, D. J. 1992.** Mass and energy transfer during lactation small phocid, the harbor seal (*Phoca vitulina*). *Physiological Zoology* **65** (4): 844-866.
- Bowen, W. D., Boness, D. J. and Iverson, S. J. 1999.** Diving behaviour of lactating harbour seals and their pups during maternal foraging trips. *Can. J. Zool.* **77**: 978-988.
- Bowen, W. D., Iverson, S. J., Boness, D. J. and Oftedal, O. T. 2001.** Foraging effort, food intake and lactation performance depend on maternal mass in a small phocid seal. *Funct Ecol* **15**: 325-334.
- Brown, R. N. R. 1915.** The seals of the Weddell Sea; notes on their habits and distribution. *Rep. Sci. Res. Voyage S. Y. "Scotia"*, Edinburgh, **4**(13): 181-198.
- Bruce, W. S. 1915a.** Measurements and weights of Antarctic seals taken by the Scottish National Antarctic Expedition. *Rep. Sci. Res. Voyage S. Y. "Scotia"*, Edinburgh, **4**(11): 159-174.
- Bruce, W. S. 1915b.** ON the skulls of Antarctic seals. *Rep. Sci. Res. Voyage S. Y. "Scotia"*, Edinburgh, **4**(12): 175-180.
- Burns, J. M. 1999.** The development of diving behavior in juvenile Weddell seals: pushing physiological limits in order to survive. *Can. J. Zool.*, **77**: 737-747.

- Burns, J. M. and Castellini, M. A. 1996.** Physiological and behavioral determinants of the aerobic dive limit in Weddell seal (*Leptonychotes weddellii*) pups. *J. Comp. Physiol.*, **166**: 473-483.
- Burns, J. M. and Testa, J. W. 1997a.** Developmental changes and diurnal and seasonal influences on the diving behaviour of Weddell seal (*Leptonychotes weddellii*) pups. *In* Battaglia, B. Valencia, J. and Walton, D. W. H., eds. *Antarctic Communities - Species, Structure and Survival* Cambridge University Press, 328-334.
- Burns, J. M., Schreer, J. F. and Castellini, M. A. 1997b.** Physiological effects on dive patterns and foraging strategies in yearling Weddell seals (*Leptonychotes weddellii*). *Can. J. Zool.*, **75**: 1796-1810.
- Burns, J. M. and Castellini, M. A. 1998.** Dive data from satellite tags and time-depth recorders: a comparison in Weddell seal pups. *Mar. Mamm. Sci.*, **14**(4): 750-764.
- Burns, J. M., Trumble, S. J., Castellini, M. A., and Testa, J. W. 1998.** The diet of Weddell seals in McMurdo Sound, Antarctica, as determined from scat collections and stable isotope analysis. *Polar Biol.*, **19**: 272-282.
- Burns, J. M., Castellini, M. A., and Testa, J. W. 1999.** Movements and diving behavior of weaned Weddell seal (*Leptonychotes weddellii*). *Polar Biol.*, **21**: 23-36.
- Burns, J. M. & Kooyman, G. L. 2001.** Habitat use by Weddell seals and Emperor penguins foraging in the Ross sea, Antarctica. *Amer. Zool.*, **41**: 90-98.
- Bury, S. J., Pinkerton, M. H., Thompson, D. R., Hanchet, S., Brown, J. and Vorster I. 2008.** Trophic study of Ross Sea Antarctic toothfish (*Dissostichus mawsoni*) using carbon and nitrogen stable isotopes. CCAMLR, document WG-EMM.
- Calhaem, I. and Christoffel, D. A. 1969.** Some observations of the feeding habits of a Weddell seal, and measurements of its prey, *Dissostichus mawsoni*, at McMurdo sound, Antarctica. *N.Z. Jl. Mar. Freshwat. Res.*, **3**: 181-190.
- Cameron M. F. and Siniff D. B. 2004.** Age specific survival, abundance, and immigration rates of a Weddell seal (*Leptonychotes weddellii*) population in McMurdo Sound, Antarctica. *Can. J. Zool.*, **82**: 601-615.
- Castellini, M. A., Davis, R. W. and Kooyman, G. L. 1991.** Annual Cycles of Diving Behavior and Ecology of the Weddell Seal. *Bulletin of the Scripps Institution of Oceanography, University of California, Volume 28.*
- Cherel, Y., Hobson, K. A., Guinet, C. and Vanpe, C. 2007.** Stable isotopes document seasonal changes in trophic niches and winter foraging specialization in diving predators from the Southern Ocean. *Journal of Animal Ecology*, **76**: 826-836.
- Clark, A., Doherty, N., De Vries, A. L. and Eastman, J. T. 1984.** Lipid content and composition of three species of Antarctic fish in relation to buoyancy. *Polar Biol.*, **3**: 77-83.
- Cline, D. R., Siniff, D. B. and Erickson, A. W. 1969.** Immobilization and collecting blood from Antarctic seals. *Journal of Wildlife Management*, **33**: 138-144.
- Costa, D. P. 1991.** Reproductive and foraging energetics of pinnipeds: implications for life history patterns *In* The behaviour of pinnipeds. Renouf D, ed., London (UK): Chapman and Hall, 300-344.
- Crawford, K., McDonald, R. A. and Bearhop, S. 2008.** Applications of stable isotope techniques to the ecology of mammals. *Mammal Rev.*, **38**(1): 87-107.
- Crocker, D. E., Le Boeuf, B. J. and Costa D. P. 1997.** Drift diving in female northern elephant seals: implications for food processing. *Can. J. Zool.*, **75**(1): 27-39.
- Dalerum, F. and Angerbjorn, A. 2005.** Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. *Oecologia*, **144**: 647-658.
- Davis, R. W., Fuiman, L. A., Williams, T. M., Collier, S. O., Hagey, W. P., Kanatous, S. B., Kohin, S. and Horning, M. 1999.** Hunting behavior of a marine mammal beneath the Antarctic fast ice. *Science*, **283**: 993-996.
- Davis, R. W., Fuiman, L. A., Williams, T. M., Horning, M. & Hagey, W. P. 2003.** Classification of Weddell seal dives based on 3-dimensional movements and video-recorded observations. *Mar. Ecol. Prog. Ser.* **264**: 109-122.

- Davis, R. W., Hagey, W. P. & Horning, M. 2004.** Monitoring the behavior and multi-dimensional movements of Weddell seals using an animal-borne video and data recorder. Mem. Natl. Inst. Polar Res., Spec. Issue **58**: 150-156.
- Dehn, L.-A., Sheffield, G. G., Follmann, E. H., Duffy, L. K., Thomas, D. L. and O'Hara, T. M. 2007.** Feeding ecology of phocid seals and some walrus in the Alaskan and Canadian Arctic as determined by stomach contents and stable isotope analysis. Polar. Biol., **30**: 167-181.
- DeNiro, M. J. and Epstein, S. 1977.** Mechanism of carbon isotope fractionation associated with lipid synthesis. Science, **197**: 261-263.
- DeNiro, M. J. and Epstein, S. 1978.** Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim Acta, **42**: 495-506.
- DeNiro, M. J. and Epstein, S. 1981.** Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim Acta, **45**: 341-351.
- De Vries, A. L. and Wohlschlag, D. E. 1964.** Diving Depths of the Weddell seal. Science, **45**: 292-293.
- Ducatez, S., Dalloyau, S., Richard P., Guinet, C. and Cherel, Y. 2008.** Stable isotopes document winter trophic ecology and maternal investment of adult female southern elephant seals (*Mirounga leonina*) breeding at the Kerguelen Islands. Mar. Biol., **155**: 413-420.
- Eastman, J.T. 2000.** Antarctic notothenioid fishes as subjects for research in evolutionary biology. Antarct. Sci., **12**: 276-287.
- Eastman, J.T. and Hubold, G. 1999.** The fish fauna of the Ross Sea, Antarctica. Antarct. Sci., **11**: 293-304.
- Eisert R., Oftedal, O. T., Lever, M., Ramdohr, S., Breier, B. H., and Barrell, G. K. 2005.** Detection of food intake in a marine mammal using marine osmolytes and their analogues as dietary biomarkers. Mar. Ecol. Prog. Ser. **300**: 213-228.
- Fenaughty, J. M., Stevens, D. W. and Hanchet, S. M. 2003.** Diet of the Antarctic toothfish (*Dissostichus mawsoni*) from the Ross sea, Antarctica (Subarea 88.1). CCAMLR Science, Vol. **10** : 113-123.
- Fuiman, L.A., Madden, K. M., Williams, T. M. & Davis, R. W. 2007.** Structure of foraging dives by Weddell seals at an offshore isolated hole in the Antarctic fast-ice environment. Deep-Sea Research II, **54**: 270-289.
- Gentry, R. L. and Kooyman G. L. 1986.** Fur seals: maternal strategies on land and at the sea. Princeton (NJ): Princeton University Press.
- Geraci, J.R. 1971.** Functional hematology of the harp seal *Pagophilus groenlandicus*. Physiological Zoology **44(3)**: 162-170.
- Giraudel, J. L. and Lek, S. 2001.** A comparison of self-organizing map algorithm and some conventional statistical methods for ecological community ordination. Ecological Modelling, **146**: 329-339.
- Goldsworthy, S. D., He, X., Tuck, G. N., Lewis, M. and Williams, R. 2001.** Trophic interactions between the Patagonian toothfish, its fishery, and seals and seabirds around Macquarie Island. Mar. Ecol. Progr. Ser., **218**: 283-302.
- Haig, H.A. 1915.** A contribution to the histology of the central nervous system of the Weddell seal (*Leptonychotes weddelli*). Rep. Sci. Res. Voyage S. Y. "Scotia", Edinburgh, **4(10)**: 137-158.
- Halsey, L. G., Bost, C. -A. and Handrich, Y. 2007.** A thorough and quantified method for classifying seabird diving behaviour. Polar Biol., **30**: 991-1004
- Hastings, K. K. and Testa, J. W. 1998.** Maternal and birth colony effects on survival of Weddell seal offspring from McMurdo Sound, Antarctica. Journal of Animal Ecology, **67 (5)**: 722-740.
- Hepburn, D. 1915.** Observations on the anatomy of The Weddell seal (*Leptonychotes weddelli*). Rep. Sci. Res. Voyage S. Y. "Scotia", Edinburgh, **4(2, 5, 6, 9)**

Hildebrand, G. V., Farley, S. D., Robbins, C. T., Hanley, T. A., Titus, K. and Servheen, C. 1996. Use of stable isotopes to determine diets of living and extinct bears. *Can. J. Zool.*, **74**: 2080-2088.

Hindell, M. A., Slip, D. J. and Burton, H. R. 1991. The diving behaviour of adult male and female southern elephant seals, *Mirounga leonina* (*Pinnipedia: Phocidae*). *Aust. J. Zool.*, **39**: 595-619.

Hindell, M. A., Harcourt, R. G., Thompson, D. and Waas, J. R. 1999. Evidence for foraging during lactation in the Weddell seal (abstract). Proceedings of the 13th Biennial Conference on the Biology of Marine Mammals held in Wailea, Maui, Hawaii, 82.

Hindell, M. A., Harcourt, R., Waas, J. R. and Thompson D. 2002. Fine-scale three-dimensional spatial use by diving, lactating female Weddell seals *Leptonychotes weddelli*. *Mar. Ecol. Prog. Ser.*, **242**: 275-284.

Hobson, K. A. 1993. Trophic relationship among High Arctic seabirds: insights from tissue-dependent stable-isotope models. *Mar. Ecol. Prog. Ser.*, **95**: 7-18.

Hobson, K. A. and Welch, H. E. 1992. Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. *Mar. Ecol. Prog. Ser.*, **84**: 9-18.

Hobson, K. A., Schell, D. M., Renouf, D. and Noseworthy, E. 1996. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. *Can. J. Fish. Aquat. Sci.*, **53**: 528-533.

Hobson, K. A., Sease, J. L., Merrick, R. L. and Piatt, J. F. 1997. Investigating trophic relationships of pinnipeds in Alaska and Washington using stable isotope ratios of nitrogen and carbon. *Mar. Mamm. Sci.*, **13**: 114-132.

Hobson, K. A., McLellan, B. N. and Woods, J. G. 2000. Using stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) to infer trophic relationships among black and grizzly bears in the upper Columbia River basin, British Columbia. *Can. J. Zool.*, **78**: 1332-1339.

Hooker, S. K. and Baird, R. W. 1999. Deep-diving behaviour of the northern bottlenose whale, *Hyperoodon ampullatus* (*Cetacea: Ziphiidae*). *Proc. R. Soc. Lond.*, **266**: 671-676.

Hooker, S. K. and Baird, R. W. 2001. Diving and ranging behaviour of odontocetes: a methodological review and critique.

Horning, M. and Trillmich, F. 1997a. Development of haemoglobin, hematocrit, and erythrocyte values in Galapagos fur seals. *Mar. Mamm. Sci.*, **13**: 100-113.

Horning, M. and Trillmich, F. 1997b. Ontogeny of diving behaviour in the Galapagos fur seal. *Behaviour*, **134**: 1211-1257.

Iverson, S. J., Bowen, W. D., Boness, D. J. and Oftedal, O. T. 1993. The effect of maternal size and milk energy output on pup growth in grey seals (*Halichoerus grypus*). *Physiological Zoology*, **66**: 61-88.

Janssen, J., Montgomery, J. C. and Tien, R. 1992. Social rotational feeding in *Pagothenia borchgrevinki* (*Pisces: Nototheniidae*). *Copeia*, **2**: 559-562.

Jenkins, S. G., Partridge, S. T., Stephenson, T. R., Farley, S. D. and Robbins, C. T. 2001. Nitrogen and carbon isotope fractionation between mothers, neonates, and nursing offspring. *Oecologia*, **129**: 336-341.

Jönsson, K. I. 1997. Capital and income breeding as alternative tactics of resource use in reproduction. *Oikos*, **78**(1): 57-66.

Jorgensen, C., Lydersen, C., Brix, O. and Kovacs, K. M. 2001. Diving development in nursing harbour seal pups. *The Journal of Experimental Biology*, **204**: 3993-4004.

Kaufman G. W., Siniff, D. B. and Reichle, R. 1975. Colony Behaviour of Weddell seals, *Leptonychotes weddelli*, at Hutton Cliffs, Antarctica. *Rapp. P.-v. Réun. Cons. Int. Explor. Mer*, **169**: 228-246.

Kelly, J. F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Can. J. Zool.*, **78**: 1-27.

- Kim, S. L., Conlan, K., Malone, D. P. and Lewis, C. V. 2005.** Possible food caching and defence in the Weddell seal: observations from McMurdo, Sound Antarctica. *Antarctic Science*, **17**: 71-72.
- Kirkwood, R. and Robertson, G. 1997.** The foraging ecology of female emperor penguins in winter. *Ecol. Monogr.*, **67**: 155-176.
- Kohonen, T. 1982.** Self-organized formation of topologically correct feature maps. *Biol. Cybern.*, **43**: 59-69.
- Kohonen, T. 2001.** Self-Organizing Maps. Third ed. Springer, Berlin.
- Kooyman, G. L. 1965.** Techniques used in measuring diving capacities of Weddell seals. *Polar Rec.*, **12**: 391-394.
- Kooyman, G. L. 1966.** Maximum diving capacities of the Weddell seal (*Leptonychotes weddelli*). *Science*, **151**: 1553-1554.
- Kooyman, G. L. 1968.** An analysis of some behavioural and physiological characteristics related to diving in Weddell seal. *Antarctic Research Series*, **11**: 227-261.
- Kooyman, G. L. 1989.** Diverse divers. *Physiology and behavior*. Burggren W., Ishii S., Langer H., Neuweiler G. and Randall D. J. (Eds.), Springer-Verlag, 143-149.
- Kooyman, G. L. 2004.** Genesis and evolution of bio-logging devices: 1963-2002. *Mem. Natl. Inst. Polar Res., Spec. Issue*, **58**: 15-22.
- Kooyman, G. L. and Drabek, C. M. 1968.** Observations on the milk, blood and urine constituents of the Weddell seal. *Physiol. Zool.*, **41**: 187-194.
- Kooyman, G. L., Castellini, M. A., Davis, R. W. and Maue, R. A. 1983.** Aerobic diving limits of immature Weddell seals. *J. Comp. Physiol.*, **151**: 171-174.
- Krafft, B. A., Lydersen, C., Kovacs, K. M., Gjertz, I. and Haug, T. 2000.** Diving Behaviour of lactating bearded seal (*Erignathus barbatus*) in the Svalbard area. *Can. J. Zool.*, **78**: 1408-1418.
- Krafft, B. A., Lydersen, C., Gjertz, I. & Kovacs, K. M. 2001.** Diving behaviour of sub-adult harbour seals (*Phoca vitulina*) at Prins Karls Forland, Svalbard. *Polar Biol.*, **25**: 230-234.
- Krahn, M. M., Pitman, R. L., Burrows, D. G., Herman, D. P., and Pearce, R. W. 2008.** Use of chemical tracers to assess diet and persistent organic pollutants in Antarctic Type C killer whales. *Marine Mammal Science*, **24**(3): 643-663.
- Kurle, C. M. 2002.** Stable-isotope ratios of blood components from captive northern fur seals (*Callorhinus ursinus*) and their diet: applications for studying the foraging ecology of wild otariids. *Can. J. Zool.*, **80**: 902-909.
- Kurle, C. M. and Worthy G. A. J. 2002.** Variation in stable carbon and nitrogen isotope ratios in northern fur seal (*Callorhinus ursinus*) tissues: implications for dietary and migratory reconstructions. *Mar. Ecol. Prog. Ser.* **236**: 289-300.
- Lajtha, K. and Michener, R. H. 1994.** Stable isotopes in ecology and environmental science. Blackwell Scientific, London.
- La mesa, M., Eastman, J. T. and Vacchi, M. 2004.** The role of notothenioid fish in the food web of the Ross Sea shelf waters: a review. *Polar Biol.*, **27**: 321-338.
- Le Boeuf, B. J., Costa, D. P., Huntley, A. C. & Feldkamp, S. D. 1988.** Continuous, deep diving in female northern elephant seals, *Mirounga angustirostris*. *Can. J. Zool.*, **66**: 446-458.
- Le Boeuf, B. J., Naito, Y., Asaga, T., Crocker D. and Costa D. P. 1992.** Swim speed in a female northern elephant seal: metabolic and foraging implications. *Can. J. Zool.*, **70**: 786-795.
- Le Boeuf, B. J., Crocker D. E., Blackwell, S. B. Morris, P. A. and Thorson, P. H. 1993.** Sex differences in diving and foraging behaviour of northern elephant seals. *Symp. Zool. Lond.*, **66**: 149-178
- Lek, S. and Guegan, J. F. 1999.** Artificial neural networks as a tool in ecological modelling, and introduction. *Ecological Modelling*, **120**: 65-73.

- Lesage, V., Hammill, M. O. and Kovacs, K. M. 1999.** Functional classification of harbour seal (*Phoca vitulina*) dives using depth profile, swimming velocity, and an index of foraging success. *Can. J. Zool.*, **77**: 74-87.
- Lesage, V., Hammill, M. O. and Kovacs, K. M. 2002.** Diet-tissue fractionation of stable carbon and nitrogen isotopes in Phocid seals. *Mar. Mamm. Sci.*, **18**(1): 182-193.
- Lindsey, A. A. 1937.** The Weddell seal in the Bay of Whales, Antarctica. *J. Mammal.*, **18**: 127-144.
- Lidersen, C. and Kovacs, K. M. 1999.** Behaviour and energetics of ice-breeding, North Atlantic phocid seals during the lactation period. *Marine Ecology Progress Series*, **187**: 265-281.
- Mansfield, A. W. 1958.** The breeding behaviour and reproductive cycle of the Weddell seal (*Leptonychotes weddellii*, Lesson). *Falkland Isl. Depend. Surv. Sci. Rep.*, **18**: 1-41
- Manzoni, M. 2001.** I ghiacci marini. *In* La natura dell'Antartide. Springer-Verlag Italia, 171-180.
- Minagawa, M. and Wada, E. 1984.** Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between ^{15}N and the animal age. *Geochim. Cosmochim. Acta*, **48**: 1135-1140.
- Mintenbeck, K., Brey, T., Jacob, U., Knust, R. and Struck, U. 2008.** How to account for the lipid effect on carbon stable-isotope ratio ($\delta^{13}\text{C}$): sample treatment effects and model bias. *Journal of Fish Biology*, **72**: 815-830.
- Miron M., L. L., Herrera M., L. G., Ramirez P., N. and Hobson, K. A. 2006.** Effect of diet quality on carbon and nitrogen turnover and isotopic discrimination in blood of a New World nectarivorous bat. *J. Exp. Biol.*, **209**: 541-548.
- Nelson, D. E., Angerbjorn, A., Liden, K. and Turk, I. 1998.** Stable isotopes and the metabolism of the European cave bear. *Oecologia* (Berl.), **116**: 177-181.
- Oftedal, O. T. 1993.** The adaptation of milk secretion to the constraints of fasting in bears, seals and baleen whales. *Journal of Dairy Science*, **76**(10): 3234-3246.
- Oftedal, O. T., Boness, D. J. and Tedman, R. A. 1987.** Ch. 6: The behavior, physiology, and anatomy of lactation in the Pinnipedia. *In* Current Mammalogy, Plenum Press, New York, **1**: 175-245.
- Owens, N. J. P. 1987.** Natural variations in ^{15}N in the marine environment. *Adv. Mar. Biol.* **24**: 389-451.
- Pankhurst, N. W. 1990.** Growth and reproduction of the Antarctic notothenioid fish *Pagothenia borchgrevinki*. *Polar Biol.*, **10**: 387-391.
- Park, Y. S., Lek, S., Scardi, M., Verdonschot, P. F. M. and Jorgensen, S. 2006.** Patterning exergy of benthic macroinvertebrate communities using self-organizing maps. *Ecological Modelling*, **195**: 105-113.
- Parker, P. L. 1964.** The biogeochemistry of the stable isotopes of carbon in a marine bay. *Geochim. Cosmochim. Acta*, **28**: 1155-1164.
- Phleger, C. F., Nichols, P. D., Erb, E. and R. Williams 1999.** Lipids of the notothenioid fishes *Trematomus* spp. and *Pagothenia borchgrevinki* from East Antarctica. *Polar Biol.*, **22**: 241-247
- Pinnegar, J. K. and Polunin, N. V. C. 1999.** Differential fractionation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among fish tissues: implications for the study of trophic interactions. *Functional Ecology*, **13**: 225-231.
- Plötz, J., Bornemann, H., Knust, R., Schroder, A. and Bester, M. 2001.** Foraging behaviour of Weddell seals, and its ecological implications. *Polar Biol.*, **24**: 901-909.
- Polischuk, S. C., Hobson, K. A. and Ramsay, M. A. 2001.** Use of stable-carbon and -nitrogen isotopes to assess weaning and fasting in female polar bears and their cubs. *Can. J. Zool.*, **79**: 499-511.

- Ponganis, P.J., Kooyman, G. L. and Castellini, M.A. 1993.** Determinants of the aerobic dive limit of Weddell seals: Analysis of diving metabolic rates, postdive end tidal PO_2 's, and blood and muscle oxygen stores. *Physiol. Zool.*, **66**(5): 732-749.
- Ponganis, P.J. and Stockard, T. K. 2007.** The Antarctic toothfish: how common a prey for Weddell seals? *Antarctic Science*, **19**(4): 441-442.
- Post, D. M. 2002.** Using stable isotope to estimate trophic position: models, methods and assumptions. *Ecology*, **83**: 703-718.
- Pütz, K. and Cherel, Y. 2005.** The diving behaviour of brooding king penguins (*Aptenodytes patagonicus*) from the Falkland Islands: variation in dive profiles and synchronous underwater swimming provide new insights into their foraging strategies. *Mar. Biol.*, **147**: 281-290.
- Ramsay, M.A. and Hobson, K.A. 1991.** Polar bears make little use of terrestrial food web: evidence from stable-carbon isotope analysis. *Oecologia*, **86**: 598-600.
- Rau, G. H., Hopkins, T. L. and Torres, J. J. 1991.** $^{15}N/^{14}N$ and $^{13}C/^{12}C$ in Weddell Sea invertebrates: implication for feeding diversity. *Mar. Ecol. Prog. Ser.*, **77**: 1-6.
- Rau, G. H., Ainley, D. G., Bengtson J. L., Torres, J. J. and Hopkins, T. L. 1991.** $^{15}N/^{14}N$ and $^{13}C/^{12}C$ in Weddell Sea birds, seals, and fish: implications for diet and trophic structure. *Mar. Ecol. Prog. Ser.*, **84**: 1-8.
- Reijnders, P.J. H., Plötz, J., Zegers, J. and Gräfe, M. 1990.** Breeding biology of Weddell seals (*Leptonychotes weddellii*) at Drescher Inlet, Riiser Larsen Ice Shelf, Antarctica. *Polar Biol.*, **10**: 301-306.
- Riedman, M. and Ortiz, C. L. 1979.** Changes in milk composition during lactation in the northern elephant seal. *Physiological Zoology*, **52**: 240-249.
- Robbins, C.T., Felicetti, L.A. and Sponheimer, M. 2005.** The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. *Oecologia*, **144**: 534-540.
- Robert-Coudert, Y., Sato, K., Kato, A., Charrassin, J. B., Bost, C. A., Le Maho, Y. and Naito, Y. 2000.** Preliminary investigations of prey pursuit and capture by king penguins at sea. *Pol. Biosci.*, **13**: 101-112.
- Sare, D.T.J., Millar, J. S. and Longstaffe, F.J. 2005.** Nitrogen- and carbon-isotope fractionation between mothers and offspring in red-backed voles (*Clethrionomys gapperi*). *Can. J. Zool.*, **83**: 712-716.
- Sato, K., Mitani, Y., Cameron, M. F., Siniff, D. B., Watanabe, Y. and Naito, Y. 2002.** Deep foraging dives in relation to the energy depletion of Weddell seals (*Leptonychotes weddellii*) mothers during lactation. *Polar Biol.*, **25**: 696-702.
- Sato, K., Mitani, Y., Kusugaya H. & Naito, Y. 2003.** Synchronous shallow dives by Weddell seal mother-pup pairs during lactation. *Mar. Mamm. Sci.*, **19**(2): 384-395.
- Schreer, J. F. and Testa, J. W. 1995.** Statistical classification of diving behavior. *Mar. Mamm. Sci.*, **11**(1): 93-96.
- Schreer, J. F. & Testa, J. W. 1996.** Classification of Weddell seal diving behavior. *Mar. Mamm. Sci.* **12**(2): 227-250.
- Schreer, J. F. and Kovacs, K. M. 1997.** Allometry of diving capacity in air breathing vertebrates. *Can. J. Zool.*, **75**: 339-358.
- Schreer, J. F., O'Hara Hines, R. J. and Kovacs, K. M. 1998.** Classification of dive profiles: a comparison of statistical clustering techniques and unsupervised artificial neural networks. *Amer. Stat. Ass. And Intern. Biom. Soc.*, **3**(4): 383-404.
- Schreer, J. F., Kovacs, K. M. and O'Hara Hines, R. J. 2001.** Comparative diving patterns of pinnipeds and seabirds. *Ecological Monographs*, **71**(1): 137-162.
- Siniff, D. B., De Master, D. P., Hofman, R. J. and Eberhardt, L. L. 1977.** An Analysis of the dynamics of a Weddell seal population. *Ecol. Monogr.*, **47**: 319-335.

- Slip, D. J., Hindell, M. A. and Burton, H. R. 1994.** Diving behaviour of southern elephant seals from Macquarie island: an overview. In *Elephant seals: population ecology, behaviour, and physiology*. Edited by B. J. Le Boeuf and R. M. Laws. University of California Press, Berkeley, 253-270.
- Sotiropoulos, M. A., Tonn, W. M. and Wassenaar, L. I. 2004.** Effects of lipid extraction on stable carbon and nitrogen isotope analyses of fish tissues: potential consequences for food web studies. *Ecol. of Fresh. Fish*, **13**: 155-160.
- Stegall, V. K., Farley, S. D., Rea, L. D., Pitcher, K. W., Rye, R. O., Kester, C. L., Stricker, C. A. and Bern, C. R. 2008.** Discrimination of carbon and nitrogen isotopes from milk to serum and vibrissae in Alaska Steller sea lions (*Eumetopias jubatus*). *Can. J. Zool.*, **86**: 17-23.
- Stephenson, R. 2005.** A theoretical analysis of diving performance in the Weddell seal (*Leptonychotes weddellii*). *Physiol. and Biochem. Zool.* **78(5)**: 782-800.
- Stirling, I. 1966.** A technique for handling live seals. *Journal of Mammalogy* **47(3)**: 543-544.
- Stirling, I. 1969a.** Distribution and abundance of the Weddell seal in the Western Ross Sea, Antarctica. *N. Z. J. Mar. Freshw. Res.*, **3**: 191-200.
- Stirling, I. 1969b.** Ecology of the Weddell seal in MacMurdo Sound, Antarctica. *Ecology*, **50**: 574-585.
- Stull, J. W., Brown, W. H. and Kooyman G. L. 1967.** Lipids of the Weddell seal, *Leptonychotes weddellii*. *J. Mammal.*, **48**: 642-645.
- Sweeting, C. J., Polunin, N. V. C. and Jennings, S. 2006.** Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. *Rapid communications in Mass Spectrometry*, **20**: 595-601.
- Tedman, R. A. and Bryden, M. M. 1979.** Cow-pup behaviour of the Weddell seal, *Leptonychotes weddellii* (*Pinnipedia*), in McMurdo Sound, Antarctica. *Aust. Wildl. Res.*, **6**: 19-37.
- Tedman, R. A. 1980.** Lactation in the Weddell seal, *Leptonychotes weddellii*. Ph.D. thesis, University of Queensland, Australia.
- Tedman, R. and Green, B. 1987.** Water and sodium fluxes and lactational energetics in suckling pups of Weddell seals (*Leptonychotes weddellii*). *Journal of Zoology*, London, **212 (1)**: 29-42.
- Testa, J. W., Siniff, D. B., Ross, M. J. and Winter, J. D. 1985.** Weddell seal - Antarctic cod interactions in McMurdo Sound, Antarctica. In: Siegfried, W.R., P.R. Condy and R.M. Laws (Eds). *Antarctic Nutrient Cycles and Food Webs*. Springer-Verlag, Berlin: 561-565.
- Testa, J. W. and Siniff, D. B. 1987.** Population dynamics of Weddell seals (*Leptonychotes weddellii*) in McMurdo Sound, Antarctica. *Ecological Monographs*, **57**: 149-165.
- Testa, J. W., Hill, S. E. B. and Siniff D. B. 1989.** Diving Behavior and maternal investment in Weddell Seals (*Leptonychotes weddellii*). *Mar. Mamm. Sci.*, **5(4)**: 399-405.
- Thomas, D. N. 2004.** What is pack ice? In *Frozen Oceans. The floating world of pack ice*. Firefly Books, Natural History Museum, London, 6-15.
- Thomas, J. A. and DeMaster D. P. 1983.** Parameters affecting survival of Weddell seal pups (*Leptonychotes weddellii*) to weaning. *Can. J. Zool.*, **61**: 2078-2083.
- Thomson, R. B. 1915.** Osteology of Antarctic seals. *Rep. Sci. Res. Voyage S.Y. "Scotia"*, Edinburgh, **4(3)**: 13-31.
- Thompson, D., Hammond, P. S., Nicholas, K. S. and Fedak, M. A. 1991.** Movements, diving and foraging behaviour of grey seals (*Halichoerus grypus*). *J. Zool. (Lond.)*, **224**: 223-232.
- Thompson, D. R., Phillips, R. A. Stewart, F. M. and Waldron, S. 2000.** Low $\delta^{13}\text{C}$ signatures in pelagic seabird: lipid ingestion as a potential source of ^{13}C -depleted carbon in the Procellariiformes. *Mar. Ecol. Prog. Ser.*, **208**: 265-271.

- Thorson, P.H. and Le Boeuf, B.J. 1994.** Developmental aspects of diving in northern elephant seal pups. *In* Elephant seals: population ecology, behaviour and physiology. Le Boeuf B.J. and Laws R. M. (Eds.) University of California Press, Berkeley, 271-289.
- Tieszen, L. L., Boutton, T.W., Tesdahal, K. G. and Slade, N.A. 1983.** Fractionation and turnover of stable carbon isotopes in animal tissue: implication for $\delta^{13}\text{C}$ analysis of diet. *Oecologia*, **57**: 32-37.
- Tims, H.W.M. 1910.** Report on a collection of seal embryos made during the voyage of the "Discovery". National Antarctic Exp. 1901-1904, London, **5**: 1-22.
- Vacchi, M., Pisano, E. and La Mesa, G. 1999.** Ecological features of Antarctic fishes. Cold-adapted organisms: ecology, physiology, enzymology, and molecular biology. Margesin R., Schinner F (eds.), 219-238.
- Vacchi, M., Greco, S. and La Mesa, M. 1999.** The coastal fish fauna of Terra Nova Bay, Ross Sea. *In* Faranda F, Guglielmo L., Ianora A. (eds), Ross Sea Ecology. Italian Antarctic Expeditions (1987-1995). Springer, Berlin Heidelberg New York, 457-468.
- Vanderklift, M.A. and Ponsard, S. 2003.** Sources of variation in consumer-diet $\delta^{15}\text{N}$ enrichment: a meta-analysis. *Oecologia*, **136**: 169-182.
- Watanabe, Y., Bornemann, H., Liebsch, N., Plotz, J., Sato, K., Naito, Y. and Miyazaki, N. 2006.** Seal mounted cameras detect invertebrate fauna on the underside of an Antarctic ice shelf. *Mar. Ecol. Prog. Ser.*, **309**: 297-300.
- Wheatley K. E., Bradshaw, C. J.A., Harcourt, R. G. and Hindell, M.A. 2008a.** Feast or famine: evidence for mixed capital income breeding strategies in Weddell seals. *Oecologia*, **155**: 11-20.
- Wheatley, K. E., Nichols, P.D., Hindell, M.A., Harcourt, R. G. and Bradshaw, C. J.A. 2008b.** Differential Mobilization of Blubber Fatty Acids in Lactating Weddell Seals: Evidence for Selective Use. *Physiological and Biochemical Zoology*, **81(5)**: pp. 651-662
- Williamns, T.M. and Kooyman G. L. 1985.** Swimming performance and hydrodynamic characteristics of harbor seals, *Phoca vitulina*. *Physiol. Zool.*, **58**: 576-589.
- Wilson, E. A. 1907.** Mammalia (seals and whales) p. 1 to 66. *In* National Antarctic expedition, 1901-04. Natural History, Zoology 4. British Museum, London.
- Wilson, R. P. 1990.** the behaviour of diving birds. *Proc. Int. Ornithol. Congr.*, **20**: 1853-1867.
- Wilson, R. P. 1995.** Foraging ecology. *In* Williams T. D. (ed.) The penguins. Oxford University Press, Oxford, 81-106.
- Wilson, R. P., Pütz, K., Benoit, J and Lage, C. J. 1995.** Artifacts arising from sampling interval in dive depth studies of marine endotherms. *Polar Biol.*, **15**: 575-581.
- Williams, T. M., Fuiman, L.A., Horning, M. and Davis, R. W. 2004.** The cost of foraging by a marine predator, the Weddell seal *Leptonychotes weddellii*: pricing by the stroke. *The Jour. of Exper. Biol.*, **207**: 973-982.
- Wilson, R. P., Putz, K., Charrassin, J. B. and Lage, J. 1995.** Artifacts arising from sampling interval in dive depth studies of marine endotherms. *Polar Biol.*, **15**: 575-581.
- Wohlschlag, D. E. 1968.** Fishes beneath Antarctic ice. *Australian Natural History*, **16**: 45-48.
- Yukhov, V. L. 1970.** New data on the distribution and biology of *Dissostichus mawsoni* Norman in Antarctic high latitudes. *J. Ichthyol.*, **10**: 422-424.
- Yukhov, V.L. 1971.** The range of *Dissostichus mawsoni* Norman and some features of its biology. *J. Ichthyol.*, **11**: 8-18.
- Zaho, L., Castellini, M.A., Mau, T. L. and Trumble, S. J. 2004.** Trophic interactions of Antarctic seals as determined by stable isotope signatures. *Polar Biol.*, **27**: 368-373.
- Zaho, L., Schell, D. M. and Castellini, M.A. 2006.** Dietary macronutrients influence ^{13}C and ^{15}N signatures of pinnipeds: captive feeding studies with harbour seals (*Phoca vitulina*). *Comp. Biochem. and Phys., Part A* **143**: 469-478.

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