

FOOD MANAGEMENT

IN

BOTTLENOSE DOLPHINS

(TURSIOPS TRUNCATUS)

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1. Introduction

The present study has been carried out in order to describe the daily management of fish in Duisburg dolphinarium, and to highlight the problems encountered. The aim of this work is also to pinpoint the most important criteria of fish quality that have to be met according to the official standards for housing bottlenose dolphins.

Nutrition represents a vital aspect in health management of marine mammals, and this from both a nutritional and sanitary point of view. The nutritional balance of the diet depends on the quality of the selected fish and on how this quality is maintained during storage and thawing processes; furthermore, the microbiological load of the fish represents an important aspect. As a matter of fact, fish used for feeding may be the prime source of import of potential pathogens into a closed water system (Overath, Gellissen and Garcia Hartmann; 1997). This is particularly true in dolphinaria working with artificial sea-water and a biological water treatment, as does the Duisburg Zoo's Dolphinarium, instead of water disinfection systems.

Dolphinaria are submitted to strict regulations and laws for the management of marine mammals, in order to ensure the best life and health conditions for the animals. The E.A.A.M. (European Association for Aquatic Mammals) is the competent organism that sets the rules in Europe. In the E.A.A.M. Standards for Establishments Housing Bottlenose Dolphins (@ 24), the section concerning the feeding of dolphins stipulates the following:

1. Food must be wholesome and of a standard fit for human consumption. It must be given to the animals in sufficient quantities and its nutritional value must be sufficient to keep the animals healthy. Any dietary additives must only be given on veterinary advice.
2. Preparation of food: All chemical and bacterial contamination must be avoided when preparing food. Frozen food must be kept in a deep-freezer below -28°C and used within four (4) months for mackerel and seven (7) months for other species.
3. Food distribution: The animals must be fed at least once a day (except other instructions from a veterinary surgeon). The diet of each individual must be determined according to its particular characteristics, such as age, size, weight, pregnancy, etc. Food must be given to animals -which are fed individually-, by a trained person who must be sufficiently competent to measure the differences and variations in the animals' eating habits in order to ensure their good health.

In order to meet these requirements, the E.A.A.M. provides some recommendations for general hygiene:

1. All waste (food remains, faeces, etc.) must be removed from the pool daily in order to prevent contamination and infection. The walls and floor of the pools must be cleaned as frequently as possible to maintain the quality of the animals' environment.
2. All utensils used in the preparation and distribution of food must be cleaned after use. The kitchens and areas for handling food must be washed down daily and treated with cleaning products (e.g. hot water, detergents, disinfectants, etc.). Chemical products used for cleaning must not be stored in areas used for storing food. Those chemicals must not be harmful to the animals.
3. Buildings and walls must be clean and in good condition. Structures must be kept in good repair. Pools must not contain any sharp or pointed objects on which the animals could injure themselves; they should be designed in such a way that there are no areas where the water remains unchanged.
4. Regular precautions must be taken against flies and other pests. Pest control products must be used under veterinary supervision.
5. The personnel must have access to washbasins, showers and lavatories to ensure cleanliness necessary for the well-being of the animals and themselves.

The fish species composing the diet of captive cetaceans are restricted, in comparison with the ones they feed upon in the wild (Worthy, 1990; Cockcroft & Ross, 1990, Barros & Odell, 1990). This is due to the commercial availability and cost of the fish. The most commonly-fed species are commercial fish meant for human consumption. Nevertheless, the number of species fed should not be too small, in order to ensure a complementary nutrient profile in the diet (White & Francis-Floyd, 1988) and to avoid dependence on one single fish species (Crissey, 1998). In the wild, a preference for mullet has been reported (Cockcroft & Ross, 1990). A balanced diet should be composed of high- and low-fat fishes (Geraci, 1978). Food uptake can be induced or stimulated by administering large doses of vitamin B12 (Wallach & Boever, 1983).

In order to ensure a fish quality equal to the one meant for human consumption, it is necessary to meet some criteria: first, the history of the catch should be ascertained and a catch date should be recorded on the fish boxes. Fish must be packaged in plastic-lined boxes and frozen as quick as possible. The optimal size for packages should be 10-20 kg to allow for proper thawing (Crissey, 1998).

Further, quality controls must be applied at the delivery stage, during the thawing process and just before feeding the dolphins. Fish should be stored in a freezer at a temperature below -28°C (E.A.A.M. standards), with 85-90% relative humidity and cold air circulation (Crissey, 1998).

As the thawing process is a main critical point for the fish's final quality, it should occur in the best conditions, i.e. in a refrigerated space where temperature does not exceed 7°C . This reduces the bacterial growth during thawing and thus restricts the final bacterial load of the fish and decomposition processes induced by bacteria which lead to a decrease in nutritional value. A second method would be to thaw under potable running water, but this method is not preferred because the water flow induces nutrient loss and available water increases the risk of bacterial growth. Fish should never be

thawed at room temperature, and the time it spends at room temperature should be kept to a minimum. Once thawed, frozen food should never be refrozen (Crissey, 1998). No fish should be fed more than 24 hours after it has been removed from the freezer (White & Francis-Floyd, 1988).

The handling of thawed fish should be carried out in such a way that it prevents any spoilage, which implies that the persons in charge of handling the fish should wash their hands properly before manipulating the fish and that all utensils should be cleaned and disinfected directly after each use (White & Francis-Floyd, 1988). During each manipulation made by competent personnel, inadequate fish should be removed from the sinks and should never be fed to the dolphins. The fish is controlled according to organoleptic criteria and can be classified in different groups, as suggested by Crissey in Table 1:

Table 1: Quality control standards

Factor	Acceptable	Inferior	Unacceptable
General appearance	shine or luster to skin; no breaks in skin; no bloating or protrusion of viscera; no dehydration	some loss of sheen	luster
Eyes	translucent, full; may be slightly sunken	dull or cloudy, slightly sunken	dull, sunken; cornea opaque (white); red-bordered eyes gone, lumpy
Gills	bright red to pink; moist	pink to slight brownish	grayish-yellow and covered with mucus
Odour	fresh odour	mild sour or “fishy” odour	medium to strong odour, fatty fish may smell rancid
Feel	firm and elastic; meat does not stay indented when touched	moderately soft, slight indentation left when touched	soft, spongy and flabby; exudes juice and easily indented when handled; may break open or skin may split when handled
Vent	normal in shape and colour	slight protrusion	noticeable discoloration
Lateral line	normal, no discoloration	pinkish tinge	red to dark red

The fish species fed to bottlenose dolphins vary from one dolphinarium to another, but there are some general considerations to be aware of. The first one is that there are different classes of fish according to their fat content: herring is an oily fish, mackerel less so, smelt and capelin are fat-poor fish, and squid – which is an invertebrate - contains even less fat (White & Francis-Floyd, 1988). The fats of fish fed to dolphins contain high amounts of polyunsaturated fatty acids (Engelhardt, Geraci &

Walker, 1975). The fattier the fish, the more energy is available; thus dolphins with a high caloric demand (debilitated, sick, lactating, etc.) should be fed fat fish species. Yet, particular attention should be dedicated to the shelf life of fatty fish (see point 2 of EAAM standards concerning the feeding of dolphins), as they are prone to oxidation and bacterial deterioration, which in severe cases could lead to food-borne intoxication. Furthermore, the fat composition of fish varies from one period to the other in function of the location, reproductive status and nutritional status of the fish (Geraci, 1978; Crissey, 1998). These fluctuations should be taken into account when composing the diets, which would require a nutritional analysis of each new fish arrival.

The proteins found in fish are of high quality and their amount varies less from one species to another. On the other hand, the carbohydrate content of fish is rather low (Geraci, 1978).

Both human and marine mammal lethal intoxications by biological toxins have been reported (O'Shea & Geraci, 1999). These intoxications were due to toxins produced by marine dinoflagellates, which most frequently induce neurologic and gastrointestinal symptoms. These toxins are produced during monospecific blooms in certain regions, so it is interesting to investigate the history of the catch of fish fed to marine mammals. Many studies have been carried out about the presence of toxic compounds in wild marine mammals, but there are still some doubts about their impact on marine mammal populations (O'Shea & Geraci, 1999). Some parasites e.g. the nematode *Anisakis simplex* are known to infest cetaceans through the ingested fish (Raga, 1997). But as the fish fed to dolphins in most delphinaria has been deep-frozen, the larvae do not survive and there is little potential risk (García Hartmann, personal communication, April 2000).

Erysipelas is an important disease in captive cetaceans and is supposed to be caused by marine *Erysipelothrix rhusiopathiae* (García Hartmann, 1997). "Cetaceans are the more susceptible aquatic mammals to erysipelas" (Bauwens, Cnops & De Meurichy, 1992). All cetaceans should be vaccinated against *erysipelas* (White, 1971) - which is or used to be the case in many delphinaria - because the organism has frequently been isolated from fish (Bauwens, Cnops & De Meurichy, 1992). Commercially available swine erysipelas vaccine show satisfactory results (White, 1971). Non vaccinated animals presenting erysipelas show either the characteristic rhomboid skin lesions or the peracute septicemic form which is more frequent and usually ends tragically (García Hartmann, 1997).

The energy requirement of marine mammals has long been thought to be considerably higher than that of terrestrial mammals. But recent results suggest that small odontocetes have the same metabolic rate as other mammals. The energy requirement varies in function of body size, activity level, reproductive state, thermoregulatory expenses of the animal and whether or not it is actively growing (Worthy, 1990). White (1971) gives 4,54-7,26 kg of fish (diet composed of 60% bluerunner, 20% butterfish and 20% herring) as food requirement for porpoises in function of the variations cited higher. Geraci (1978) gives the following requirements:

- young growing dolphins 9-15 % of body weight (BW)
- young whales 5-9 % BW
- older dolphins 4-9 % BW

Worthy (1990) gives precise formulas for the calculation of gross energy requirements. Vitamin requirements will be discussed under **point 5.3**.

2. MATERIAL AND METHODS

2.1 Fish species composing the diet

The different fish species fed in Duisburg are:

- Atlantic herring (*Clupea harengus*):
 - o Fr - Hareng de l'Atlantique;
 - o Germ - Hering;
 - o Sp - Arenque del Atlántico.
- Atlantic mackerel (*Scomber scombrus*):
 - o Fr - Maquereau commun;
 - o Germ - Makrele;
 - o Sp - Caballa del Atlántico.
- Norway pout (*Trisopterus esmarkii*):
 - o Fr - Tacaud norvégien;
 - o Germ - Stintdorsch;
 - o Sp - Faneca noruega.
- Sprat (*Sprattus sprattus*):
 - o Fr - Sprat;
 - o Germ - Sprotte;
 - o Sp - Espadín.
- Whiting (*Gadus merlangus*):
 - o Fr - Merlan;
 - o Germ - Wittling;
 - o Sp - Pescadilla .
- Squid (*Loligo patagonica or gahi*): Patagonian Inshore Squid
 - o Fr - Calmar;
 - o Germ - Tintenfisch;
 - o Sp.- Calamar patagónico; Arg - Calamarete .

Due to market availability some species are fed only during specific periods of the year, e.g. whiting (*Merlangius merlangus*) and Norway pout (*Trisopterus esmarkii*). The proportion of each type of fish in the diet is established in function of the respective nutritional composition and of the requirements of each single dolphin in respect of its physiological status. The details of fish composition and of dolphin requirements will be discussed later.

The following sheets contain specific information on the different fish species:

Atlantic herring (*Clupea harengus harengus*):



Figure 1: *Clupea harengus* (@9)

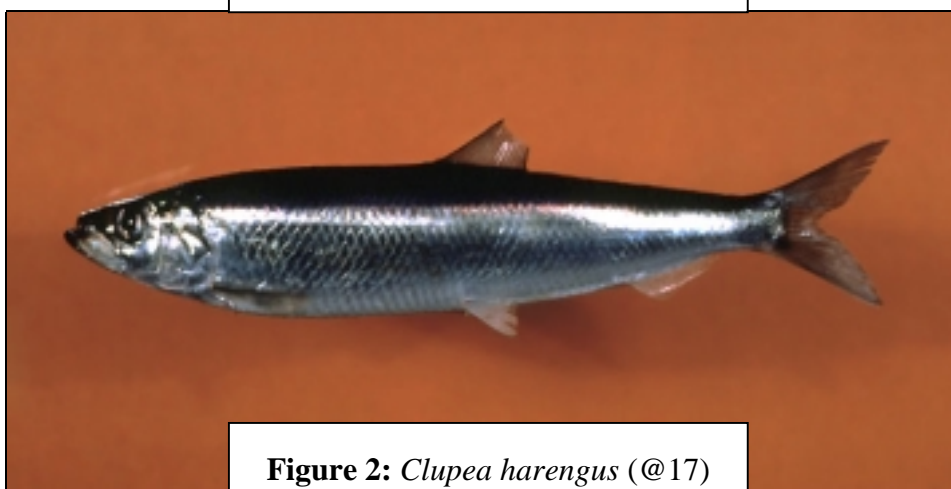


Figure 2: *Clupea harengus* (@17)

Identification: body elongate and fairly slender, belly rather rounded, scutes without prominent keel. No median notch in upper jaw (cf. *Alosa*). Gill cover (operculum) without radiating bony striae (cf. *Sardinops*, which has dark spots along the flank). Hind border of gill opening evenly rounded (with two fleshy outgrowths in *Sardinella*). Pelvic fin insertion behind vertical from dorsal fin origin (below or in front in *Sprattus*) pelvic finrays: 8 (rarely 7 or 9) (only *Alosa* and some species of *Sardinella* have this count; all other clupeids have 6 or 7). No distinctive dark spots on body or fins. Overlaps *C. pallasii* in White Sea, but distinguished by more vertebrae and post-pelvic scutes (usually 55 to 57 and 12 to 16; cf. usually 52 to 55 and 10 to 14).

Geographical distribution: Eastern Atlantic (northern Bay of Biscay northward to Iceland and southern Greenland, eastward to Spitzbergen and Novaya Zemlya, also Baltic; western Atlantic (south-western Greenland, Labrador, southward to South Carolina).

Habitat and biology: coastal, pelagic down to 200 m. Feeds on small planktonic copepods in the first year, and thereafter mainly copepods (especially *Calanus finmarchicus* and *Temora longicornis*), but also hyperid amphipods, euphausiids, mysid shrimps, small fishes, arrow-worms, ctenophores and pteropods).

Size: to 40 cm standard length, usually 20 to 25 cm.

Atlantic mackerel (*Scomber scombrus*):



Figure 3: *Scomber scombrus* (@8)

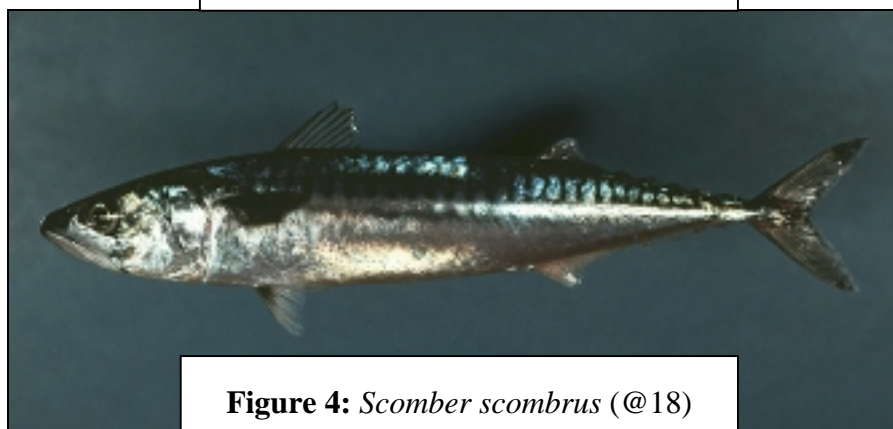


Figure 4: *Scomber scombrus* (@18)

Identification: palatine wide, teeth in two widely spaced rows. Space between first dorsal fin groove and second dorsal fin clearly greater (approximately 1.5 times) than length of groove; anal fin origin opposite that of second dorsal fin or nearly so; anal fin spine conspicuous, joined to the fin by a membrane but clearly independent of it. Swimbladder absent. Vertebrae 13 precaudal plus 18 caudal; first haemal spine anterior to first interneural process; 21 to 28 interneural bones under first dorsal fin. Colour: markings on back oblique to near vertical, with relatively little undulating; belly unmarked.

Geographical distribution: North Atlantic Ocean, including the Baltic Sea; eastern Atlantic including the Mediterranean and the Black seas; and western Atlantic from Labrador to Cape Lookout.

Habitat and biology: an epipelagic and mesodemersal species, most abundant in cold and temperate shelf areas. Juvenile Atlantic mackerel feed on zooplankton (fish larvae, small crustaceans, pteropods). As they grow, they are in turn preyed upon by tunas, sharks and dolphins.

Size: maximum fork length is 50 cm, common to 30 cm.

Norway pout (*Trisopterus esmarkii*):



Figure 5: *Trisopterus esmarkii* (@10)



Figure 6: *Trisopterus esmarkii* (@14)

Identification: Very similar body plan as *Merlangius merlangus*. No chin barbel, with the lower jaw slightly longer than the upper jaw, and the eyes are large in proportion to head size. Three dorsal fins (*Gadidae*) all widely spaced, with the first being slightly pointed in profile. Of the two anal fins, the first has a long base, in fact longer than the distance from the snout to the anus. Reported age: 5 years.

Habitat: benthopelagic, marine.

Food: diet consists of crustaceans and small fish

Geographical distribution: Northeast Atlantic: south-west Barents Sea, sometimes at Bear Island, south to the English Channel, around Iceland, and at the Faeroe Islands.

Size: max. 35cm.

Sprat (*Sprattus sprattus*):

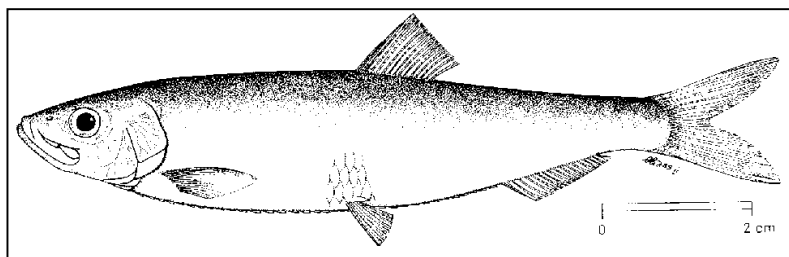


Figure 7: *Sprattus sprattus* (@25).

Identification : fusiform body, slightly compressed; scutes form a well-pronounced keel on the belly, from the throat to the anus. Lower jaw slightly larger than upper jaw, without median notch; smooth operculum. Origin of dorsal fin at mid-length of the body, long anal fin beginning behind the dorsal; origin of pelvic fins under or slightly in front of the dorsal fin. Colour: back is blue-greenish; sides are silver.

Three sub-species can be distinguished thanks to small differences, namely the number of post-pelvic scutes.

- *S. s. sprattus* : Atlantic Ocean and North Sea (on average over 11.5 post-pelvic scutes).
- *S. s. phalerinus* : Mediterranean and North Sea (on average under 11.3 post-pelvic scutes).
- *S. s. balticus* : Baltic Sea (on average under 11.5 post-pelvic scutes).

Habitat and biology: pelagic coastal species, often living very close to the shores, their tolerance to low salinity (4 ‰) even enabling them to enter estuaries. Remains close to the sea bottom during the day and comes close to the surface at night; can create large concentrations. Large migrations between feeding areas occur during winter, and migrations between egg-laying areas occur in the spring. Can breed all year round, close to the shores or as far as 100 kilometres into the open sea, but mainly breeds from May to June in the Black Sea and from December to April in the Mediterranean. First sexual maturity around 1 year. Adults mainly feed from plankton, young diatoms and copepod eggs and larvae. Also to be found in the eastern Atlantic Ocean, from Lofoten Islands and the Baltic Sea to the Moroccan coasts.

Size : max. 16 cm; on average 8 to 12 cm.

Whiting (*Gadus merlangus*):

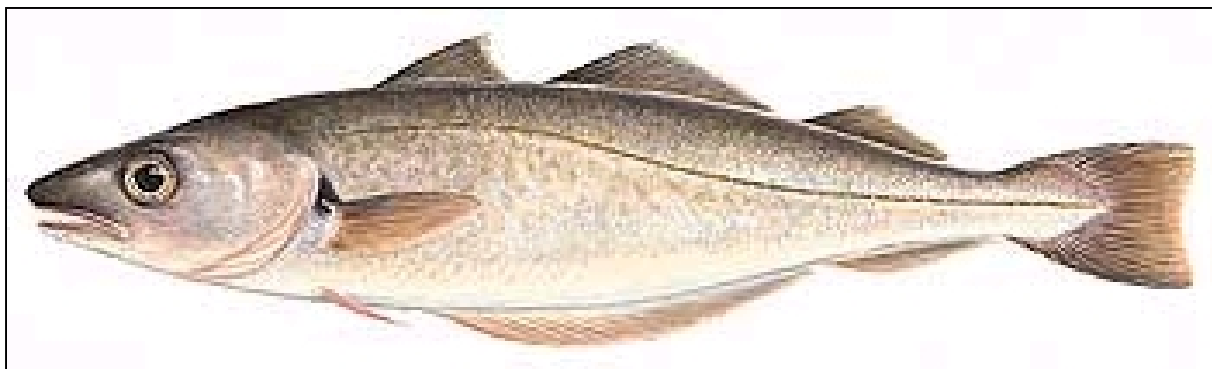


Figure 8: *Gadus merlangus* (@25)

Identification: as it belongs to the *Gadidae* family (cods and haddocks), the whiting has three dorsal fins. The abdominal fins at the level of the gill cover also remind of that belonging, as well as both anal fins. The dark spot near the abdominal fin is a particularity of the species, as well as the prominent upper jaw with pointed teeth. The back is grey-olive, the sides are silvery and the abdomen is white.

Food: feeds on shrimps, crabs, molluscs, small fish, polychaetes and cephalopods.

Geographical distribution: Northeast Atlantic: from the south-eastern Barents Sea and Iceland to Portugal, also in the Black Sea, Aegean Sea, Adriatic Sea and adjacent areas. Rare in the north-western Mediterranean.

Size: max 40 cm; max. reported age: 10 years.

Squid (*Loligo patagonica* or *L. gahi*):

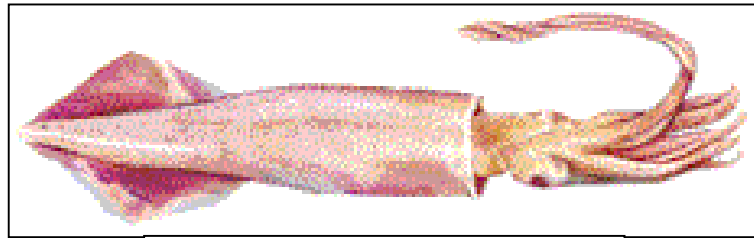


Figure 9: *Loligo gahi* (@11)

Identification : long fusiform body, very large eyes, has eight short and two long tentacles. Mantle cylindrical, rather slender, slenderer in large males. Triangular fins covering half the body length are joint at the posterior end; head slender. Funnel-mantle rocking apparatus a simple, straight groove. Eyes covered with transparent skin (corneal membrane). Arms: suckers in 2 rows; sucker rings with 5-9 square teeth on distal half and basal half smooth; left arm of mature male hectocotylished by modifications of the distal third to fourth of arm, bases of the suckers enlarged to nipple-like papillae in 2 rows, suckers in dorsal row minute or lost. Tentacles shorter than mantle; club short, suckers in 4 rows and enlarged in medial 2 rows; the largest sucker ring with 32-38 blunt small teeth. Colour may vary but usually pink or beige with darker spots.

Food: feeds on crustaceans, benthic and pelagic fish.

Geographical distribution: known from Peru to Chile and Argentine. Common on the continental shelf from Tierra del Fuego to the Golfo San Jorge in the Argentine waters.

Size: attains 33 cm.

2.2 Daily work at the dolphinarium kitchen (from 7 February to 31 March 2000)

Fish and squid are delivered every two months by the *Dieter Farell company* (address in appendix). The delivery takes place early in the morning in order to avoid the warmest hours of the day. Fish is deep-frozen and packed in bulk, each pack weighing about 20 to 25 kg, and consisting of a plastic inner layer and a cardboard crate. Upon delivery the nature, the quantity and the quality of the fish are carefully controlled by the dolphinarium staff. The goods are then directly stored in the deep-freezer, the new crates being placed behind the less recent ones, in order for the latter to be used first. The deep-freezer is equipped with a blower guaranteeing a constant temperature of about -28°C to -30°C .

The deep-frozen fish is taken out of the freezer in the afternoon of the day before consumption. It is then taken out of the cardboard crate and stored in the kitchen on a tilted stainless-steel working surface equipped with a drain. The dolphinarium staff daily prepares one pack of blue whiting, one of mackerel, another one of herring and squid, and a half pack of sprat. However, all that fish is not meant to be eaten by the dolphins. A portion is indeed set apart for the beluga (*Delphinapterus leucas*), the Commerson dolphin (*Cephalorhynchus commersonii*) and the fur seals¹ (*Arctocephalus pussillus*). The fish is then left to thaw in its plastic wrapping; the process occurs at room temperature, i.e. 17 to 22 °C, and lasts until the next morning. At 8 A.M. the fish is then taken out of its plastic wrapping and sorted out by the dolphinarium staff, according to the organoleptic criteria mentioned under point 2.4. The different dolphins' portions are subsequently prepared according to **Table 1**. To each dolphin corresponds a large and a small stainless steel bucket, both equipped with a coloured plastic ring which is different for each dolphin. The daily ration is weighed as accurately as possible and is then put into the large bucket, which has a capacity of 10 to 12 litres. For each meal a portion of fish is taken out of the large bucket and placed into the small one, which has a capacity of 6 litres. The small buckets are then left under streaming cold water, in order speed up the thawing process and to eliminate possible gravy and impurities from the fish. Once the fish has been sorted out, the buckets are stored at room temperature until the end of the day.

Dolphins	Fish in Kg					Total	Bucket
	<i>T.esmarkii</i>	<i>S.scombrus</i>	<i>S.sprattus</i>	<i>L.gahi</i>	<i>C.harengus</i>		
Pepina	2	3,5	1	0,5	1	8	yellow
Delphi	2	1	1	1	2	7	green
Daisy	2	1,5	0,5	/	1	5	orange
Ivo	2,5	/	1	1	1,5	6	pink
Iris	1	1,5	1	1	1,5	6	blue

Table 1: Diet of the five bottlenose dolphins² on the week of the 20th to the 27th of March.

¹ This was the situation until the fur seals were removed from the dolphinarium on 17.4.2000.

² Pepina: 19-year-old female bottlenose dolphin; Delphi: 8-year-old female bottlenose dolphin
Daisy: 3-year-old subadult female bottlenose dolphin; Ivo: 21-year-old male bottlenose dolphin
Iris: 31-year-old female bottlenose dolphin

After the fish has been prepared, the kitchen and all the tools are cleaned and disinfected. In the meantime, the fish buckets are temporarily placed in the shower room next to the kitchen, in order to avoid any contamination of the fish with detergents or disinfectants.

All surfaces are first cleaned with a jet of cold water under pressure; then they are disinfected using a Lysoformin spezial® solution (guanidin derivat + didecyl-dimethylammonium-chloride); they are subsequently rinsed with streaming hot water at approximately 65°C. After dissipation of the steam, the surfaces are scraped and the buckets brought back in the kitchen; they will be placed in a stainless-steel sink of about 2 metres length equipped with 8 taps.

2.3 Vitamin and mineral supplements

The exact amount of vitamin and mineral supplements dispensed at Duisburg for an adult dolphin (220-250 kg) is:

- 4 Sea Tabs® pills daily (official dose = 1 pill / 56,75 kg body weight, or 1 pill / 2,27-4,54 kg pounds of fish).
- 1 Plastulen®N pill daily; contains 324 mg iron sulphate (equivalent to 100 mg Fe) and 0.5 mg folic acid.
- 1 E1000® pill three times a week (Tuesday, Thursday and Saturday).
- Vitamin C: on request of the veterinarian.

These pills are incorporated into the fish by introducing them under the gills and through a thin membrane into the abdominal cavity; fish prepared in this way is fed singly during the morning feeding, in order to avoid vitamin destruction by antivitamin enzymes of the fish.

2.4 Organoleptic criteria for fish quality control

Organoleptic criteria are applied at the dolphinarium; they are quite subjective, yet experienced trainers directly recognise inappropriate fish without focusing precisely on the part of the fish that is not normal.

The fish is subject of several controls during its handling; first of all, before paperwork is signed at the delivery, the outer aspect and the amount of boxes is checked by dolphinarium employees who are familiar with this control. One box of each type of fish is opened and checked for the right size, frozen aspect and general aspect. Particular attention is dedicated to the presence of freeze brands and thawing signs (wet aspect of the cardboard crate, presence of water, flabby fish,...). The fish must preferably be packaged in plastic lined boxes in order to avoid air contact.

A second control is carried out when the fish is thawed and prepared for daily feeding. Every single fish is taken in the hand and checked before being put in the respective dolphins' buckets. At this point, only acceptable fish –i.e. only high-quality fish- is retained, and if the slightest doubt of the fish's quality remains, it is put aside.

The major criteria used in the dolphinarium for determining the rejection of fish are:

- Damaged fish.
- Freeze-brands.
- Colour changes in the skin, gills and eyes.
- Protrusion of viscera.
- Rancid odour.
- Flabby feeling.
- Dehydrated skin.
- Sharp or pointed fins.
- Unwanted fish species.

Further, the trainers will check the fish once again when selecting it for each feeding throughout the day and again during the feeding itself.

2.5 Bacteriological control

Samples are taken by the veterinarian on fish thawed in the same way as the fish fed to the dolphins. The veterinarian wears gloves and uses a scalpel and sterile swabs for sample collection. Before incising the fish, the scalpel blade is sterilised over the flame of a Bunsen burner (gas burner) in order to avoid secondary contamination.

Skin, gills, muscles and abdominal cavity are sampled for each type of fish. The sampling zones in squid differ a little bit due to its anatomical differences with fish: skin, inner sac, outer sac and top of sac. In addition some samples are taken from the cutting boards, the sink and the knives in the fish kitchen.

The samples are sent to BIOGNOST GmbH (address in appendix) for routine bacteriological examination.

2.6 Fish nutrients analysis

Samples of each fish species are sent to the Staatliches Veterinäruntersuchungsamt Krefeld (address in appendix) where total microbial count, energy, fat and protein contents are determined. This type of analysis was carried out twice a year in the past but there is a trend towards analysing every new delivery.

3. Results

3.1 Organoleptic control of fish quality

From more or less 112.5 kg of thawed fish, most frequently only 12 to 15 kg of fish are inappropriate for consumption, i.e. 10,6 to 13,3%. The biggest part of the unsuitable fish consists of damaged or freeze-branded fish. The amount of inappropriate fish was weighed on 5 occasions and was appreciated during a two-week period. Considering that the fish was selected according to very strict criteria (that means that the slightest imperfection leads to rejection), the percentage of rejected fish indicates that the quality of the fish after thawing is quite good.

On one occasion it happened that a bigger amount (20-22%) of fish was not good enough for consumption; this was due to a storage problem that had led to large freeze-brandings on the peripheral fishes.

3.2 Bacteriological findings

(+) = few

(++) = moderate

(+++)= massive

Species	Part	Date of analysis		
		23.03.1999	25.11.1999	22.2.2000
Atlantic Herring (<i>Clupea harengus</i>)	SKIN	<i>Pseudomonas stutzeri</i> +++ <i>Pseudomonas fluorescens</i> + <i>Ochrobactrum anthropi</i> + <i>Micrococcus sp.</i> + <i>Staphylococcus epidermis</i> + <i>Shewanella putrefeciens</i> ++ <i>Acinetobacter anitratus</i> +	<i>Pseudomonas alcaligenes</i> ++ aerobic sporeformers +++ <i>Micrococcus sp.</i> +	<i>Serratia liquefaciens</i> + <i>Staphylococcus hominis</i> +
	GILLS	No samples taken	<i>Aeromonas hydrophila</i> + <i>Micrococcus sp.</i> +	<i>Serratia liquefaciens</i> +
	ABDOMEN	<i>Shewanella putrefaciens</i> ++ <i>Acinetobacter sp.</i> ++ <i>Pseudomonas fluorescens</i> + <i>Pseudomonas stutzeri</i> +	aerobic sporeformers +	<i>Serratia marescens</i> +
	MUSCLE	No bacterial growth	No bacterial growth	No bacterial growth

Norway pout (<i>Trisopterus esmarkii</i>)	SKIN	<i>Shewanella putrefaciens</i> ++ <i>Acinetobacter anitratus</i> ++ <i>Aeromonas hydrophila</i> + <i>Staphylococcus epidermis</i> ++ <i>Micrococcus sp.</i> ++	aerobic sporeformers +	No bacterial growth
	GILLS	No samples taken	aerobic sporeformers +++	<i>Serratia liquefaciens</i> +++
	ABDOMEN	<i>Aeromonas hydrophila</i> +++ <i>Shewanella putrefaciens</i> ++ <i>E.coli</i> + <i>Staphylococcus epidermis</i> +		<i>Serratia liquefaciens</i> +++ <i>Acinetobacter lwoffii</i> ++
	MUSCLE	No bacterial growth	<i>Aeromonas hydrophila</i> + aerobic sporeformers +	No bacterial growth

Sprat (<i>Sprattus sprattus</i>)	SKIN	<i>Pseudomonas stutzeri</i> +++ <i>E.coli</i> ++ <i>Micrococcus sp.</i> +	<i>Moraxella sp.</i> ++ <i>Staphylococcus warneri</i> ++ <i>Micrococcus sp.</i> +	No bacterial growth
	GILLS	No samples taken	<i>Aerococcus viridans</i> +	<i>Serratia liquefaciens</i> +
	ABDOMEN	<i>Staphylococcus epidermis</i> +	No bacterial growth	No bacterial growth
	MUSCLE	No bacterial growth	No bacterial growth	No bacterial growth

Atlantic mackerel (<i>Scomber scombrus</i>)	SKIN	<i>Shewanella putrefaciens</i> ++ <i>E.coli</i> + <i>Acinetobacter sp.</i> +++ <i>Ochrobactrum anthropi</i> + <i>Micrococcus sp.</i> ++ <i>Staphylococcus epidermis</i> +	aerobic sporeformers ++ <i>Micrococcus sp.</i> +	<i>Staphylococcus epidermis</i> +
	GILLS	No samples taken	aerobic sporeformers ++ CDC GR.IV C-2 ++	<i>Serratia liquefaciens</i> +
	ABDOMEN	<i>Shewanella putrefaciens</i> ++ <i>Acinetobacter sp.</i> +++ <i>Staphylococcus epidermis</i> +	<i>Aeromonas hydrophila</i> +	No bacterial growth
	MUSCLE	No bacterial growth	No bacterial growth	No bacterial growth
Whiting (<i>Gadus merlangus</i>)	SKIN	<i>Pseudomonas stutzeri</i> +++ <i>E.coli</i> + <i>Acinetobacter anitratus</i> + <i>Staphylococcus epidermis</i> + <i>Micrococcus sp.</i> +	No samples taken	No samples taken
	GILLS	No samples taken	No samples taken	No samples taken
	ABDOMEN	<i>Ochrobactrum anthropi</i> ++ <i>Micrococcus sp.</i> + <i>Staphylococcus epidermis</i> +	No samples taken	No samples taken
	MUSCLE	No bacterial growth	No samples taken	No samples taken

Squid <i>(Loligo patagonca</i> <i>or L. gahi.)</i>	SKIN	<i>Acinetobacter sp</i> +++ <i>Enterobacter amnigenus</i> ++ <i>Shewanella putrefaciens</i> ++ <i>Staphylococcus epidermis</i> ++	<i>Acinetobacter sp</i> + aerobic sporeformers +	No bacterial growth
	ANTERIOR MANTLE	<i>Shewanella putrefaciens</i> + <i>Pseudomonas fluorescens</i> + <i>Pseudomonas cepacia</i> + aerobic sporeformers +	<i>Proteus vulgaris</i> + <i>Micrococcus sp.</i> +	<i>Pseudomonas stutzeri</i> + <i>Corynebacterium propinquum</i> + aerobic sporeformers +
	POSTERIOR MANTLE	<i>Pseudomonas fluorescens</i> ++ <i>Acinetobacter sp.</i> ++ aerobic sporeformers ++ <i>Ochrobactrum anthropi</i> +	No bacterial growth	<i>Corynebacterium propinquum</i> + aerobic sporeformers +
	TOP OF MANTLE	No samples taken	<i>Proteus vulgaris</i> +++	<i>Pseudomonas diminuta</i> ++

3.3 Nutrient composition of the diet

28.2.2000	Protein (%)	Fat (%)	Energy (kcal)
Herring	17.5	5.5	119.9
Mackerel	17.5	18.1	232.9
Blue whiting	15.7	2.9	160
Squid	14.7	1.2	69.5
Sprat	15.6	18.6	229.5

Table 2: Results of the fish nutrient analyses.

4. DISCUSSION

4.1 General

4.1.1 Importance of fish handling

As the fish fed to marine mammals has to be first quality, it is of great importance to prevent any spoilage during the handling. This does not only imply the cleaning and disinfection of the utensils after each preparation, but it also includes a good general hygiene, especially of the hands. This is not only crucial to avoid premature decomposition of the fish, but also to reduce the risk of transferring potential pathogen organisms to the dolphins, as humans can be non-symptomatic carriers of many organisms. The best way to reduce this risk is to proceed to an efficient hand and fingertip washing before starting to handle the fish, and after any other occupation, e.g. going to the lavatories. As a matter of fact, as there are no performance standards for toilet paper, no one should assume that toilet paper provides an effective barrier to keep fingertips free of faecal pathogens (@ 9). A fingernail brush and non-toxic soap should always be ready for use in the kitchen to keep fingernails clean.

The hygiene of the kitchen's floor is quite important as well, because during the cleaning of the kitchen with water under pressure, particles suspended in aerosol, so that the walls and the material that had previously been disinfected might be spoiled again. To reduce this spoilage and also to avoid contamination of the pools, the dolphinarium staff wears rubber boots only used in the dolphinarium and there are shoe disinfection devices at the dolphinarium entrances. These disinfection devices contain a Lysoformin spezial® solution. For occasional visitors who do not possess boots stored at the dolphinarium one-way plastic overshoes are available right next to the disinfection device.

The location of the kitchen at Duisburg Zoo is not the best one from a hygienic point of view, and this for two reasons. First, the kitchen is located on the main personnel entrance to the dolphinarium, that means that the dolphinarium or zoo staff and the backstage visitors entering the dolphinarium have to go right through the kitchen. Even if there is not much traffic, this could be avoided by favouring another existing entrance, or in further transformations or even constructions, to isolate the kitchen from the passage.

Finally, the kitchen's roof is directly exposed to the sun, that means that temperatures tend to rise too high during the summer. This is of course fatal to the fish quality, as it is thawed at room temperature in the kitchen. To avoid this, several solutions are possible: a ventilation system air conditioner could be installed, but this would be of little help in real hot summer days. A roof protection could be built in order to protect the kitchen roof from direct sun. Yet the best solution would be to create a thawing room where the fish would be stored until the feeding and where it would be allowed to thaw slowly and at low temperatures. This will be discussed in the next point.

For a complete disinfection of the kitchen, ultraviolet lamps could be installed and used over night, but there is a potential danger of water versus electricity..

4.1.2 Thawing process

As discussed hereabove, thawing at room temperature can be dangerous, especially during the hot season, but also in normal circumstances, because at room temperature bacteria proliferate very fast. The safest way to thaw fish is in a refrigerated space (Crissey, 1998) where the temperature does not exceed 7°C. Not only the fish will then have the possibility to thaw slowly, in order to prevent massive bacterial multiplication, but the quality would be maintained once thawed, because the metabolism of most of the bacteria does not work very fast at such temperatures.

Fish thawed for longer than 24 hours should never be fed to the dolphins (Crissey, 1998). This rule should be followed quite strictly, especially when fish is thawed at room temperature, because the bacterial load rises exponentially and the risk of a food-borne disease becomes evident. In Duisburg dolphinarium the last fish is fed 24 to 27 hours after it has been taken out from the deep-freezer. It would be very interesting to perform bacteriological analysis on fish that has been kept aside for the last feeding at 17h00 during the week, or even 18h00 on Sundays, and compare it to samples taken earlier in the day.

4.1.3 Organoleptic versus laboratory analysis

The organoleptic analysis is a quick, easy, cheap and method reliable –when made by qualified personnel- to check the quality of fish before feeding marine mammals. Another advantage is that it is possible to make it during the whole thawing process until the last fish that is fed at the end of the day. This is particularly interesting knowing that the bacterial populations are not the same throughout time. As a matter of fact, some bacteria will grow faster at low temperatures and will leave room for others that are more effective at higher temperatures. In addition, some bacteria require a particular pH to be able to grow, and here, too, very often there are different bacteria that follow each other, each one preparing the field for the next one.

The results of laboratory analysis only reflect the situation at the moment when the sample was taken, they are expensive and the results require some days to be delivered. But laboratory results have the immense advantage that they show exactly what agent we are dealing with and they can provide the pattern of antibiotic resistance of the bacteria involved, what can be very helpful in case of clinical symptoms.

4.2 Water consumption

Dolphins can be compared to desert animals in the way that they live in an environment where there is no available freshwater and also because they are able to concentrate their urine to the extreme. Many studies have been carried out in order to find out whether dolphins do drink saltwater or not, but the results differ. The authors agree on the fact that if they do consume saltwater, then it is in very small amounts (Worthy, 1990).

Their main freshwater source is fish; as a matter of fact, fish consists of 60-80 % pre-formed water and in addition, the fat and protein contents provide metabolic water, respectively 1.07g of freshwater per gram of fat and 0.4g of freshwater per gram of protein. Consequently, the fatter the fish, the more energy and freshwater are available

(Worthy; 1990). Dolphins are also capable of deriving freshwater from their fat reserves during fasting periods.

As an example, the freshwater uptake through the diet is calculated in the following table for Ivo, the adult male dolphin :

Table 3:

	Quantity (g)	preformed water(%)	fat (%)	protein (%)	total water (ml)
Blue whiting	2500	70	2,9	15,7	1984,575
Sprat	1000	70	18,6	15,6	961,42
Squid	1000	70	1,2	14,7	771,64
Herring	1500	70	5,5	17,5	1243,275
Total=	6000			Sum =	4960,91

It is therefore easier to understand why prolonged fasting can lead to critical dehydration with all its clinical implications. The solution to this problem can be to inject freshwater into the abdominal cavity of the fish (White & Francis-Floyd, 1988) and to (force-) feed the dolphin with this prepared fish. Freshwater can also be administered via gastric intubation (Geraci, 1978; García Hartmann, 2000).

In Duisburg the freshwater used for this purpose is MILUPA WASSER usually meant for baby food. The reason why this type of water was chosen is that it is low in sodium (< 0,8 mg / 100 ml) and that it has been boiled (García Hartmann, 2000).

In physiological conditions, water loss occurs only via urine, faeces and respiration, as bottlenose dolphins do not possess sweat glands.

4.3 Choice of vitamin and mineral supplements

Vitamin supplementation is a necessity in marine mammal management, even though the diet is balanced and the fish of excellent quality. The reason for this is that frozen fish loses vitamins and some fish species contain anti-vitamins which destroy the vitamins normally available (e.g. thiaminases). These problems are discussed in **point 5.3**.

Two vitamin and mineral supplements are compared in the Appendix under point **5.2**. The Duisburg dolphinarium chose the Sea Tabs® for several reasons:

- Some important vitamins like thiamine, vitamin B2, vitamin B12, vitamin C are in higher dosage than in the Mazuri® supplements.
- The Sea Tabs® contain kelp in traces and as it is recommended to feed both carnivorous and algae-eating fishes (García Hartmann, personal communication, April 2000), the kelp can compensate for a lack of algae-eating fish in the diet.
- Sea tabs® have a higher iron content.

4.4 Significance of the bacteriological findings

Numerous different bacteria have been isolated from the fish samples, but few of them are synonymous with pathology. Actually, most of the bacteria isolated are environmental contaminants -e.g. *Acinetobacter anitratus* or *Enterobacter amnigenus*- or commensal of the skin and of the mucosa -e.g. *Micrococcus sp.* and many *Staphylococcus hominis, capitis, auricularis*.

Nevertheless, some well-known pathogens have also been isolated -e.g. *Pseudomonas cepacia* and *Proteus vulgaris*-, which confirms that fish is the prime vehicle for potential pathogens into dolphinarium (Overath, Gellissen and Garcia Hartmann; 1997). Many other bacterial isolates are not known as specific pathogens, but are capable of using an opportunity -e.g. a weakened or immunodeficient animal- to display their pathological effect.

Bacteria	Characteristics	Significance
<i>Acinetobacter lwoffii</i>	Gram negative (G-) , Found in water and soil, strict aerobic, resistant to Penicillin. Parasite or commensal on mucosa of man and animals (@19; Rolle & Mayr, 1984) .	Possible opportunistic pathogen
<i>Acinetobacter anitratus</i>	G- , Found in water and soil, non pathogen (@19) .	Unknown
<i>Acinetobacter sp.</i>	G- , Opportunistic pathogen essentially isolated in intensive care units in patients with local or general immunodeficiency (@19).	Possible opportunistic pathogen
Aerobic sporeformers	G+ to labile, Bacillus = Zoonosis, <i>Bacillus anthracis</i> (anthrax: exotoxin with oedema and tissular necrosis, lethal in high concentration; treatment penicillin, erythromycin or tetracyclin) and <i>B. cereus</i> , resistant to heat and cell-destructive chemicals (Bisping, 1979).	Probably no significance
<i>Aerococcus viridans</i>	G+ , typical tetrad-forming coccal bacteria (0.8-1.1 µm), non-motile, catalase negative, beta-haemolytic, facultative anaerobic. <i>Aerococcus viridans</i> (var.) <i>homari</i> causes the disease called Gaffkemia or Red tail disease, a fatal infection of lobsters (genus <i>Homarus</i>) (=Gaffkya <i>homari</i>) (@12).	Probably no significance

<i>Aeromonas hydrophila</i>	G-, found in water, <i>A. hydrophila</i> has frequently been found in fish and shellfish. Some strains of <i>A. hydrophila</i> are capable of causing illness in fish and amphibians as well as in humans who may acquire infections through open wounds or by ingestion of a sufficient number of the organisms present in food or water. <i>A. hydrophila</i> may cause gastro-enteritis in healthy individuals or septicaemia in individuals with impaired immune systems or various malignancies. Since little is known about the virulence mechanisms of <i>A. hydrophila</i> , it is presumed that not all strains are pathogenic, given the ubiquity of the organism. Naturally resistant to cephalosporines, oxacillin and imipenem (@18).	Possible pathogen
<i>Alcaligenes faecalis</i>	G-, wet soils, water, wet places in hospitals (Viehmann, 1998).	Unknown
CDC Gr.IV C-2	Related to <i>Pseudomonas</i> non fermentative, found in water (Biognost laboratory, May 2000).	Probably no significance
<i>Corynebacterium propinquum</i>	G+, parasite and pathogen for man and animal, frequent in nature: soil, plants water and animal's skin (Rolle & Mayr, 1984).	Unknown
<i>Enterobacter amnigenus</i>	G-; found in water and soil, unimportant (Viehmann, 1998).	Probably no significance
<i>Escherichia coli</i>	G-, Enterobacteria, many strains are pathogenic, facultative pathogen, resistant to colistine (@21).	Depends on the type of E.coli and on the concentration
<i>Lactococcus lactis lactis</i>	G+	Probably no significance
<i>Micrococcus sp.</i>	G+, commensal of skin and mucosa (Rolle & Mayr, 1984).	Unknown
<i>Moraxella sp.</i>	G-, <i>M. bovis</i> : keratoconjunctivitis in cattle (Bisping, 1979); septicaemia in other animals.	Known pathogen
<i>Ochrobactrum anthropi</i>	G-, Motile strictly aerobic, found in environment, water and hospital environment only rarely reported as human pathogen (nosocomial meningitis); resistant to many antibiotics (@3; @4).	Unknown

<i>Proteus vulgaris</i>	G-, Enterobacteria, facultative anaerobic, motile; exists in man and animal (commensal of intestinal tract) and environment: mostly non pathogen, seldom infection in animals. In man: pyogen infection, nosocomial infection, septicaemia, urogenital infection, food-borne disease, naturally resistant to colistine and third-generation cephalosporines; important to ask for antibiogram (@23; Bisping, 1979).	Possible pathogen
<i>Pseudomonas stutzeri</i>	G- aerobic, facultative pathogen for man and animal, seldom cause of illness (@22; Rolle & Mayr, 1984).	Possible pathogen
<i>Pseudomonas diminuta</i>	G-, found in sediments, capable of crude oil degradation (Bisping, 1979).	Probably no significance
<i>Pseudomonas alcaligenes</i>	G-, found in water and wet environment, occasionally reported in human infections (@22) .	Possible pathogen
<i>Pseudomonas acidovarans</i>	G-, found in wet environment, occasionally isolated in samples (@22) .	Unknown
<i>Pseudomonas fluorescens</i>	G-, Found in water; food hygiene, psychrotroph, often isolated in hospitals, seldom important infection in immunocompromised patients (@22).	Possible pathogen

<i>Pseudomonas cepacia</i>	G- . Facultative pathogen for man and animal.; agent to promote crop growth. Found in soil and moist environment. Particular predilection for the lung of human patients with cystic fibrosis. Inherently resistant to multiple antibiotics. Important opportunistic human pathogen in hospitalised and immunocompromised patients (@2).	Possible pathogen
<i>Serratia marescens</i> Synonym: <i>Bacillus marescens</i>	G- , facultative anaerobic, belongs to the family of the <i>Enterobacteriaceae</i> . Produces a red pigment. Very frequent in the environment (soil, water, plants and animals), one of the most frequent contaminants of Petri plates in the lab. More recently it has been found to be pathogenic to some people (immunocompromised) and has been involved in nosocomial infections (contamination of blood product). In veterinary medicine <i>S. marescens</i> causes clinical, subclinical and chronic mastitis often caused by contaminated antiseptic solutions. In rare cases it has caused abortion in cows, septicaemia and endocarditis in horses, septicaemia in dogs and septic choc in cats. Insect pathogen. Resistant to many antibiotics (first generation cephalosporines, colistine, polymyxine B, ampicillin, carbenicillin, tetracyclins, aminosides, chloramphenicol, sulfamides, trimethoprim,...)(@5; @6; @7; Viehmann, 1998).	Possible pathogen
<i>Serratia liquefaciens</i> Synonym: <i>Aerobacter</i>	G- , facultative anaerobic, belongs to the family of the <i>Enterobacteriaceae</i> . Not as pathogen as <i>S. marescens</i> , opportunistic infections reported. Found in the environment. Rare cases of subclinical mastitis in cows. Insect pathogen (@5; @6; @7).	Possible pathogen
<i>Shewanella putrefaciens</i>	Previously classified as <i>Alteromonas putrefaciens</i> and <i>Pseudomonas putrefaciens</i> . Associated with human cases of intra-abdominal sepsis, meningitis and bacteraemia. Reported susceptibilities: ampicillin, cefotaxime, gentamicin and chloramphenicol (@1).	Possible pathogen
<i>Staphylococcus auricularis</i>	G+ , belongs to the normal flora of the human skin, outer ear conduct (Rolle & Mayr, 1984).	Unknown
<i>Staphylococcus capitis</i>	G+ , belongs to the normal flora of the human skin, mainly head (Rolle & Mayr, 1984).	Unknown

<i>Staphylococcus caprae</i>	G+, no reported pathogenicity.	Unknown
<i>Staphylococcus epidermis</i>	G+, belongs to the normal flora of the human skin, non pathogen for humans (Rolle & Mayr, 1984).	Unknown
<i>Staphylococcus hominis</i>	G+, belongs to the normal flora of the human skin (Rolle & Mayr, 1984).	Unknown
<i>Staphylococcus warneri</i>	G+, no reported pathogenicity.	Unknown

5. APPENDIX

5.1 Composition of fish

Chemical composition and energetic value of fish and squid per 100g of edible part

	Per 100g	<i>Loligo patagonica</i>	<i>Scomber scombus</i>	<i>Sprattus sprattus</i>
	% Edible part	65	80	
	Water (g)	80,0	69,8	64,9
	Proteins (g)	15,58	17,0	16,7
	Lipids (g)	1,38	11,1	16,6
Glucids	Available (g)			
	Starch (g)			
	Soluble			
	Fibres			
Energy	Kcal	92	168	215
	kJ		703	
Minerals	Na (mg)	44	130	100
	K (mg)	246	360	300
	Fe (mg)		1,2	1,9
	Ca (mg)	32	38	85
	P (mg)	221	264	200
	Mg (mg)	33		30
Vitamins	Thiamine(mg)		0,10	0,00
	Riboflavin (mg)		0,25	0,3
	Niacin (mg)		7,60	
	Vitamin A (mg)		45	
	Vitamin C (mg)		0	0,2
	Vitamin E (mg)			1,5

5.2 Composition of different vitamin and mineral complements

	Mazuri Zoo Foods: Fish Eater Tablets ³		Sea Tabs: Marine Mammals ⁴ per pill = 1,05 g	
Fat	%	21,30	/	/
Protein	%	16,00	/	/
Fibres	%	1,00	/	/
Ashes	%	16,80	/	/
Starch	%	1,30	/	/
Glucids	%	23,10	/	/
Total energy	MJ/kg	19,10	/	/
Digestible energy	MJ/kg	15,60	/	/
Metabolic energy	MJ/kg	14,10	/	/
Linoleic acid	%	0,12	%	0,95
Linolenic acid	%	0,03	/	/
Ca	%	0,11	/	/
P	%	0,31	/	/
Phytin-Phosohor	%	/	/	/
Na	%	0,03	/	/
Cl	%	1,40	/	/
K	%	0,38	/	/
Mg	%	0,66	%	2,38
Fe	mg/kg	41,03	mg/kg	23809,52
Cu	mg/kg	6,15	mg(CuSO4)	1,5
Mn	mg/kg	1,03	mg/kg	57,14
Zn	mg/kg	20,51	µg(ZnSO4)	250
Co	µg/kg	41,03	/	/
I	µg/kg	2,05	µg/kg	142857,14
Se	µg/kg	/	/	/
F	mg/kg	1,05	/	/
Vit. A	IU/kg	12820512,00	IU/kg	11904761,90
Vit. D3	IU/kg	2564103,00	IU/kg	380952,38
Vit. E	mg/kg	225641,00	IU	50
Vit. B1	mg/kg	63815,38	mg/kg	238095,24
Vit. B2	mg/kg	1548,72	mg/kg	4761,90
Vit. B6	mg/kg	1543,59	mg/kg	2,85

³ Mazuri Zoo Foods
PO Box 705 Withem,
Essex CM 8 3AD England

⁴ Sea Tabs: vitamin & mineral supplement formulated for marine mammals
Pacific Research Lab, Inc
1951 Willow Glen Drive
El Cajon, California 92019 USA

Vit. B12	µg/kg	1538,46	mg/kg	9523,80
Vit. C	mg/kg	60,31	mg/kg	119,04
Vit. K3	mg/kg	512,82	/	/
Folic acid	mg/kg	211,28	mg/kg	47619,05
Nicotinic acid	mg/kg	7282,05	/	/
Panthenic acid	mg/kg	8548,72	mg/kg	4,76
Choline	mg/kg	2748,72	/	/
Inositol	mg/kg	1435,90	mg/kg	4,76
Biotin	µg/kg	36205,13	mg/kg	95238,09
Kelp	/	/	/	trace

5.3 Nutritional disorders of cetaceans

5.3.1 Thiamine deficiency

Causes: induced by eating raw fish that contains thiaminase (Geraci, 1981), i.e. mackerel, herring, alewife, anchovies, and smelt (Worthy, 1990). Thiaminase is a mixture of heat labile and heat stable compounds, some of which are enzymes (Geraci, 1974) that inactivate vitamin B1 by splitting it (Wallach & Boever, 1983).

Clinical signs: they are non-specific: refusal to perform and anorexia were observed in one case of thiamine deficiency in an Atlantic bottlenose dolphin (White, 1988). In seals the most significant diagnostic feature is the finding of abnormal red cell transketolase activity, which requires the apoenzyme thiamine pyrophosphate (Geraci, 1981).

Treatment: in early stages, the oral or parenteral therapy is efficient. 100 mg of thiamine IM produce noticeable effect within one hour (Geraci, 1981).

Deficiency can be avoided by, on the one hand, feeding assorted fish in order to dilute the thiaminase, and on the other hand, the diet can be supplemented with vitamin B1 if fish is known to contain the antivitamin. There are two ways to supplement the diet (Geraci, 1986):

- 2 to 4 mg thiamine / kcal of food / day, given two hours before feeding
- 25 to 35 mg thiamine / kg fish, at the time of the meal

5.3.2 Vitamin E deficiency

Causes: animals fed improperly stored or prepared fish, especially dark-fleshed, oily fish, such as mackerel and tuna. Marine fish contain high levels of unsaturated fatty acids, which are rapidly transformed into toxic peroxides and polymerised products when in contact with oxygen. Vitamin E is a natural anti-oxidant; in its absence, in dietary inadequacy, or destruction through oxidation, peroxides accumulate. Transformation is accelerated by light, heat and heavy metals (Geraci, 1981).

Clinical signs: there is no characteristic manifestation of vitamin E deficiency in marine mammals. In terrestrial mammals, steatitis, muscular degeneration, liver necrosis, anaemia metals (Geraci, 1981) and impaired reproductive capacity (Alexander, 1989) are reported.

Diagnostic: a definitive diagnostic is difficult to make due to the complex effects of vitamin E. The following symptoms might help orientating the diagnostic (Alexander, 1989) :

- non-supplemented diet of fatty fish
- hyponatraemia
- plasma Vit. E below 7 to 10 µg/ml
- histological lesions of myopathy and fat necrosis
- increased serum muscle-enzyme concentration
- renal insufficiency due to myoglobinuria

Treatment: feed only high-quality fish, store the fish at -30°C and only during maximal periods of 4 months for the mackerel, 6 to 7 months for herring, and 9 months for smelt and capelin (Geraci, 1981).

Store at the highest humidity, thaw rapidly and immediately feed. Feed only whole fish (not eviscerated), eliminate stressful conditions. Vit. E supply should be established on basis of the fat content of the fish and its storage duration. A recommended dose would e.g. be 100 IU Vit. E/kg of fish daily, or weekly if shorter storage. Increase Vit. E supply in lactating females in order to increase the transfer to the young (Alexander, 1989).

5.3.3 Vitamin C deficiency

In all species, adrenals and liver contain high concentrations of Vit. C; only cetaceans have considerable amounts in the epidermis. Fish contains enough Vit. C even after storage (mainly in the viscera). The cetaceans' ability to synthesise Vit. C is rather controversial.

One case of speculative diagnosis in a bottlenose dolphin responsive to Vit. C therapy was reported. The dolphin showed gingivitis, glossitis, pharyngitis, severe necrotic stomatitis and anorexia; the symptoms responded to daily dosages of 200 to 250 mg of dietary ascorbic acid (Geraci, 1981).

5.3.4 Fish-induced anaemia

This disorder is reported in terrestrial mammals eating gadoid fish, e.g. pollack (*Gadus virens*), whiting (*Gadus merlangus*) and hake (*Gadus productus*). One or more heat labile substances interfere with normal iron absorption; causing anaemia. The suspected substances are trimethylamine oxide or formaldehyde. The disorder appears only when thawed raw gadoid fish is fed, and not with fresh raw fish (Geraci, 1981).

Fish-induced anaemia has not been demonstrated in marine mammals, but non-specific anaemia are a common finding in captive cetaceans, which might indicate a possible relationship between the two. However, with some exceptions, these do not respond to orally-supplemented iron (White, 1988; Geraci, 1981).

5.3.5 Scombroid poisoning

Causes: consumption of poorly-preserved scombroid fish, e.g. Atlantic mackerel, Atlantic herring, sprat, tuna and related dark-fleshed species (Stratton & Taylor, 1991). This poisoning is caused by histamine and related compounds. dark-fleshed fish contain high concentrations of histidine (up to 200 mg/100 g) (Geraci, 1981; Worthy, 1990).

When inadequately preserved or handled, histidine becomes decarboxylated to histamine (time and temperature dependent). This occurs by autolysis and by the action of histamine producing bacteria such as *E. coli* and *Aerobacter aerogenes*. The toxin is present without evidence of putrefaction fish (Geraci, 1981; Stratton & Taylor, 1991).

Symptoms: there is yet no evidence of scombroid poisoning in marine mammals but this is probably due to a failure to recognise the problem (Worthy, 1990). Some suspected cases have been reported. The symptoms go from refusal to perform, lethargy

(Geraci, 1986), pulmonary oedema, irritable behaviour, inappetance and debilitation to peptic ulcers in the first and second stomach chambers (Geraci, 1981).

Prevention: avoid using scombroid fish species that have been stored beyond their shelf time; fish should be stored at -30°C , thawed in a cool environment and fed as soon as possible after thawing. Histamine has a bitter taste, this might be a reasonably good criteria for detection during fish preparation (Geraci, 1981).

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