

# **The European Association of Aquatic Mammals**

---



## **European Association for Aquatic Mammals**

Devoted to marine mammal  
conservation since 1972.

---

**Standards and Guidelines for the management of aquatic  
mammals under human care (version March 2019)**

## Table of contents

Preamble.....	3
1. Acquisition and Disposition of Aquatic Mammals.....	4
2. Animal Training.....	6
3. Education standards.....	8
4. Scientific research and Conservation.....	10
5. Enclosures and Spatial requirements.....	12
6. Husbandry.....	18
7. Veterinary care .....	21
8. Breeding.....	22
9. Water and Environmental Quality.....	24
10. Interactive programs.....	28
11. Record Keeping.....	30
12. Transportation.....	32
Appendix I: Handling Fish Fed to Fish-Eating Animals .A Manual of Standard Operating Procedures.....	35
Appendix II: EAAM Fish Quality Parameters .....	61

## Preamble

Public display facilities are resource centres that help people to expand their knowledge about: the importance of marine conservation, responsible human behaviour, and the principles of ecology. Increasing public awareness of aquatic mammals and the marine ecosystem through lectures, exhibits, courses and conservation programs for adults and children makes a contribution to the preservation of aquatic mammals and their environment. Providing the opportunity for 20 million people to view aquatic mammals at public display facilities arguably prevents much harassment of aquatic mammals in the wild. Many facilities have programs to assist stranded or sick aquatic mammals, which communicate the importance and commitment with conservation.

Much of what has been learned about aquatic mammal behaviour, biology, and physiology has been derived from scientific research on captive aquatic mammals, and is important in better understanding how to sustain aquatic mammal populations in the wild. Greater knowledge about aquatic mammals improves efforts to help the animals cope with natural and anthropogenic risks and threats. Aquatic mammals are difficult to observe in the wild, and aquarium settings offer opportunities to develop and adjust field research techniques.

Education of the public about aquatic mammals has made people feel strongly about protecting the animals and their environment. The various presentations, lectures, exhibits, and courses at public display facilities are all part of their education programs. Public display increases peoples understanding of aquatic mammals and the marine ecosystem. Many people who live away from the coasts might never be exposed to these animals if they did not have the opportunity to visit a public display facility. You cannot protect what you don't know and respect.

These Standards and Guidelines reflect present-day practices, which are based on current scientific data and the cumulative experience of the membership of this association. They will be updated and improved as the knowledge base expands. **These Standards and Guidelines will be reviewed regularly** under the direction of the Board of the EAAM, thereby assuring the goal of the EAAM institutional members to lead the aquatic mammal display community in the integration of advancing science and technologies.

The Standards and Guidelines reflect the commitment of the EAAM members to hold and display our aquatic mammal collection under state of the art conditions. They are available on demand in order that our commitment is transparent and controllable. The relevant authorities can use this Standards and Guidelines as a scientifically based standard regarding the housing of cetaceans and other aquatic mammals in zoos.

## 1. ACQUISITION AND DISPOSITION OF Aquatic MAMMALS

### 1.1. General introduction

Acquisition and disposition of aquatic mammals by member institutions reflect policies that maximize the educational, research, and conservation potential of the collections. Living animals require a substantial commitment of resources and cannot be managed using strategies based on short-term interests. Furthermore, living animals have intrinsic needs both as individuals and as members of species. These must be of primary concern in animal management.

Each institution must have in place an **Animal Collection Plan**

### 1.2. Definitions

1.2.1. **Acquisition** is the addition of an animal(s) to a collection through trade, transfer, donation, loan, purchase, collection from wild, rescue, or birth. Any collection from the wild must be justified by a clear need for conservation, population management (supported by the EAZA Marine Mammal TAG) or rescued individuals that cannot be released back to the wild.

1.2.2. **Disposition** is the removal of an animal(s) from a collection through trade, transfer, donation, loan, sale, escape, reintroduction, or death.

1.2.3. **Lawful purposes** refer to purposes, which are in accord with all applicable local, state/provincial, regional, national, and international laws and regulations.

1.2.4. **Marine mammal park, aquarium, marine life park, zoo**, refers to a permanent institution that owns and maintains aquatic mammals and, under the direction of a professional staff, provides its collection with appropriate care; is open to the public on a regularly scheduled, predictable basis; conforms to all applicable laws and regulations; and whose mission and purposes are consistent with those of the EAAM.

1.2.5. **Collection Plan** refers to the 3-5 year plan of the institution regarding its aquatic mammal collection; from the amount of individuals the facility wants to have and breed to what species want to be acquired/disposed of or maintained based on objective parameters such as for instance conservation, education or research importance.

1.2.6. **Acquisition and Disposition Policy** refers to written policies by the institutions where it should be defined that animal(s) should be acquired and disposed of only following the EAAM's S&G.

### 1.3. Standards and Guidelines for Acquisition of aquatic mammals

1.3.1. Members must only acquire or accept a aquatic mammal for lawful purposes.

- The animal should be acquired for purposes consistent with the mission, programs, and activities of the member.

- Members must be capable of providing for the animal's proper care and management according to EAAM standards.

1.3.2. All animals must be acquired using humane methods consistent with professional practices.

## EAAM Standards and Guidelines

1.3.3. Acquisitions from the wild through direct collections must only be undertaken under authority from the appropriate governmental regulatory agency managing the source population, following the CITES regulations and the IUCN guidelines where appropriate, and in agreement with the EAZA's Marine Mammal Tag.

1.3.4. Members acquiring aquatic mammals from the wild must be able to demonstrate that they previously evaluated that such removal will not compromise the sustainability of the stock or population from which the animals were taken.

### 1.4. Standards and Guidelines for Disposition

1.4.1. Members must only remove living animals from their collections to a qualified marine mammal park, aquarium, marine life park, zoo, or comparable institution.

- Animals should only be placed in an institution or collection whose purposes are consistent with those of the EAAM.

- Members should incorporate as standard practice a determination that the recipient will provide care and management for the animal to EAAM standards.

- Animals should be disposed of in a manner consistent with humane practices and applicable governing law.

1.4.2. Living aquatic mammals must only be loaned to other aquatic mammal facilities for purposes of exhibition, breeding, education or research, provided the holding institution is satisfied that the recipient can provide adequate care and treatment.

- Such disposition should not be detrimental to the animal or its species.

- Such loans should be documented by written agreement declaring the nature of the loan and its terms.

1.4.3. Aquatic mammals maintained as part of a collection must only be released to the wild if part of an authorized and proper, scientifically-based experimental reintroduction program that is anchored in the principles of conservation biology and has the ultimate goal of sustaining a threatened or endangered aquatic mammal stock and/or population. Any release must fulfil the IUCN reintroduction guidelines.

1.4.4. Euthanasia is recognized as a legitimate animal management tool but must only be utilized, when appropriate, as recommended by the attending veterinarian in accordance with the member's program of veterinary care, including but not limited to terminating animal suffering caused by illness, injury, or other medical conditions. An euthanasia policy should be established in any member institution, based on their institutional policy and on legislation in force.

1.4.5. In all cases, members must insure that the manner of disposition considers the best interests of the animal, the group and its populations .

## 2. Animal Training

### 2.1. General introduction

2.1.1. EAAM members recognize animal training as an application of behavioural science that:

- (1) Provides a means to observe, assess and enrich an animal's physical and psychological health
- (2) Assists the animal care staff in providing safe and expedient methods for preventive and clinical medical procedures
- (3) Integrates public display within husbandry regimes
- (4) Facilitates education and research objectives

### 2.2. Definitions

2.2.1. **Animal Training** is the deliberate application of learning principles, operant and classical conditioning to shape an animal's behaviour in order to facilitate husbandry, public display and research.

2.2.2. **Experienced Competent Trainer** is defined as an individual that has actively participated in the training and husbandry of aquatic mammals with sufficient knowledge and experience.

2.2.3. **Animal Training Program** is a defined and managed functional activity that is comprised of animals, equipment and personnel who are responsible for developing and administering animal training plans to meet the goals and objectives of the organization.

2.2.4. **Professional organizations** are formal membership associations established to promote the exchange of information among professionals in the field of animal behavioural science, management, training, husbandry, enrichment, or other related disciplines; and wherein progress is monitored and evaluated.

2.2.5. **Professional journals** refer to periodicals, magazines, or other publications, which contain material relevant to animal behavioural science, management, training, husbandry, enrichment or other related disciplines.

2.2.6. **Student trainer** refers to a period of practical experience and training for a student that is supervised by an expert or specialist in a particular field.

### 2.3. Standards and Guidelines for Training Programs

2.3.1. Members must have a policy on animal training. For best practice is recommended that this policy meets the standardized training guidelines of the International Marine Animal Trainers Association (IMATA) and the EAZA guidelines on the use of animals in public demonstrations.

2.3.2. The animal training policy should describe the organization's view of the animal training program, its role in the organization and how management interfaces with it, typically including:

- a. Focus of the animal program
- b. Philosophy of animal training
- c. Application of animal training
- d. Statements of animal care and treatment principles

## EAAM Standards and Guidelines

### e. Management review and accountability

2.3.3. Members must have a behaviour development and Management plan.

The behaviour development and management plan should describe the animal training program and its objectives, methods of accomplishment and success criteria. It should address training oversight, standard *and* emergency procedures, and record keeping, typically including:

- a. Animal training decision authority
- b. Categorized list of behaviour goals and objectives
- c. Animal training plan for individual behaviours
- d. Criteria for measuring success of animal training plans
- e. Schedule of frequency to maintain criteria
- f. Contingency plans

2.3.4. Members must provide for adequate animal training, staff that is appropriately sized and qualified to meet all program requirements.

The on-site animal training staff should be under the direction of an experienced, competent trainer. The size of the staff should be based on the number of animals, husbandry and training requirements, activities with guests, and the physical facility. Animal training responsibilities should be clearly defined and the positions described by proficiency requirements. The organization should have and support a continuing professional development programme, typically including:

- a. Organizational flow chart
- b. Animal training position(s) responsibilities
- c. Requirements of animal training positions
- d. Position descriptions for animal training positions
- e. Qualifications of animal training personnel

2.3.5. Members must participate in animal training information exchange activities.

The organization should participate in information exchange activities to enhance their program and contribute to the collective knowledge of the community, thereby advancing the science of animal husbandry and training.

Suggested considerations include:

- a. Membership in professional organizations
- b. Animal trainer/zoo keeper exchange programs
- c. Internships and student trainers
- d. Subscriptions to professional journals
- e. Professional conference attendance

### **3. Education standards**

#### **3.1. General introduction**

EAAM members must conduct educational activities related to aquatic mammals. Such actions will provide information on the biology, physiology, ecology and husbandry of aquatic mammals in zoological parks and aquariums as well as on the role of these institutions regarding nature and biodiversity conservation. Ultimately, these activities will be focused in raising awareness about the threats faced by aquatic mammals in the wild, and inspiring the audiences to reduce their impact on the aquatic environments. Members are encouraged to share and communicate their educational activities within the association and in professional forums.

#### **3.2. Definitions**

3.2.1. Educational programmes refer to written protocols, procedures, and guidelines identifying educational strategies, goals, target groups, feed back and the main educational resources of the park. The concept of marine mammal park education programme is broader than a program for schools; it should be targeted at the entire marine mammal park visitation and even beyond the park limits and adapted to the characteristics of the different audiences.

3.2.2. Educational goals might include such topics as: raising awareness about the main global threats affecting the ocean, threats to species, basic information on aquatic mammals, the role of marine mammal parks in conservation, sustainability and a respect for and appreciation of wildlife and what visitors can do to contribute to it.

3.2.3 Target groups refer to how the visitor audience is constituted in order to target the education programme. Examples might include: general visitors, locals, tourists, families, mixed age groups, friends, school groups, same age groups, teenagers, elderly, handicapped, literate and illiterate people.

3.2.4. Staff with a relevant academic background refers to persons with training in education, preferably with a university degree and/or teacher training qualification.

#### **3.3. Standards and Guidelines for Education**

3.3.1. Members must develop and implement an educational programme for the institution that takes into account, at a minimum, goals, target audiences, staff, equipment, activities and funding.

3.3.2. Members are encouraged to prepare yearly activity plans and perform systematic evaluations, in order to check the efficacy of their education, and to help improving the educational programme regularly.

3.3.3. At least one member of the staff (with a relevant academic background) within the institution should be responsible for a professional implementation of the education programme. In smaller marine mammal parks, the education role might be combined with other functions and be carried out by personnel such as: the director, curator, senior keeper and or researcher. Staff involved must be familiar with education practice and ideally have some formal or informal training in education (through education conferences and regional workshops). Medium-sized marine mammal parks should employ at least one member of



## EAAM Standards and Guidelines

staff with overall responsibility for education. Larger marine mammal parks should also have additional education staff.

3.3.4. Animal species must be clearly and correctly identified at their enclosures. Threatened species and species in regional, national and international coordinated breeding programmes should be highlighted.

3.3.5. Marine mammal park animals must be exhibited in the best conditions in enclosures that enable them to live as natural as possible and to participate in natural behaviours as far as possible.

3.3.6. Interpretation/education should be an integral part of marine mammal park exhibits, demonstrations and any other animal activities and the educator should be incorporated in the exhibit planning and collection planning process, as well as in development of presentations and encounter programs, to ensure the communication of a proper conservation message.

3.3.7. A reference library appropriate to the size and complexity of the marine mammal park should be maintained and made available to all staff members, and possibly to the public where practical.

3.3.8. Resource material/education information should be made available to the general public and marine mammal park audience. This might include: leaflets, guide books, teacher's notes, resource packs, work sheets and should be displayed and available for purchase or for free.

3.3.9. Education programs about aquatic mammals should include institution experts as a marine science resource to professional groups and the education community when appropriate and practicable.

- Public display facilities employ and collaborate with many highly knowledgeable and experienced aquatic mammal experts, such as animal behaviourists, veterinarians, research scientists, trainers, marine educators, and other specialists. When appropriate and practicable, facilities should encourage and facilitate opportunities for these specialists to serve as marine science resources and share their expertise with interested professional groups and the education community.

## **4. Scientific Research and Conservation**

### **4.1. General introduction**

4.1.1. EAAM members must conduct and/or support scientific research and/or conservation projects related to aquatic mammals. Such projects provide information important to the conservation of species, habitats, and biodiversity in the wild, as well as help improve husbandry and welfare for animals in zoological parks and aquariums. These projects contribute to the scientific understanding of aquatic mammals in the wild and under human care and benefit their protection, care and survival, including the rehabilitation of stranded animals. Members are encouraged to facilitate responsible research and conservation projects and to communicate findings in scientific journals and forums.

4.1.2. EAAM members must provide opportunities to scientifically study the various aspects of aquatic mammal biology that cannot be conducted in the wild, and/or that will primarily benefit the research or conservation of wild populations of aquatic mammals or the welfare of populations under human care.

### **4.2. Definitions**

4.2.1. Research programmes refer to written protocols, procedures, and guidelines governing the various aspects of a facility's research activity.

4.2.2. Animal Care and Welfare Committee (ACWC) refers to a committee established by a facility, including at least one independent expert, for the purpose of evaluating research proposals and the participation of collection animals in research, as well as any other activities that could significantly impact animal welfare including some husbandry or educational activities.

4.2.3. *Bona fide* research is that which is conducted with earnest intent to advance knowledge through application of the scientific method. It is most convincingly evidenced by participation in the peer review process, such that findings are shared openly through presentation at professional meetings and publications, particularly in refereed texts.

4.2.4. Conservation projects refer to formal, organized projects with goals and objectives designed to support, directly or indirectly, the conservation of aquatic mammals in the wild. These programs should be scientifically based, including results-oriented evaluation and peer review, and the findings should be shared openly through presentation at professional meetings and publications.

### **4.3. Standards and Guidelines for Scientific Research and Conservation**

4.3.1. Members must develop a research and conservation programme for the institution that takes into account, at a minimum, the wellbeing of the animals and the availability of resources, including animals, staff, equipment, and funding.

4.3.2. Members are encouraged to establish an Animal Care and Welfare Committee that would be supervising any activities requiring the use of animals including research and education programs as well as husbandry decisions that could have significant impact over animal welfare.

- At a minimum, the composition of the committee should include the facility's veterinarian and the aquatic mammal curator or head of the animal care staff. Ideally an independent external professional expert from the animal care/welfare field (e.g. university professor) should be included in the committee.

## EAAM Standards and Guidelines

- Meetings should be occurring on a regular basis to approve and/or reject proposed activities/research lines and minutes with the conclusions of the different aspects discussed should be registered and stored.

4.3.3. Members may participate in *bona fide* research and conservation projects by providing biological samples and/or access to records, animals, equipment, or staff time. Institutional support will be guided by the priorities set forth by the facility's research and conservation programmes.

4.3.4. Members, when possible, should contribute to the body of aquatic mammal scientific literature by sharing findings from their research and conservation projects through publication in peer-reviewed journals and presentations at professional meetings.

4.3.5. Financial contributions to *bona fide* research and conservation projects must be guided by a conservation programme, including a mission statement, which describes the facility's goals in supporting, evaluating, and collaborating with outside, aquatic mammal related studies.

## 5. Enclosures and Spatial requirements

### 5.1. Definitions

5.1.1. **Marine mammal parks** shall refer to all establishments having a valid license under their respective authorities, open to and administered for the public to promote nature conservation and to provide education, information and recreation through the presentation and conservation of wildlife.

5.1.2. **Welfare** shall refer to the physical, behavioural and social well-being of animals through the provision of appropriate conditions for the species involved, including but not necessarily limited to housing, environment, diet, medical care, enrichment and social contact where applicable:

- **Enclosure** means any accommodation provided for animals in marine mammal parks.
- **Enclosure barrier** means a barrier to contain an animal within an enclosure.
- **Stand-off barrier** means a physical barrier set back from the outer edge of an enclosure barrier designed to prevent public access to the latter.

### 5.2. The Standards

#### 5.2.1. Routine observation of aquatic mammals

- The condition and health of all aquatic mammals in the marine mammal park is to be checked daily by the persons in charge of their care for that particular day.
- Any aquatic mammals which are noted to be unduly stressed, potentially sick or injured, must receive immediate veterinary attention and, where necessary and based on the diagnosis, and adequate treatment.

#### 5.2.2. Accommodation - Space, Exercise and Grouping

5.2.3. Aquatic mammals must be provided with an environment, space and furniture sufficient to allow the exercise and behavioural activity that is needed for their welfare (For examples refer to the EAZA and EAAM guidelines).

5.2.4. Enclosures to be of sufficient size and structure to be so managed

- To avoid aquatic mammals within groups being unduly dominated by individuals.
- To avoid the risk of persistent and unresolved conflicts between group members or between different species in mixed exhibits.
- To provide areas and barriers to allow for escaping should it be necessary and to maintain steady groups to promote social stability.
- To ensure that the physical carrying capacity of the enclosure is not overburdened.
- To prevent an unacceptable build-up of parasites and other pathogens.

### 5.3. Minimum pool sizes

Pool sizes are set to satisfy the need of aquatic mammals for physical exercise, opportunity to enrol in natural behaviours and constructive social interaction. The minimum standards are set for periods of five years.

If a facility does not meet newly set standards in already built in pools, it has ten years to adapt. However any new pools should be built under the last approved standards.

Facility designers are therefore well advised not to take minimum standards for new to build enclosures. Inspiration can be obtained from modern facilities.

5.3.1. The Pinnipeds enclosure sizes (Tables 1 and 2) are the minimum sizes under this S&G and are based on the 2018 EAZA Regional Collection Plan and EAZA-EAAM pinniped guidelines, compared with US and Canadian legislation. For the walrus the draft Husbandry guidelines is used. The bottlenose dolphins minimum enclosure sizes (Table 3) are based on the 2009 EAAM Standards and Guidelines. The pool depth is determined with help of the Average Sizes of the different species. A presentation pool that is not available for the animals outside the demonstration, shall not be included in the minimum space calculation.

Table 1: EAAM main pool minimum space requirements for Pinnipeds. For definitions and clarification see the “Pinniped Minimum requirements” by the EAZA Marine Mammal TAG that can be found at <https://www.eaza.net/conservation/programmes/>.

Species with similarly range size	Average length (m)	Group size	Land area (m <sup>2</sup> )	Additional land area per extra animal (m <sup>2</sup> )	Pool area (m <sup>2</sup> )	Additional pool area per extra animal (m <sup>2</sup> )	Minimum Volume (m <sup>3</sup> )	Minimum Depth (m)
<i>P. hispida</i>	1.6	1-6	18	3	72	12	153	2,1
<i>P. vitulina</i>	1.9	1-6	24	4	92	15	233	2,5
<i>H. grypus</i>	2.3	1-6	30	5	120	20	367	3,0
<i>A. tropicalis</i> <i>A. australis</i>	1.9	1-6	20	3,3	80	13	202	2,5
<i>A. pusillus</i> <i>Z. californianus</i> <i>O. flavescens</i>	2.6	1-4 to max 1-6	36	6	144	24	500	3,5
<i>E. jubatus</i>	3.3	1-4 to max 1-6	54	9	216	36	948	4,4

## EAAM Standards and Guidelines

Table 2: EAAM secondary pool minimum space requirements for Pinnipeds. For definitions and clarification see the “Pinniped Minimum requirements” by the EAZA Marine Mammal TAG that can be found at <https://www.eaza.net/conservation/programmes/>.

Species with similarly range size	Average species length (m)	Land area / each individual (m <sup>2</sup> )	Pool area / each individual (m <sup>2</sup> )	Minimum depth (m)
<i>P. hispida</i>	1.6	3	12	1,6
<i>P. vitulina</i>	1.9	4	15	1,9
<i>H. grypus</i>	2.3	5	20	2.3
<i>A. tropicalis</i> <i>A. australis</i>	1.9	3,3	13	1,9
<i>A. pusillus</i> <i>Z. californianus</i> <i>O. flavescens</i>	2.6	6	24	2,6
<i>E. jubatus</i>	3.3	9	36	3.3

Table 2: EAAM pool minimum space requirements for manatees. For definitions and clarification see the “EAZA best practice guidelines for the Antillean Manatee” by the EAZA Marine Mammal TAG that can be found at <https://www.eaza.net/conservation/programmes/>.

EAAM minimum space (Antillean manatees)				
Number of animals	Land surface (m <sup>2</sup> )	Pool surface area (m <sup>2</sup> )	Pool volume (m <sup>3</sup> )	Pool depth (m)
Antillean manatee (2 animals)	N/A	150	270	3
Per additional animal	N/A	25	50	

Table 4: EAAM minimum space requirements (bottlenose dolphins)

EAAM minimum space (bottlenose dolphins)				
Number of animals	Land surface (m <sup>2</sup> )	Pool surface area (m <sup>2</sup> )	Pool volume (m <sup>3</sup> )	Pool depth (m)
Bottlenose dolphin basis (1-6 animals)	N/A	550	2.000	3,5 ≥ 50%PS
Per additional animal	N/A	75	300	

## EAAM Standards and Guidelines

### 5.4. Accommodation - Comfort and Well-being

5.4.1. Temperature, humidity, ventilation and lighting of the enclosures to be suitable for the comfort and wellbeing of aquatic mammals at all times, and in particular:

- Consideration to be given to the special needs of pregnant and newly- born aquatic mammals.

- Newly arrived imported aquatic mammals to be fully acclimatized bearing in mind that this may be only a gradual process.

5.4.2. Aquatic mammals in outdoor enclosures to be provided with sufficient shelter from inclement weather or excessive sunlight where this is necessary for their comfort and well being. All the animals need to be able to access the sheltered area at the same time and at any time if they want to.

5.4.3. Aquatic mammals should not be unnaturally provoked for the benefit of the viewing public.

5.4.4. Aquatic mammals in visibly adjoining enclosures should be kept in a way they do not interact in an excessively stressful way.

5.4.5. Separate accommodation for pregnant aquatic mammals and aquatic mammals with young has to be available, if necessary, in the interests of avoiding unnecessary stress or suffering for mothers or calves.

5.4.6. For gregarious and social species (specially cetaceans), only isolate aquatic mammals when strictly necessary, and in that case always provide appropriate accommodation and attention while being temporarily separated from a group.

### 5.5. Enclosure requirements

5.5.1. At least one lifting platform, or another way to approach a sick, a new born or young cetacean quickly, should be available for each cetacean and manatee enclosure.

5.5.2. There should be at least 3 connected pools available (primary, secondary and medical) in cetacean facilities, or two pools with enough accesses to prevent animals from being cornered.

5.5.3. Pinnipeds should be kept in outside enclosures whenever possible provided their environmental temperature meets their physiological and natural requirements. The enclosures are defined as follows:

- \* Main housing facility: an enclosure that houses animals during a long period of the day and is usually an outside facility.

- \* Secondary housing facility: an enclosure that is used for housing animals for a certain period of time which can be used as separation and/or quarantine facility (if an independent filtration system exists or if it can be isolated from the main system). Its dimension can be smaller than the main, since they will not be used for prolonged period of time, although it should meet the minimum and considered as a whole without internal subdivisions.

## EAAM Standards and Guidelines

\* Main enclosures used for interactive programs must have an area of the enclosure forbidden to public entrance that allows the animals to choose where to go in every moment of the day

\* A presentation pool, that is not accessible to the animals apart from the presentation/demonstration, shall not be included in the minimum. Channels between pools have to be excluded from the calculation

5.5.4. The colours utilized to paint the different parts of the exhibits, pools and holdings should be as natural as possible, by using colours that avoid light reflection.

5.5.5. Rotating animals between enclosures that meet the minimum dimensions and other enclosures that do not, is not an acceptable mean of complying with the minimum space requirements.

5.5.6. In pinnipeds, enclosures housing two or more sexually mature males should have separate areas with sufficient visual barriers (such as fences, gates and/or rock work) to provide relief from aggressive animals, especially during breeding season.

5.5.7. Enclosures must have floor areas between the beach and the backdoors to allow correct and safe management for the trainers and veterinarians. In case of utilization of sand, its type should be safe for ingestion.

5.5.8. Enclosures and barriers to enclosures to be maintained in a condition that presents no likelihood of harm to aquatic mammals, and in particular:

- Any defect noted in a aquatic mammal barrier or in any appliances or equipment within aquatic mammal enclosures to be repaired or replaced without delay.
  
- Any defect likely to cause harm to aquatic mammals to be rectified at once or, if this is not possible, the aquatic mammals to be removed from the possibility of any contact with the source of the danger.
  
- Any vegetation capable of harming aquatic mammals to be kept out of reach.

5.5.9. All plants and fixed equipment, including electrical apparatus, to be installed in such a way that it does not present a hazard to aquatic mammals and its safe operation cannot be disrupted by them.

5.5.10. Rubbish in aquatic mammals enclosures to be cleared regularly to avoid any possibility of harm to aquatic mammals.

## 5.6. Prevention of Stress or Harm to aquatic mammals

5.6.1. Aquatic mammals to be handled only by, or under the supervision of, competent trained authorised staff; and this to be done with care, in a way which will avoid unnecessary discomfort, behavioural stress or actual physical harm to animals.

5.6.2. Smoking is prohibited in aquatic mammal enclosures, in any areas of the buildings where aquatic mammal's enclosures are located and in areas where food is stored or prepared.



#### EAAM Standards and Guidelines

5.6.3. Any direct physical contact between animals and the visiting public only to be under the control of the parks staff and for periods of time and under conditions consistent with animal welfare and not leading to their discomfort.

5.6.4. Any external workers accessing aquatic mammal facilities with direct access to the animals should be informed about pertinent animal care and safety operational rules and policies and supervised at all times by marine mammal park staff.

## 6. Husbandry

### 6.1 Definitions

6.1.1. **Enrichment** is a dynamic process for enhancing animal environments within the context of the animals' behavioural biology and natural history. Environmental changes are made with the goal of increasing the animal's behavioural choices and its control over its environment and drawing out their species-appropriate behaviours, thus enhancing animal welfare.

### 6.2. Nutrition

Nutrition standards refer to the 1998 USDA document *Handling Fish Fed to Fish-Eating Animals*, found in appendix I and the EAAM *Fish quality parameters*, found in appendix II.

6.2.1. The food for aquatic mammals must be wholesome, palatable, and free from contamination and must be of sufficient quantity and nutritive value to maintain aquatic mammals in a state of good health. The diet must be individually prepared with consideration for factors such as age, species, condition, and size of the aquatic mammal being fed.

6.2.2. Food, when given to each aquatic mammal individually, must be given by an employee or attendant responsible to management who has the necessary knowledge to assure that each aquatic mammal receives an adequate quantity of food to maintain it in good health. Such employee or attendant is required to have the ability to recognize deviations from a normal state of good health in each aquatic mammal so that the food intake can be adjusted accordingly.

6.2.3. Public feeding may be permitted only in the presence and under the supervision of a sufficient number of knowledgeable identified employees or attendants. Such employees or attendants must assure that the aquatic mammals are receiving the proper amount and type of food.

6.2.4. Food preparation, handling and analysis must be conducted so as to assure the wholesomeness and nutritive value of the food. For fish eating species, minimum analysis should include organoleptic and caloric values, as well as microbiology and physicochemical parameters as listed in Appendix II.

6.2.5. Frozen fish or other frozen food must be stored in freezers that are maintained at a maximum temperature of  $-18^{\circ}\text{C}$  ( $0^{\circ}\text{F}$ ). The length of time food is stored and the method of storage, the thawing of frozen food, and the maintenance of thawed food must be conducted in a manner that will minimize contamination and that will assure that the food retains nutritive value and wholesome quality until the time of feeding. For fish and seafood products, thawing temperatures on refrigeration should ideally not go over  $5^{\circ}\text{C}$  and for a maximum of 24h to minimize bacterial overgrowth.

6.2.6. Storage programs, thawing procedures and food preparation processes should be designed to prevent loss of nutrients and bacterial contamination. Fish that has not being consumed within 24 hours post thawing should be discarded.

6.2.7. Vitamin supplementation and other medication prescribed by the attending veterinarian must be individualized for each aquatic mammal.

### 6.3. Sanitation

#### 6.3.1. Enclosures:

- Animal and food waste in areas other than the pools must be removed from the primary enclosures at least daily, and more often when necessary in order to provide a clean environment and minimize health and disease.
- In particular animal and food waste, trash, or debris that enters the primary enclosure (pool) must be removed at least daily, or as often as necessary, to maintain the required water quality and to minimize health and disease hazards to the aquatic mammals.
- The wall and bottom surfaces of the pools must be cleaned as often as necessary to maintain proper water quality. Natural organisms (e.g. algae or molluscs) that do not degrade water quality, prevent proper maintenance, or pose a health or disease hazard to the animals are not considered contaminants.

#### 6.3.2. Food preparation:

- Equipment and utensils used for food preparation must be cleaned and sanitized after each use.
- Food preparation areas and other food handling areas where animal food is prepared must be cleaned at least once daily and sanitized at least once every week. Sanitizing must be accomplished by washing with hot water and soap or detergent in a mechanical dishwasher, or by washing all soiled surfaces with a detergent solution followed by a safe and effective disinfectant, or by cleaning all soiled surfaces with steam.
- Substances such as cleansing and sanitizing agents, pesticides, and other potentially toxic agents must be stored in properly labelled containers in secured cabinets designed and located to prevent contamination of food storage preparation surfaces and food itself.

#### 6.3.3. Housekeeping:

- Buildings and grounds, as well as exhibit areas, must be kept clean and in good repair.
- Fences must be maintained in good repair.
- All enclosures housing aquatic mammals must not have any loose objects or sharp projections and/or edges which may cause injury or trauma to the aquatic mammals contained therein.
- All people entering an enclosure with aquatic mammals must follow the disinfection protocols.

#### 6.3.4. Pest control:

- A safe and effective program for the control of insects, ectoparasites, and avian and mammalian pest must be established and maintained. Insecticides or other such

## EAAM Standards and Guidelines

chemical agents must not be applied in primary enclosures housing aquatic mammals except when deemed essential by an attending veterinarian.

### 6.4. **Enrichment**

6.4.1 EAAM members must provide appropriate environmental and behavioural enrichment for all animals of the collection.

6.4.2. An enrichment plan needs to be established and evaluations done on a regular basis, followed by readjustments should those be deemed necessary.

6.4.3. The enrichment plan should be in writing and plans and evaluations stored for at least 5 years.

## 7. Veterinary care

7.1.1. Members must have in place a comprehensive program for veterinary medical care that is integrated with husbandry, research and management functions.

7.1.2. Each facility must have a qualified attending veterinarian who oversees a program of preventive medicine and clinical care, and who supports all other programs to ensure the health of the facility's aquatic mammals.

7.1.3. Animal health assessment program should include:

- Regular veterinary rounds.
- Daily monitoring by husbandry staff of each animal's physical appearance, activity, temperament and changes in behaviour. Any significant change must be immediately communicated to the attending veterinarian.
- Complete physical examinations must be performed at regular intervals on each aquatic mammal in the collection. Physical examinations should include:
  - a. Determination of weight change (weekly).
  - b. Comparison of food intake and body weight.
  - c. At least one complete physical exam including one blood work per year in any aquatic mammal except for animals that are not that easily trained and where anesthesia poses a risk.
  - d. Other laboratory tests as needed.
  - e. Parasite screening and treatment must be conducted where indicated by the attending veterinarian for each aquatic mammal in the collection.

7.1.4. Animals can be kept in a smaller enclosure if compelling veterinary reasons justify such holding. A written statement of the attending veterinarian explaining the temporary keeping of an animal under different conditions should be available which:

- Outlines the veterinary reason for the different holding condition.
- Clarifies what actions are taken to end this situation.
- Clarifies how long this exceptional situation is expected to persist.

7.1.5. Members must have a euthanasia policy.

7.1.6. Aquatic mammals that die must be subjected to a post-mortem examination as determined by the attending veterinarian, with a permanent record generated indicating the disposition and/or results of the necropsy.

- Maximum use should be made of dead aquatic mammal specimens with priority given to those that enhance animal husbandry or conservation of the species in the wild.

- Second priority should be given to placing dead specimens in suitable museum collections or other bona fide scientific research programs, or in an educational facility.

- Dead aquatic mammals not disposed of by any of the above-named methods should be destroyed by incineration, burial, or in a manner deemed suitable by the attending

EAAM Standards and Guidelines  
veterinarian and curator in accordance with law.

## 8. Breeding

### 8.1. General introduction

7.1.1. The EAAM is committed to the goal of increasing the contribution of captive breeding to groups held in all institutions. The continued development and improvement of current breeding techniques by EAAM members will substantially benefit these efforts. It will also benefit rare and endangered species whose populations are increasingly threatened by diminishing habitat and other anthropogenic factors.

### 8.2. Definitions

8.2.1. **Current breeding techniques** refer to up-to-date methods and strategies utilized to maximize the potential for successful breeding efforts.

8.2.2. **Comprehensive breeding plan** is participating in cooperative breeding programs within the spatial possibilities of the exhibits.

8.2.3. **Studbook** refers to the genealogical register established to track lineage of aquatic mammal offspring.

### 8.3. Participating in breeding programs

8.3.1. Members must prioritize, through strategic planning, the selection of species for reproductive management based upon biological, demographic, genetic and conservation needs of the species. Whenever there is an existing EAZA Ex situ Program (EEP), and the facility is part of the EEP, the plan must follow its recommendations.

8.3.2. Members should participate in regional, national and/or international studbook and breeding management programs to serve present and future needs for conservation, education, and potential reintroduction of genetic material into natural populations should the need arise in the future.

8.3.3. Members should contribute to a better understanding of aquatic mammal reproductive biology and physiology by developing techniques and models that can be applied to rare and endangered species.

### 8.4. Aquatic mammal breeding

8.4.1. Members must develop a comprehensive institutional breeding management plan maximizing the potential for success before actively pursuing aquatic mammal breeding.

8.4.2. Personnel should possess or have access to expertise concerning aquatic mammal reproduction.

8.4.3. Members should provide maternity pools that are of a size and configuration to facilitate nursing, calf/pup rearing, and separation from other animals if necessary.

8.4.4. A plan to monitor pregnancy, calf/pup delivery and rearing should be in place and overseen by a specialized veterinarian.

8.4.5. Members should consider the reproductive and physical condition of participating animals.

## EAAM Standards and Guidelines

8.4.6. Breeding, pre-parturient and lactating animals should be maintained in social environments encouraging successful rearing of offspring.

8.4.7. Contingency plans should be developed, protocols recorded, and resources for implementation should be in place for: emergency intervention before, during, and after delivery; weaning; illness; pathological examination of mortalities.

8.4.8. Consideration should be given to species-specific needs.

8.4.9 The plan should prioritize acquisition of aquatic mammals for their collections through managed breeding programs; loans, exchanges or purchases from other qualified aquatic mammal park institutions; or, programs providing non-releasable orphaned or injured and rehabilitated individuals from wild populations.

The plan should include a commitment to partnerships for future maintenance and, wherever possible, breeding of the collections.

The plan should include species-specific rationale for situations where breeding of wild-caught animals is not part of the long-term plan for their management.



## 9. Water and Environmental Quality

### 9.1. General introduction

Wild aquatic mammals live in a medium in which organic and inorganic waste is quickly diluted or readily dissipates. In most zoological settings, pool water is recycled through filtration and water treatment systems, with only a small percentage replenished daily to make up for losses due to splash-over or filter backwash discharge. To ensure optimum quality, aquatic mammal pool water is usually subjected to biological disinfection, mechanical filtration, and chemical treatment of both dissolved and particulate organic matter. These processes are not exclusive and the efficacy of one method of treatment is usually dependent on that of another, as well as the physical and chemical parameters of the medium. The design of water treatment systems varies considerably between member institutions. In all operations, however, the establishment of optimum water parameters must be based on both the physiological needs of the animals and the efficiency of the water processing techniques involved.

The quality of aquatic mammal's environment can have significant effects on its health and welfare. The exhibit setting must be designed to meet species-specific needs as well as the physical condition of the individual animals.

Regardless of the design of the water treatment systems, in all operations, to ensure optimal water quality and animal welfare, an effective program must be in place for monitoring physical, chemical and biological parameters, with multiple sampling points along the system.

### 9.2. Definitions

9.2.1. **Aquatic mammal pool** refers to any structure or enclosure containing water designed to house aquatic mammals, including natural lagoon, bays and tidal basins, as well as man-made structures.

9.2.2. **Laboratory techniques listed in standard methods** refers to analytical methods as outlined in standards for water quality.

9.2.3. **Adequate ventilation** refers to an ample flow of fresh air necessary to minimize the accumulation of chlorine fumes, other gases, and noxious odours.

9.2.4. **Vertical air space** refers to the space between the surface of a aquatic mammal pool and the overhead ceiling or canopy, usually pertaining to an indoor facility.

9.2.5. **Acoustic monitoring** refers to a system for detecting sounds and noise could be affecting to aquatic mammals.

9.2.6. **ORP**: Oxygen Reduction Potential or Redox Potential is a measurement of water's ability to oxidize contaminants.

9.2.7. **Life support systems**: (LSS) constitutes the configuration of the system, type of filters, type of disinfection system, whether the system is automated or manually controlled. The components and materials of construction may vary considerably.

9.2.8. **Turn over rate**: time it takes for the whole volume to go through the LSS

### 9.3. Standards and Guidelines for Water Quality

Aquatic mammal pool water must be monitored for selected physical, chemical and biological

## EAAM Standards and Guidelines

parameters of water to ensure a healthy aquatic environment as appropriate for closed or open circulation systems.

9.3.1 Aquatic mammal pool water should be tested and recorded at least daily and treated as necessary to maintain pH values not less than 7.2 or more than 8.4.

9.3.2. Aquatic mammal pool water should be tested minimum twice daily for concentration of chlorine and/or ORP in case of other oxidizing agents. Total free and combined chlorine should never exceed 1.0 mg/l. It is recommended to keep Free chlorine under 0,8 mg/l, Combined chlorine under 0,2 mg/l. The use of chlorine is not acceptable in sea otter exhibits due to fur damage.

9.3.3. Aquatic mammal pool water, whenever applies, should be free of residual dissolved ozone. ORP should be maintained within a relatively narrow zone range to minimize the risk of producing excess residual oxidants. Multiple sampling points along the system are recommended.

9.3.4. Cetaceans and all other marine mammals (excluding manatees) maintained in closed water systems should have the water treated with sodium chloride or a combination of sodium chloride and other naturally occurring sea salts so as to maintain a salinity of not less than 28 ppt. All EAAM pinniped facilities must comply with this regulation before 2029.

9.3.5. For closed water systems, whatever the system of water treatment, it is important to discard and replace some water at regular intervals.

9.3.6. Members should test the concentration of bacteria in aquatic mammal pool water as an indicator of disinfection capacity. As reference total coliform counts should not exceed 1000 colonies/100 ml. E. Coli counts are also an adequate indicator for water quality. For best practice it is recommended to keep total coliform counts below 500 colonies/100ml. Other bacteria, fungi and yeast can also be measured routinely for water quality evaluation.

9.3.7. Members should minimize disinfection by-products through flocculation, foam fraction, etc.

9.3.8. Food waste should be regularly removed from the water body, to maintain water quality and pool hygiene as well as to minimize contamination.

9.3.9. Members should implement a program of daily facility cleaning and maintenance that minimizes the risk of animal exposure to pathogenic microorganisms.

9.3.10. Water quality records should include: maintenance logs, added chemicals controls, bacterial culture and filtration operating log. All records should be maintained on site for at least 5 years.

9.3.11. Pool surfaces should be constructed of non-abrasive materials impervious to liquid penetration and resistant to disinfection.

9.3.12. Water temperature should be monitored at least daily. Where conditions outside of the ranges noted in 9.4 occur for longer than one whole week, members should take

## EAAM Standards and Guidelines

protective actions so as to prevent adverse animal health consequences.

9.3.13. Members with facilities incorporating water that is open to the ambient sea environment must monitor their water source to be sure that conditions remain compatible with updated animal management.

9.3.14. For keeping cetaceans, salinity should not fall below 2.8% salt (28 ppt, parts per thousand) for a period of longer than one week. Salinity should be monitored daily.

9.3.15. With the exception of manatees, if salinity falls below 28 ppt for a period of time longer than one week, animals should be maintained under enhanced veterinary supervision and an expanded schedule of water quality monitoring should be in place to assess environmental impact associated with increased freshwater runoff or other causes.

9.3.16. Bacteria should be monitored at least weekly for total coliform. Additionally, it is recommended that total bacteria/100 ml of water be tested as an indicator of overall bacterial concentrations within the water body. Evaluations of total bacteria concentrations are made on a relative scale utilizing a baseline standard for the test of the water body itself.

9.3.17. Members should be aware of, and be prepared to test for, potential sources of water borne toxins that could adversely impact animal health. Potential toxin sources include pollutants from agricultural and industrial sources, as well as naturally occurring toxins.

9.3.18. Turn over rate should be ideally be below 2 hours in most aquatic mammal species. Walrus should be kept below 90 minutes. Ideally none should exceed 4 hours.

## 9.4. Standards and Guidelines for Environmental Quality

9.4.1. Bottlenose dolphins should be maintained in water temperatures between 14°C and 30°C - the lower range could reach 10°C in those facilities that hold Black Sea dolphins, *Tursiops truncatus ponticus*. Bottlenose dolphin calves should be maintained in water temperatures between 14°C and 28°C.

Killer whales should be maintained in water temperatures between 12-16 °C

Seals should be maintained in water temperatures between 0-25 °C

Sealions and fur seals should be maintained in water temperatures between 5-26 °C

Manatees must be maintained in water temperatures between 25-30 °C

Provide access to shelter to afford protection from adverse weather and direct sunlight. Members must provide adequate heating or chilling for pool systems, whenever extreme temperatures are achieved.

9.4.2. All aquatic mammal pool waters should be free of ice, except for seals and walrus.

9.4.3. For indoor facilities provide ample lighting by either natural or artificial means or both.

## EAAM Standards and Guidelines

9.4.4. All aquatic mammal enclosures must be provided with adequate ventilation.

9.4.5. Indoor housing facilities should be ventilated by natural or artificial means to provide a flow of fresh air that minimizes the accumulation of chlorine or other fumes and noxious odours. The ventilation should be  $\geq 10$  air changes per hour.

9.4.6. A vertical air space averaging at least eight foot (2.5 meters) should be maintained in all primary enclosures, including pools of water.

9.4.7. Members must minimize exposure of aquatic mammals to noises of such high intensity or type to cause auditory discomfort or distress.

9.4.8. A plan of acoustic monitoring for aquatic mammals enclosures should be in place. This monitoring should be performed yearly or when the LSS has been submitted to significant changes, major maintenance operations or reparations. Efforts should be made to acoustically isolate sound-generating mechanical equipment located in close proximity to aquatic mammal enclosures.

9.4.9. A contingency plan needs to be written that ensures emergency sourcing of water and electric power in the event of failure.

## 10. Interactive programs

### 10.1. General introduction

An interactive program must comply with all requirements for the care and maintenance of aquatic mammals as defined by relevant laws and regulations

### 10.2. Definitions

10.2.1. **Interactive Program** refers to a program in which members of the public participate in an activity that includes close contact with a aquatic mammal under trainer control.

10.2.2. **Controlled Interaction** refers to an interactive program in which the movements and interactions of both aquatic mammals and public participants are maintained under stimulus control.

### 10.3. Standards and Guidelines for aquatic mammals Interactive Programs

10.3.1. Primary enclosures used for interactive programs must meet relevant government regulations, must have an area of the enclosure established for animals participating in interactive activities that the public may not enter, and have the restricted area not configured in any way that is uninviting to the animals.

10.3.2. Interactive activities programming must include educational information about the aquatic mammal species and promote an improved understanding of and an appreciation for the conservation of the animals and their ecosystems.

- Such educational presentations should include oral and written procedures and rules outlining appropriate behaviour for the protection of both the animals and the guests.

- All programs should include information that feeding, touching, harassing and swimming with wild aquatic mammals is not advised, that it is illegal in some countries, and that swimming with wild aquatic mammals can be harmful to both the animals and the people involved.

10.3.3. Aquatic mammals participating in interactive programs must be properly trained and conditioned, and appropriate action must be taken to maintain a controlled interaction.

- If a member of the public refuses to participate responsibly in an activity, that guest should be immediately removed from the interactive session.

10.3.4. A facility must have a behaviour development and management plan specific to each type of interactive activity offered to the public and must meet the standardized training guidelines of the International Marine Animal Trainers Association.

- The behaviour development and management plan should describe the animal training program, its objectives, and methods of accomplishment.

- The amount of time each aquatic mammal is asked to participate in interactive

## EAAM Standards and Guidelines

activities should be specific to the individual animal and based on behavioural criteria compiled for that animal.

- Ratios of public participants to animals should be appropriate to the type of interactive activity offered. Approval of the ratio by the ACWC (including at least both the attending veterinarian and the supervising trainer) is required, based on their observations of the specific interaction.
- Aquatic mammals undergoing medical treatment may only participate in interactive programs with the approval of the facility's attending veterinarian.
- Supervisory staff overseeing interactive programs must have actively participated in the training and husbandry of aquatic mammals in interactive programs for at least three years accumulated over a period of no longer than five years prior to current employment.
- Each animal must have one period of at least 12 continuous hours without public interaction within a 24-hour period.
- All incidents resulting in injury or damage to either aquatic mammals, trainers, keepers or the public as a result of an interaction, as defined above, requiring veterinary or medical care must be recorded and kept at the facility for at least ten years and made available to the EAAM accreditation team.

## **11. Record Keeping**

### **11.1. General introduction**

The EAAM recognizes the need to maintain standardized, comprehensive and accurate records concerning the humane and healthful care of the aquatic mammals in our collections. Whereas certain record maintenance is required by various laws, up-to-date records will: (1) assist all members in providing the latest in appropriate care for the animals; (2) enable all members to share their collective knowledge about health and behaviour concerns; and, (3) facilitate reproduction programs through accurate recording of activities and with appropriate management of related animals through studbooks. The EAAM therefore recommends that members maintain the following data in each category. It is recognized that individual members may collect and maintain more data, where appropriate, based on individual member circumstances and needs.

### **11.2. Standards and Guidelines for Record Keeping**

11.2.1. Records must be protected from fire, flooding and other natural or human created hazards.

11.2.2. Duplicate records, as appropriate, must be kept in either a separate location or a fire proof case.

11.2.3. It is recommended that all records are kept indefinitely to allow retrospect analysis.

11.2.4. Acquisition and disposition records must include: date and location of acquisition; method of acquisition (wild caught, captive birth, transfer, loan, temporary holding); sex; genus and species; progeny; identification (genus, species, sex, id #. For example: TT-M1562, name, studbook number); date and location of disposition; method of disposition; all applicable CITES documentation relating to the animal; transportation records. Acquisition and disposition records must be kept indefinitely. The use of ZIMMS is encouraged as a best practice for record keeping.

11.2.5. Food and nutrition records must include: type, and/or species of food; caloric value; analysis; freezer rotation; dates on food packets; and, freezer temperature. Food and nutrition records must be kept for at least five years.

11.2.6. Health and medical records must include: date of examination; veterinarian's name; reason for examination; action taken; diagnostic test results, medications; supplements and prophylactic treatments; individual nutritional requirement; body measurements; necropsy findings; photographs (when appropriate), physical characteristics; subjective and objective findings. Health and medical records must be kept indefinitely.

11.2.7. Health and medical records should always remain with the individual animal. Duplicate records must be kept indefinitely at the originating facility.

11.2.8. Environmental quality records must include: test parameters for water quality; tests for added chemicals; microbiological culture test results; amount of added chemicals;

## EAAM Standards and Guidelines

facility maintenance log; and, filtration operation log. Environmental quality records must be kept at least five years.

11.2.9. Daily records must include: behavioural observations; anomalies and patterns;

outside factors; types and quantities of food consumed; and, amount and type of interaction. Daily records must be kept at least five years.

11.2.10. Facility descriptions must include: enclosure dimensions and location; water system/filtration type, and protocols for LSS functioning.

11.2.11. All incidents resulting in injury or damage to either aquatic mammals, trainers, keepers or the public as a result of a training session, demonstration or interaction, as defined in section 9 of these standards and guidelines, that require veterinary or medical care must be recorded and kept at the facility at least for ten years.

11.2.12. Inspection records of any professional or governmental organisation should be kept at least ten years.



## 12. Transportation

### 12.1. General introduction

EAAM members must ensure that aquatic mammals under their care are transported between facilities in a manner that is both safe and humane. Implicit in the transport of an aquatic or semi-aquatic mammal is the fact that the animal will be restricted from access to its normal environment, an environment that provides physical support, protection from extremes of temperatures, and freedom of normal postural adjustments. For this reason, the movement of a aquatic mammal between facilities must be executed in an efficient manner by experienced staff.

### 12.2. Definitions

12.2.1. **Aquatic mammal transportation** refers to the relocation or movement of aquatic mammals by any method or mode of transport that requires more than two hours from the time of removal from current housing until arrival at destination housing.

12.2.2. **Initial health assessment** refers to a preliminary physical exam including review of animal records conducted by attending veterinarian or other qualified veterinarian in order to determine that the animal is of sufficient health and physical condition necessary to be safely transported.

12.2.3. **Transport plan** refers to a thorough, written plan of action designed to insure a safe, humane, and efficient move of a aquatic mammal from one location to another.

### 12.3. Standards and Guidelines for aquatic mammal Transportation

12.3.1. An initial health assessment must be conducted by the attending veterinarian or another experienced aquatic mammal veterinarian on each animal between three to ten days preceding transport. The health assessment will include the evaluation of behavioural, feeding, and medical records.

12.3.2. A transport plan must be in place.

12.3.3. A final transport planning meeting should be held by a designated transport coordinator not more than 24 hours prior to transport to ensure the aquatic mammal's health and well being. Emergency contingency plans should be outlined and approved at this meeting.

12.3.4. Aquatic mammals (except manatees) should be fasted for between 8 and 24 hours prior to transport. However, animals can be reinforced with limited amount of fish if voluntarily accepting food during transport.

12.3.5. If transported via air, the transporting crate must follow container requirement of the IATA Live Animals Regulations. By land either the IATA LAR or the Cites guidelines for the Non-air Transport of Live Wild Animals and Plants should be followed.

12.3.6. A facility must have an emergency plan and emergency equipment ready, including animal transport devices e.g. in case of filtration emergency, fires, tornados, flooding's or other natural hazards.

## EAAM Standards and Guidelines

### 12.4. **specific guidelines for cetacean transport**

12.4.1. Cetaceans must be transported and positioned so as to avoid contact with hard or abrasive surfaces, to prevent harmful restrictions in blood flow, and with sufficient attendants to provide for physical and medical needs.

12.4.2. Cetaceans should be transported, whenever possible, within properly secured, open-top containers with sufficient water to provide body support and to facilitate thermoregulation.

12.4.3. When transporting cetaceans in aircraft, cabin pressure should be maintained at less than 8,000 feet (2,400 meters), with 6,000 feet (1,800 meters) or less being optimal for most individuals.

12.4.4. Cetacean transports should always be accompanied by the attending veterinarian or another licensed veterinarian experienced in cetacean transports.

12.4.5. One attendant per each bottlenose dolphin is recommended on transports of four or less animals with a minimum of two attendants per transport. On transports of five or more bottlenose dolphins, additional attendants may be added at the discretion of the veterinarian and/or transport coordinator.

12.4.6. Cetaceans must be monitored throughout transport.

EAAM Standards and Guidelines

## **Appendices**

### **Appendix I: USAD Handling Fish Fed to Fish-Eating animals**

FAAM Standards and Guidelines



United States  
Department of  
Agriculture

Agricultural  
Research  
Service

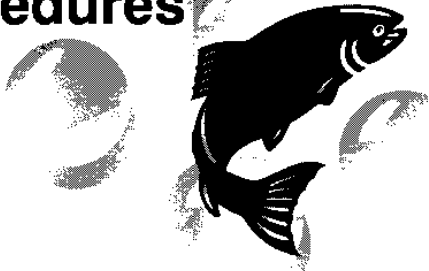
National  
Agricultural  
Library

Animal and  
Plant Health  
Inspection  
Service

June 1998

# Handling Fish Fed to Fish-Eating Animals

## A Manual of Standard Operating Procedures



United States Department of Agriculture  
Agricultural Research Service

EAAM Standards and Guidelines  
National Agricultural Library  
Animal and Plant Health Inspection Service

# **Handling Fish Fed to Fish-Eating Animals A Manual of Standard Operating Procedures**

**Susan D. Crissey, Ph.D.**

**With the assistance of Susan Bunn Spencer**

Crissey is director, Nutrition Services, Daniel F. and Ada Rice Center, Chicago Zoological Society, Brookfield Zoo, Brookfield, IL 60513.

## EAAM Standards and Guidelines

### Abstract

Crissey, S.D. 1998. Handling Fish Fed to Fish-Eating Animals: A Manual of Standard Operating Procedures. U.S. Department of Agriculture, Agricultural Research Service, National Agricultural Library.

Most captive fish-eating animals are fed frozen, thawed fish that are received in bulk and have been stored for a period of time before being prepared by cutting and chopping prior to being fed to the animals. Since it is important that nutrient loss and bacterial load in this food source be kept to a minimum, proper handling is essential. This document provides background and guidance for the handling of fish fed to captive fish-eating animals. All points of fish handling are discussed, from ordering, purchasing, and receipt through storage, thawing, and feeding—including cleaning and sanitation—to validating procedures and sampling. Using these guidelines, along with the appropriate documentation as presented in the text and the sample forms, should allow institutions that feed fish-eating animals to meet or exceed regulations current at this time.

**Keywords:** fish, fish-eating animals, fish handling, piscivores, sanitation.

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not recommended.

Cover photograph by Mike Greer, ©1991 by Chicago Zoological Society.

While supplies last, single copies of this publication can be obtained at no cost from S. Crissey, Brookfield Zoo, Brookfield, IL 60513.

Copies of this publication may be purchased from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161; telephone (703) 605-6000.

*The United States Department of Agriculture (USDA) prohibits discrimination in all its programs and activities on the basis of race, color, national origin, gender, religion, age, disability, political belief, sexual orientation, and marital or family status. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (Braille, large print, audiotape, etc.) should contact USDA's TARGET Center at 202-720-2600 (voice and TDD).*

*To file a complaint of discrimination, write USDA, Director, Office of Civil Rights, Room 326-W, Whitten Building, 14th and Independence Avenue, SW, Washington, DC 20250-9410 or call 202-720-5964 (voice or TDD). USDA is an equal opportunity provider and employer.*

June 1998

## EAAM Standards and Guidelines

### Contents

Introduction .....	1
Ordering and purchasing .....	2
Inspection of shipment .....	3
Storage .....	4
Transportation .....	5
Thawing .....	5
Handling thawed fish .....	6
Feeding .....	7
Cleaning and sanitation .....	8
Validating procedures .....	10
Sampling for microbial and nutrient content .....	11
Forms	
Checklist for inspecting a fish shipment .....	12
Quality control standards .....	13
Refrigerator/freezer temperature chart .....	14
Feeding container temperatures .....	15
Fish consumption chart .....	16
Temperature log for single box of fish .....	17
Appendix A. Properties of freezing .....	18
Appendix B. Infections and toxins of foods .....	19
References .....	21

### **Introduction**

This document provides background and guidance for the handling of fish fed to captive fish-eating animals. Wherever possible, official U.S. Department of Agriculture (9 CFR §3.105, §3.107) or state sanitation or fish-handling documents were used to provide these guidelines. The guidelines in this document were designed to meet, or in some cases be more stringent than, official guidelines. Thus the use of these guidelines, along with the appropriate documentation as presented in the text and the sample forms, should allow an institution that feeds fish-eating animals to meet or exceed regulations.

Local sanitation regulations may vary from state to state. Therefore, care should be taken to review any relevant state or local regulations with respect to instituting or modifying the guidelines presented in this document. As more information on fish contamination, diseases, and sanitation becomes available, it should be used to update and augment these guidelines.

Most captive fish-eating animals (including cetaceans, pinnipeds, and a variety of bird and fish species) are fed frozen, thawed fish. Since daily food availability is crucial to any captive program, most fish purchases are made in bulk. This requires the items to be frozen and stored until use. Given the perishable nature of fish, appropriate food-handling procedures are crucial to the nutritive quality of the food and consequently to the successful management and welfare of the animals.

The term "fish" is used throughout this document to mean all fish, including freshwater and saltwater fish, and other seafood items (squid, clams, etc.) that may be fed to fish-eating animals. Types of fish selected for use by an institution are chosen for specific nutrient content, quality, availability, price, and animal preference. The nutrient value of fish varies considerably due to several factors: species differences, individual differences due to season of capture, age, and sex (Stoskopf 1986).

Nutrition and quality must be considered major factors in fish selection. Care must be taken to ensure that food for captive marine animals is of the highest quality. USDA regulations state that "food for marine mammals shall be wholesome, palatable, and free from contamination, and shall be of sufficient quality and nutritive value to maintain all of the marine mammals in a state of good health" (9 CFR §3.105). Purchasing inferior-quality fish is wasteful (Oftedal and Boness 1983). More importantly, consumption of fish that are contaminated with high levels of bacteria is a serious health problem for animals as well as for handlers processing the food.

In order to avoid ultimate dependence on one particular food item, it is prudent to offer a variety of fish to the animal. It is possible for an animal to become imprinted on a specific food item. If that item becomes unobtainable, it may be very difficult to coax the animal to eat a new species. In addition, offering a variety of food items helps to ensure a complementary nutrient profile in the diet. Geraci (1978) emphasizes the need to feed more than one food type, including high- and low-fat fishes, in order to help ensure a balanced diet.

Practically, there are two basic approaches to offering fish as food: offering one species of fish on a seasonal basis or offering several species of fish throughout the year. If one fish species is offered seasonally, a new species should be rotated into the diet. The rotation may occur as often as



### EAAM Standards and Guidelines

quarterly. The advantage of the seasonal approach is that relatively fresh fish would be fed. The disadvantages include-

- Offering only one type of fish at a time may provide a nutrient profile that is not nutritionally balanced (for example, too fatty or too lean, low or high in a nutrient).
- The quality of the one type of fish may happen to be poor or unacceptable, and there is no backup supply.
- The supply may run out with no backup supply.
- The fish may be unpalatable to one or more of the animals in the collection.
- Some species are harvested only at specific times or once a year.

Given the goal of a balanced diet, it would be preferable to offer more than one species of fish, but holding stored fish for prolonged periods may cause nutrient losses. The objective is to provide a balanced diet utilizing the freshest fish possible.

Another approach is to obtain the catch seasonally, but store the fish for up to a year and spread usage evenly throughout the year. The advantages include-

- Several fish species are available and fed simultaneously for a nutritional balance.
- Backup supplies are on hand in case of palatability problems or poor quality.

The disadvantage is that frozen fish may lose nutrients over time.

Uncertainties in the future availability of fish stocks, reliance on farm fish, and the development of technologies such as a fish substitute for marine mammal diets: These factors make selection of appropriate fish and their handling of utmost importance. Such uncertainties and possibilities require an awareness and evaluation of the nutritional content and quality of diets.

### **Ordering and Purchasing**

To determine the freshness and wholesomeness of fish, the history of the catch should be ascertained. This history should include knowledge of precapture conditions. Epidemiological data such as local and periodic occurrences of pesticide and heavy metal pollution also are useful (Stoskopf 1986). The broker or fishery can be contacted for this information. Also, for information about current fish supplies, status, or contamination problems, newspapers and fisheries reports may be helpful. Additionally, request that a catch date be recorded on the boxes received to provide an indication of freshness of fish. The date can provide a link between the catch and environmental events that may have affected it.

In order to meet USDA standards, all fish should be of the same quality as that intended for human use (9 CFR, Subpart E, §3.105). Therefore, fish fed to animals should be supplied from fisheries that have caught, processed, and stored the fish as if they were intended for human use. The primary difference between fish for human use and those for captive fish-eating animals is that whole fish are usually fed to animals. Therefore, it is not required that the product be deboned and cleaned of internal organs.

## EAAM Standards and Guidelines

The packaging of fish by a processor can play a significant role in fish quality. Fish must be packaged in plastic-lined boxes with date of catch printed on the box. Fish may be block frozen,

individually quick frozen (IQF), or in a shatter pack. The optimal size for packages should be 10-20 kg to allow for proper thawing. It is suggested that package size provide 1 day's supply without leftovers (Stoskopf 1986). Package size is also determined by the type and usage of fish. Those fish used in smaller quantities should be purchased in smaller packages or should be prepared in a manner that allows for easy access to smaller quantities (by using IQF or shatter pack).

Size of the individual fish may be important to avoid the necessity of cutting (some species are naturally smaller than others; younger fish also may be smaller). Cutting fish causes a greater nutrient loss, as well as increased hours for preparation (Stoskopf 1986). If the entire fish is not fed, the nutrient content is altered. There may be a substantial decrease in the calcium content of the fish if the heads are removed, for example.

### **Inspection of Shipment**

Ideally, to ensure that fresh fish are handled appropriately throughout processing by the fisheries, the fisheries should be visited during processing and the fish inspected at that time. Since this may be impractical for most institutions, they should concentrate on a thorough inspection when the product arrives at the storage facility.

The first step in quality control is at the delivery stage. Since products should be inspected and processed immediately, schedule deliveries during business hours. An inspection should occur at the place of receipt (storage site) before or possibly during unloading of the shipment so that a representative number of boxes can be examined. Inspection must be performed by one of the zoo's or aquarium's employees who is familiar with proper inspection techniques and fish quality. A thorough inspection should include looking for signs of pests around and inside containers, maintenance of proper temperatures during shipment, and signs of thawing and freezing (Crissey et al. 1987).

*Every* lot or shipment of fish must be inspected before paperwork is signed to officially receive it from the supplier. Form 1 is designed to assist with inspection of the fish shipment. When inspecting a shipment (U.S. Navy 1965)-

- Check the supplier's documents to ensure that the fish shipment corresponds to the fish ordered- type, size, quantity, price.
- Observe the overall condition of the shipping vehicle and its contents. Sometimes shippers may transport other items in the same truck as the fish order to save freight costs. There should be no nonfood items shipped with the fish. This is to avoid possible contamination with items not intended for consumption.
- Check the temperature gauge in the storage area of the vehicle, since it indicates temperature of the vehicle's contents. If there is any question concerning the appropriate shipping temperature of the fish, use a portable thermometer to check the temperature inside several of the containers of fish.
- Visually inspect the contents of enough packages in the shipment to ensure that the entire load is suitable. The number of packages to be checked depends on the size of the shipment, but at a

## EAAM Standards and Guidelines

minimum open and examine at least three packages- one each from the front, middle and end of the load.

- Visually inspect the fish to make sure that the product is the species and size of fish, as well as the size and type of packaging, ordered.
- Look for evidence that the fish have been frozen, thawed, and then frozen again: water or ice buildup on the boxes or floor beneath the boxes; wrappings that are moist, slimy, or discolored; fish with soft flabby flesh, a sour odor, and an “off” color. If any of these indicators are present, do not accept the order.

When thawed, fresh fish have bright red gills, prominent clear eyes, and firm, elastic flesh. Old or thawed and refrozen fish are dull in appearance, have cloudy and red-bordered eyes, and have soft flesh, and finger impressions are made easily and remain (U.S. Navy 1965).

If the quality is questionable, it is wise to thaw a few fish from several packages for a better determination (form 2). Again, try to do this before officially accepting the shipment. If the order is acceptable, a sample of fish should be taken for nutrient analyses at this time.

If the fish have been found to be unsatisfactory for any reason, refuse to take receipt, even if that means reloading the vehicle. The shipper should take the load back. If there is any disagreement as to the quality of the product or what the shipper is to do with it, contact the supplier. Bad fish are unusable, unpalatable, and a health hazard and may cause a significant economic loss due to illness or death of the animals.

In other words, sign any documents officially receiving fish only after the shipment has been inspected and found satisfactory.

### **Storage**

Once a fish shipment has been accepted, it should be placed immediately in the institution's storage facility. This facility should be designed to adequately protect supplies from deterioration or contamination. It is crucial that the length and conditions of storage minimize contamination and ensure that the product retains its nutritive value and wholesome quality.

Prior to storing a new shipment, inspect the storage freezer to ensure that it is in good working order. There should be no potential for contamination by chemicals or other items that may also be stored in the freezer. Any older stock remaining in the freezer should be placed so that it will be used before the new stocks—a "first in, first out" basis. Always rotate shipments of the same species of fish to help ensure freshness. Optimally, the date received should be stamped or written on a box or pallet of boxes (Crissey et al. 1987).

Because they can support microbial populations, fish are included in the definition of potentially hazardous foods: "any food that consists in whole or in part of ... fish, shellfish, edible crustacea ... in a form capable of supporting rapid and progressive growth of infectious or toxigenic microorganisms" (IDPH 1993). To decrease or inhibit growth of such micro-organisms, proper storage temperatures are required (appendix B).

### EAAM Standards and Guidelines

Several sources cite optimum freezer temperatures ranging from -30 to -18 °C (-22 to 0 °F) (Geraci 1978, Stoskopf 1986, Crissey et al. 1987, Shinaburger 1992, IDPH 1993; also 9 CFR 3.105). Desrosier (1978) reports that in the United States commercial frozen-storage temperature is -18 °C (0 °F), but lower temperatures may be better. Desrosier further states that temperatures above -9 °C (-16 °F) but below the average freezing point for foods

of -2 °C (28 °F) cause critical damage to appearance and losses of nutrients and that long-term storage at 6 °C yields unacceptable foods. It is recommended that fish stored for prolonged periods (up to a year) should be in a freezer with temperatures maintained at -23 °C (-10 °F) or lower.

Refrigeration is used only for thawing of fish and short-term storage until fish are fed (9 CFR 3.105). Once removed from the freezer for thawing or thawed under refrigeration, fish must be used within 24 hours. Several authors report a refrigerator temperature requirement for storing potentially hazardous foods of 0 to 10 °C (32 to 50 °F) (Geraci 1978, Shinaburger 1978, Crissey et al. 1987). USDA cites a refrigerator temperature of less than 4 to 6 °C (40 to 43 °F) as optimal (Pond 1987).

There are no studies reporting shelf-life recommendations for particular species of whole fish. However, following the procedures below will help to ensure that contamination conditions are minimized and that the fish retain their nutritive value and wholesome quality.

- Refrigerators and freezers designated for fish storage must be used for perishable food only. No substances that are known to be or may be toxic or harmful to marine animals should be stored or maintained in the animal-food-storage areas.
- Adequate and proper cold air circulation is required for maintaining the desired uniform temperature in all areas of the freezers and refrigerators where fish are stored. Check to be sure that cold air ducts are not blocked when items are placed in the storage area. Allowing at least 2 feet between the top of stacks and opening of air ducts usually provides the circulation needed to maintain the proper range of temperature.
- Proper temperatures in refrigerated and freezer spaces should be:
  - Freezer:* -30 to -18 °C (-22 to 0°F) or lower
  - Refrigerator:* 4 to 6 °C (40 to 43 °F)
- Set up a schedule for routinely checking temperatures in several locations in the refrigerator and freezer. Document the temperatures in writing. Form 3 is provided for recording temperature.
- Relative humidity should be maintained at 85 to 90 percent in refrigerated spaces. A high humidity in the freezer helps to decrease dehydration of the frozen items (Stoskopf 1986).

### Transportation

It is necessary to transport fish from bulk freezer storage to a location used for storing smaller quantities and subsequent thawing and processing (kitchen preparation area). Such transportation must be accomplished in a manner that keeps the fish solidly frozen. The vehicle should be cooled or insulated. If this is not possible, procedures must be taken to cover or insulate the load while in transit, depending on outside environmental conditions. The length of transportation time necessary to move stock from storage to the appropriate short-term storage or preparation area should be minimized.

## EAAM Standards and Guidelines

It is recommended that the temperature of fish in transit be monitored by placing a thermometer in one or more of the boxes during transport. This could be a maximum/minimum thermometer or another temperature-sensing or -recording device. If temperature is monitored, it should be documented. Any boxes thawed or partially thawed during transportation should be used immediately and not refrozen.

### Thawing

The thawing process is critical to the product's final quality. Therefore, it must be carefully controlled (appendix A). Incorrect thawing increases the potential for nutrient loss, lipid peroxidation (rancidity), microbial buildup, and loss of palatability (appendix B). The safest and most preferable way to thaw fish is in a refrigerated space (Stoskopf 1986). Freezing tends to break down tissues, making the food much more susceptible to bacterial invasion after thawing.

USDA regulations state "thawing of frozen food shall be conducted in a manner that will minimize contamination and which will assure food retains nutritive value and wholesome quality" (9 CFR 3.105). Thawing in cold water is not preferable but is allowed by some regulations.

IDPH (1993) outlines three methods by which fish may be thawed:

1. The preferred method is "in refrigerated units so the temperature of the fish themselves does not exceed 7 °C (45 °F)." This method can be documented by placing a thermometer with the fish.
2. "Under potable running water at a temperature of 21 °C (70 °F) or below, with sufficient water velocity to agitate and float off loose particles into the overflow." This is not as desirable as method 1 because running water over thawing fish increases nutrient loss, especially of water-soluble nutrients, and therefore is not recommended. Thawing in standing water is not recommended because of loss of nutrients and the possibility of increased microbial buildup and contamination.
3. "In a microwave oven set to defrost." This method is used only when the food will be immediately transferred for consumption but is not preferred.

Fish should never be thawed at room temperature.

Different regulations and guidelines exist regarding ideal temperatures for fish thawing. Selecting the most stringent conditions ensures appropriate conditions for fish quality. IDPH (1993) guidelines state that the temperature of the fish should not exceed 7 °C (45 °F). Pond (1987) cites USDA recommendation of "thawing any meat product at a temperature not to exceed 4 °C (40 °F)."

Frozen foods should not be thawed by exposure to excessive heat or thawing in standing water. These methods cause an increased loss of nutrients (Stoskopf 1986). The use of fans to speed thawing causes loss of fluid through dehydration.

Ideally during thawing, fish should be kept in wrapping or a container, which provides insulation and allows the fish to thaw uniformly. The container may include the original shipping box or a covered plastic container.

All fish should be fed to the animals within 24 hours following removal from freezers for thawing (9 CFR 3.105). While this may be ideal, fish packed in large boxes may not thaw in 24 hours. To

## EAAM Standards and Guidelines

promote uniform thawing, the block of fish may be cut (sawed) or broken up while still frozen and the smaller portions thawed. If a large block of fish is to be thawed, it is advisable to remove the outer, thawed fish as the block defrosts. This helps to ensure thawing of the inner fish while protecting the outer fish from thawing for a prolonged period.

### **Handling Thawed Fish**

The thawed product should be kept iced or refrigerated until a reasonable time before feeding (9 CFR 3.105). The term "reasonable" can be interpreted many ways. It is best to document the temperature of the fish frequently before feeding. Frozen foods, once thawed, should never be refrozen. If not fed, fish must be discarded 24 hours after removal from freezer or, if thawed under refrigeration, 24 hours after being thawed.

The objective of handling or preparing the thawed fish before feeding is to inspect its quality once again and possibly to process the fish in order to facilitate consumption and training. Processing of thawed fish may consist of removing fins with potentially harmful spines and cutting whole fish into chunks for use in animal training or because whole fish are too large for an animal to consume. Again, the goal is to perform these processes while minimizing bacterial contamination and assuring wholesomeness and nutritive value.

Even with exact care in handling, most uncooked foods harbor some microorganisms (Frazier and Westhoff 1988; see appendix B). The growth of these organisms can be prevented or retarded through proper temperature control, cleaning, and sanitation. Utensils and processing surfaces to be used must be cleaned and sanitized prior to fish processing (see "Cleaning and Sanitation" below).

Fish must be processed immediately upon removal from the thawing stage and as close as possible to the feeding time. Minimize the time the fish spend at room temperature. Please note that fish can be processed while still frozen just prior to thawing (Stoskopf 1986).

There usually is a span of time between processing and feeding. Care must be taken to minimize this time while continuing to store fish under cool conditions. Feeding frozen fish is undesirable because they may not be palatable, they are rigid in physical form, and the availability of nutrients to the animals may be decreased.

The term "cool conditions" refers to the final temperature of the fish being fed. Fish should be fed cold but not frozen. Environmental conditions affect the final temperature of fish, as the following examples illustrate.

Example A. If the animals are fed outside in hot, humid, sunny weather, it is important to keep the fish iced or in a poolside cooler to avoid microbial buildup, nutrient loss, or contact by disease-spreading pests. If held in iced conditions, care should be taken to avoid standing water. For example, place fish in a plastic bag before immersing in ice (or melting ice) or place the fish in a covered insulated container that has a spigot or drain to allow water to run off.

Example B. If the animals are fed inside under relatively unchanging conditions and room temperature is about 18 °C (65 °F), it may be possible to feed fish directly from cooled containers with no water or ice. This is possible if the fish are fed in a timely manner and the process has been validated to ensure that the fish maintain refrigerated temperatures

## EAAM Standards and Guidelines

when feeding begins and ends. However, to ensure that fish remain cool throughout the feeding period, hold them in iced or cold water.

Example C. If the animals are fed outside in cool or cold conditions, no extra cooling precautions are needed. A covered container will keep the fish cool and free of contamination. Again, the objective is to ensure that the fish stay cool.

Adequacy of the procedure chosen for poolside feeding should be validated before it becomes a standard procedure (see "Validating Procedures" below). In addition, temperature of the fish at feeding time should be periodically documented in writing (see form 4). Once the procedure for feeding has been validated it should be written and added to this manual as a standard operating procedure.

## Feeding

Food must be wholesome, palatable, free from contamination, and of sufficient quantity and nutritive value to maintain the animals in good health (9 CFR 3.105). USDA also requires that marine mammals be fed at least once daily unless otherwise indicated by veterinary treatment or accepted practices.

USDA stipulates that food receptacles, if used, must be accessible to all marine animals in the same primary enclosure and placed to minimize contamination. All food and feeding receptacles must be cleaned and sanitized after each use.

When animals are fed individually, USDA requires an employee or attendant responsible for management to perform or directly oversee the feeding. The age, species, condition, and size of each animal should be considered when feeding. The employee must be able to recognize alterations from a normal state of health in order to adjust food intake. The quantity and type of food consumed by each animal should be documented (see form 5) and kept on record for at least 1 year.

Actual feeding by members of the public is allowable only if the food is provided by the holding institution and held under proper conditions and feeding is supervised by an adequate number of qualified employees or attendants. Again, the quantity and type of food consumed by each animal should be estimated, documented (see form 5), and kept on record for at least 1 year.

Diet of marine animals often includes vitamin or mineral supplements to make up for nutrient losses during storage and thawing of the food. Table 1 illustrates some of the factors affecting loss of selected nutrients. It can be inferred that any nutrient affected by leaching will be affected by thaw water in addition to thawing losses in juices originating from the fish themselves. Vitamin E is destroyed during fat breakdown (oxidation). The extent to which this occurs depends on the fat content of the fish. Enzymes (thiaminases) naturally present in fish tissue may destroy thiamin during storage. Addition of supplements is beyond the scope of this document, but use and consumption of supplements should be documented in order to track nutritional status.

## Cleaning and Sanitation

Equipment, including all utensils, cutting boards, food containers, and tables, can harbor pathogens and should be properly cleaned and sanitized (Stoskopf 1986; see appendix B). USDA specifies that "containers such as buckets, tubs, and tanks, as well as utensils, such as knives and cutting boards or any other equipment that have been used for holding, thawing or preparing food for

### EAAM Standards and Guidelines

marine animals must be cleaned and sanitized after each feeding, if the marine mammals are fed once a day, and at least daily if the marine mammals are fed more than once a day" (9 CFR 3.105). Fish prepared with utensils, stored in containers, or prepared on surfaces that have not been cleaned and sanitized may be contaminated by this unclean equipment, rendering the fish unfit for consumption.

USDA further requires that kitchens and other food-handling areas where animal food is prepared must be cleaned at least once daily and sanitized at least once weekly. This includes surfaces within the preparation area not directly in contact with fish such as floors, table tops, freezer doors and handles, and refrigerator doors and handles.

Sanitizing by washing with hot water of 82 °C (180 °F) or higher and soap or detergent in a mechanical dishwasher or by washing all soiled surfaces with a detergent solution followed by a safe and effective disinfectant is required by USDA. Manual sanitation can be accomplished by one of the following methods using a final sanitizing rinse (IDPH 1993, CDHS 1994; also 9 CFR 3.105):

- Contact with a solution of 100 parts per million (ppm) available chlorine for 20 seconds or 50 ppm for at least a minute.
- Contact with a solution of 25 ppm available iodine for 1 minute.



## EAAM Standards and Guidelines

**Table 1. Stability and factors affecting loss of selected nutrients**

<b>Vitamin</b>	<b>Stability</b>	<b>Sensivities</b>	<b>Factors Affecting Loss</b>
C (ascorbic acid)	very unstable	oxygen, heat, alkaline pH, water	leaching into water, especially from cut surfaces
B1 (thiamin)	very unstable	heat, alkaline pH, water	leaching, exposure to light
B2 (riboflavin)	somewhat unstable	alkaline pH, water	leaching, exposure to light
Niacin	stable	water	leaching
Pantothenic acid	somewhat unstable	heat, alkaline pH, acidic pH, water	leaching, heat destruction
B6 (pyridoxine)	somewhat unstable	water	leaching
Folic acid	somewhat unstable	heat, alkaline pH, acidic pH, oxygen	heat destruction
B12	somewhat unstable	heat, alkaline pH, oxygen	leaching
Biotin		oxygen	
A	somewhat unstable	heat, oxygen, light	Exposure to light
E	somewhat unstable	oxygen, light	oxidation
K	stable	oxygen, lighth	exposure to lighth, oxidation

Source: Kutsk 1981.

## EAAM Standards and Guidelines

- Contact with a solution of 200 ppm quaternary ammonium for 1 minute.
- Contact with water of at least 77 to 82 °C (170 to 180 °F).
- Use of a dishwashing machine with approved sanitizing methods (chemical or hot water).
- Washing all surfaces with a detergent solution followed by a safe and effective disinfectant.

Only those poisonous or toxic materials necessary for cleaning and sanitizing equipment, utensils, and the kitchen area, or for controlling insects and rodents, may be present in a food preparation area (IDPH 1993). CDHS (1994) states that "insecticide, rodenticide or other poisonous substances" should not be stored in any food preparation area, "except in a separate enclosure provided for that purpose." IDPH states that to prevent possible contamination, such substances not be stored above or with any food, food equipment, or preparation utensils. Substances such as cleansing and sanitizing agents, pesticides, and other potentially toxic agents must be stored in properly labeled containers away from food preparation surface areas (IDPH 1993; 9 CFR 3.107). It is recommended that any potentially hazardous materials be stored in a separate room, away from any fish preparation or storage area. This greatly limits potential contamination conditions.

Provisions need to be made for the removal and disposal of food wastes, trash, and debris. Disposal facilities must be provided and operated in a manner that minimizes vermin infestation, odors, and disease hazards (IDPH 1993). IDPH and CDHS (1994) cite that garbage and refuse in the food preparation area should be in a container that is rodent- and insect-proof, as well as leak-proof.

### **Validating Procedures**

In order to ensure that conditions are appropriate and therefore that the methods for storage and handling fish are proper, validation of the conditions and procedures is needed. Validation must occur (1) before procedures become policy and practice, (2) at periodic intervals to ensure compliance, and (3) when the procedure is changed.

The nutritional quality and wholesomeness of fish can be determined by periodic scheduled sampling of the fish before they are fed (which is the time of primary interest) or at any stage in the handling process. Fish can be examined for nutrient content and microbial load. These tests indicate the effectiveness of the handling procedures. Sampling methods for analysis are covered in the following section.

Validating the procedures employed in all of the fish-handling processes is a determining factor in setting or modifying any procedure. Documentation can serve not only as validation that fish are held under specified conditions, but also as a record to highlight potential problems.

Validating fish-handling procedures can be done by documenting temperatures of the fish and storage compartments at key points in the process. One method of process validation is to take samples of fish at key points (receipt, storage, thawing, preparation, and before feeding) to determine the temperature of the fish themselves (see forms 2 and 4). Documentation of freezer and refrigeration temperatures should be included (see form 3). As stated previously, it is best to document cold-storage conditions at several locations within the storage area to ensure uniform temperatures.

## EAAM Standards and Guidelines

Another method of validation uses a maximum/minimum thermometer placed in a container of fish. The thermometer travels with the fish from frozen state to feeding (see form 6). A maximum/minimum thermometer should be placed in a sealed plastic bag (in case of breakage) before being placed in the container of fish. The thermometer is exposed to the same conditions as the fish. A thermometer shows temperatures in the immediate vicinity of the device, so be sure to place it somewhere other than the outside section of the container. For example, if thawing fish under running water, the thermometer registers the temperature of the water, not the thawing fish. However, the thermometer will show temperatures to which the fish have been exposed. Freezer and refrigerator temperatures should be documented periodically.

Validation requires careful documentation. Everything must be recorded in pen. Forms in this manual may be used or modified for documentation. Initially, validation of a procedure should be performed more than once. Procedures used for validation, a schedule for validation and temperature monitoring, and documentation of such should be maintained and continually updated. Data can be transcribed to a computer spreadsheet and graphed if needed.

### **Sampling for Microbial and Nutrient Content**

Fish can be sampled for microbial buildup (total plate count) of any number of specific organisms as well as some nutrients (Crissey et al. 1987; see appendix B). Red blood cells can be examined by microscope for rupture to determine whether the fish have been frozen, thawed, and refrozen (Stoskopf 1986). Although not all facilities have the instrumentation or money to perform these tests, all should strive to make them a part of the regular procedures. The information can become very important when investigating animal or facility problems.

Sampling for microbial buildup can be performed at various stages of fish thawing and handling. If microbes increase substantially (above the counts recognized as safe for humans), the storage and handling procedures must be reviewed and changed. This is important because certain microbes can cause illness. Also, as microbes increase, nutrients may decrease (appendix B).

Fish should be sampled for protein, fat, and energy content in order to provide a nutritious diet (Stoskopf 1986). Fish can be analyzed for the content of some vitamins and minerals. In addition to using the data to balance the diet, an analysis can further quantify the quality of the fish (Stoskopf 1986). If possible, the basic analyses (protein, fat, and energy) should be performed with every lot of fish. Sampling periodically during long-term storage to determine nutrient losses is also advisable.

Fish should be sampled for common toxins or heavy metal contamination to monitor the food source or determine if there may be a problem. The catch must be immediately tested if any problem is suspected such as a report of contamination that links the fish to environmental or other potentially compromising events. If feasible, testing should be performed with every lot of fish or once per year per type of fish offered. Once per year is a minimum, although that may not be enough to detect potential hazards.

A written protocol for sampling, including procedures for sampling, a sampling schedule, and procedures for reporting results (who is notified about results) should be established. Results of analyses should be recorded and maintained. Protocols should be reviewed yearly and continually updated.

## EAAM Standards and Guidelines

### Form 1

#### *Checklist for Inspecting a Fish Shipment*

- |  |     |    |
|--|-----|----|
| 1. Are the documents in order?   | YES | NO |
| A. Type and size of fish   |     |    |
| B. Size of entire shipment: number of boxes/containers                               |     |    |
| C. Quantity: total quantity by weight of shipment                                    |     |    |
| D. Freezing method: block - IQF - shatter pack                                       |     |    |
| E. Pricing   |     |    |
| 2. Is the packaging size correct?  | YES | NO |
| 3. If required, are the boxes dated?   | YES | NO |
| 4. If required, is there a history of the catch included?                            | YES | NO |
| 5. Are there any nonfood items in the shipping vehicle?                              | YES | NO |
| 6. Does the temperature gauge of the vehicle indicate frozen conditions inside?      | YES | NO |
| 7. Do the contents appear frozen?  | YES | NO |
| 8. Is there any evidence of thawing (and refreezing)?                                | YES | NO |
| A. Are there areas of ice under the boxes?   | YES | NO |
| B. Are any of the boxes stained or distorted?  | YES | NO |
| 9. Examine three boxes (make appropriate comments).                                  |     |    |
| A. Quality of fish. (Fish need to be thawed for a thorough inspection.) (See form 2) |     |    |
| B. Size of fish.   |     |    |
| C. Method of freezing.   |     |    |

The above list may be copied and laminated for use when inspecting a shipment or can be filled out and filed for documentation.

## EAAM Standards and Guidelines

### Form 2

#### *Quality Control Standards*

Quality control factors are used to determine fish quality during inspection and preparation. Although there is no ultimate test to determine the quality of fish, below is a compilation of descriptions of acceptable, inferior, and unacceptable fish (Frazier and Westhoff 1988, Oftedal and Boness 1983, Stoskopf 1986).

<b>Factor</b>	<b>Acceptable</b>	<b>Inferior</b>	<b>Unacceptable</b>
General Appearance	shine or luster to skin; no breaks in skin; no bloating or protrusion of viscera; no dehydration	some loss of sheen	luster gone, lumpy
Eyes	translucent, full; may be slightly sunken	dull or cloudy, slightly sunken	dull, sunken; cornea opaque (white); red-bordered eyes
Gills	bright red to pink; moist	pink to slight brownish	grayish-yellow and covered with mucus
Odor	fresh odor	mild sour or "fishy" odor	medium to strong odor, 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 color	slight protrusion	noticeable discoloration
Lateral line	normal, no discoloration	pinkish tinge	red to dark red











## **Appendix A**

### **Properties of Freezing**

Oftedal and Boness (1983) report that poor quality of fish may be caused by delays in freezing, slow rates of freezing, and inadequate freezer temperatures. Physical, chemical, and biological changes occurring during freezing are complex and not fully understood (Desrosier 1978).

The freezing point of a substance is "that temperature at which the liquid is in equilibrium with the solid" (Desrosier 1978). Many foods, including fish, have a high water content and freeze between temperatures of 0 and 3 °C (32 and 37 °F); fish freeze, on average, at about 2 °C (36 °F) (Desrosier 1978).

There are several methods of freezing, including cold air blasts, direct immersion in a cooling medium, contact with refrigerated plates in a freezing chamber, and freezing in liquid air, nitrogen, or carbon dioxide (Desrosier 1978).

Changes in flavor and color and losses in nutrients and texture occur fairly rapidly at temperatures above 9 °C (48 °F) (Desrosier 1978). Because of the physical nature of fish, the method of freezing affects quality and nutrient loss upon thawing (Desrosier 1978). Fish frozen rapidly to 0 °C (32 °F) have less "drip" (nutrient loss due to water loss from cells) when thawed (Desrosier 1978). Length of time for fish to freeze depends on temperature of freezing chamber, temperature of food upon entering the freezing chamber, and type, shape, and size of packaging (Desrosier 1978). Freezer burn by dehydration can be reduced by the method of packaging. Unprotected items are subject to a constant moisture loss as water is removed by circulating air. Damage caused by freezer burn is irreversible and causes changes in color, texture, flavor, and nutritive value (Desrosier 1978).

Freezing kills some microbes, but others not killed will grow upon thawing (Frazier and Westhoff 1988). Although most microorganisms do not grow well at temperatures below 0 °C (32 °F), some yeasts and molds can grow in nonfrozen foods with temperatures as low as 9 °C (48 °F) (Desrosier 1978). Growth of microorganisms can be greatly influenced by the temperature at which the food is thawed (Desrosier 1978).

Some nutrients can be affected by freezing. Although there is little change in the nutritive value of proteins, they can be denatured by freezing, altering appearance and quality. Proteolysis can occur while animal tissue is frozen if the enzymes are not inactivated (Desrosier 1978). Freezing only slows enzyme activity, which is usually optimum at higher temperatures (Desrosier 1978).

Fish naturally contain considerable quantities of long-chained unsaturated fats and oils. These fats are particularly susceptible to hydrolysis and oxidation (or rancidity). Higher fat fish deteriorate more quickly than lower fat fish (Frazier and Westhoff 1988). At a temperature of about 2 °C (36 °F) there is a reduction in rancidity of fatty tissue (Desrosier 1978). Fish with rancid fats have lower nutritive value, and antioxidants like vitamin E are utilized during breakdown (Oftedal and Boness 1983). Activity of enzymes such as thiaminase destroys thiamin in fish (Oftedal and Boness 1983). Also, processing of foods, including the exposure of tissue to air and heat, allows oxidation and destruction of vitamins (Desrosier 1978).

## EAAM Standards and Guidelines

Parasites may be destroyed by freezing temperatures (Desrosier 1978). Molds and yeasts may grow at freezing or slightly below freezing temperature (Frazier and Westhoff 1988). Some bacteria that grow on fish (such as *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Alcaligenes*, and *Flavobacterium*

species) can survive freezing temperatures and will resume growth when thawed (Frazier and Westhoff 1988). At temperatures of 3 °C (37 °F) or above, spores of *Clostridium botulinum* can survive freezing and may grow and produce toxins (Frazier and Westhoff 1988).

## **Appendix B** **Infections and Toxins of Foods**

Stewart states that food infections and intoxications, often referred to as food poisonings, have largely gone undetected in zoo animals because investigations have been limited. Such infections can be caused by natural toxins found on the fish, improper handling of foods, and exposure to microbes (Stoskopf 1986, Stewart 1987).

Fish are categorized as a perishable food item and must be handled carefully to prevent spoilage (Frazier and Westhoff 1988). IDPH (1993) includes fish in "potentially hazardous food," which is defined as "any food that consists in whole or in part of ... fish, shellfish, edible crustacea ... in a form capable of supporting rapid and progressive growth of infectious or toxigenic microorganisms."

There are a variety of causes for spoilage of foods, including one or more of the following (Frazier and Westhoff 1988):

- growth and activity of microorganisms (or occasionally higher forms) present; often a succession of organisms are involved
- insects or parasites
- action of the enzymes naturally found in fish
- purely chemical reactions, that is, those not catalyzed by enzymes of the tissues or microorganisms
- physical changes, such as those caused by freezing, burning, drying, and pressure.

The type and numbers of microorganisms present on fish in the storage area, in the preparation area, on the utensils, or transferred by the handler determine the type and extent of spoilage (Frazier and Westhoff 1988). Several types of bacteria can be found in or transferred by human carriers, including *Salmonella* sp., *Staphylococcus aureus*, *Clostridium perfringens*, *Campylobacter jejuni*, *Clostridium botulinum*, *Listeria monocytogenes*, *Escherichia coli*, and *Yersinia enterocolitica* (Rehe 1990). Competition occurs among bacteria, yeasts, fungi, and molds—one organism outgrowing another due to the environmental conditions. Not all microorganisms are antagonistic. Some may be symbiotic or synergistic. Microorganism can also be metabiotic, with one organism making conditions favorable for growth of the second (Frazier and Westhoff 1988).

Stewart (1987) describes the difference between food infections and intoxications. Infections are caused by the ingestion of the organism. In healthy adult humans, infections usually are not fatal, although they can be so in weakened individuals. Food intoxications are caused by the ingestion of toxins produced by the bacteria, molds, plants, or insects. Intoxications occur less frequently but reactions may be more severe and result in severe gastroenteritis, paralysis, and possibly death.

### EAAM Standards and Guidelines

Exposure to insects and rodents may increase the microorganism load or introduce new microorganisms to the fish. Insects and other pests also can carry microorganisms to utensils, buckets, tables, and so forth, which then can contaminate the fish. Environmental conditions govern which fungus, yeast, and bacteria flourish (Frazier and Westhoff 1988).

All foods, and possibly utensils, should be kept covered in containers that are rodent- and insect-proof to prevent contamination (Stewart 1987). Equally as important is good hygiene by the staff preparing the fish (Pond 1987, Stewart 1987).

Chemical properties of the fish may affect spoilage (Frazier and Westhoff 1988). Properties of food that influence spoilage include pH (hydrogen-ion concentration), nutrient content, moisture availability, oxidation potential, and presence of inhibitory substances.

The physical state of the food—frozen, heated, moistened, or dried—can influence whether a food spoils and the type of spoilage. Organisms need water to grow. Salt dissolved in water draws water from the cells, and freezing may damage tissue, causing a release of juices when thawed (Frazier and Westhoff 1988). The emulsions of fat and water caused by the breakdown of tissue and denatured protein are more readily available for organisms.

Prevention of foodborne illness begins on the boat with proper handling at the time the fish are caught and processed (Stoskopf 1986). Zoos and aquaria can help prevent food-related illnesses with proper handling when storing and processing fish. Fish should be kept frozen until 24 hours prior to preparation and use. Proper procedures and validation processes help to ensure that contamination does not occur and that growth of those contaminants is kept to a minimum.

## EAAM Standards and Guidelines

### References

California Department of Health Services [CDHS]. 1994. Health and Safety Code, chapter 4, article 7, sections 27601, 27606, and 27613. In California Uniform Retail Food Facilities Law, rev. 1/1/94. Sacramento.

Crissey, S.D., M.E. Allen, and D.J. Baer. 1987. Food handling and commissary procedures. In T.P. Meehan and M.E. Allen, eds., Proceedings of the 6th and 7th Dr. Scholl Conferences on the Nutrition of Captive Wild Animals, Chicago, December 1987, pp. 119-123. Lincoln Park Zoo, Chicago.

Desrosier, N.W. 1978. The technology of food preservation, 4th ed. AVI Publishing Company, Westport, CT.

Frazier, W.C., and D.C. Westhoff. 1988. Food microbiology, 4th ed. McGraw-Hill, New York.

Geraci, J.R. 1978. Nutrition and nutritional disorders. In M.E. Fowler., ed., Zoo and Wild Animal Medicine, pp. 568. W.B. Saunders, Philadelphia.

Illinois Department of Public Health [IDPH]. 1993. Food Service Sanitation Code, including subpart B, section 750.140, 750.240; subpart E, section 750.820; subpart G, section 750.1290, 750.1310. Springfield.

Kutsky, R.J. 1981. Handbook of vitamins, minerals and hormones. Van Nostrand Reinhold, New York.

Oftedal, O.T., and D.J. Boness. 1983. Considerations in the use of fish as food. In T.P. Meehan and M.E. Allen, eds., Proceedings of the Third Annual Dr. Scholl Nutrition Conference, Chicago, December 1983, pp. 149-161. Lincoln Park Zoo, Chicago.

Pond, J. 1987. The results of microbiological culture of a commercial frozen meat based animal food and whole frozen chicken thawed by various methods. In T.P. Meehan and M.E. Allen, eds., Proceedings of the Third Annual Dr. Scholl Nutrition Conference, Chicago, December 1983, pp. 69-72. Lincoln Park Zoo, Chicago.

Rehe, S. 1990. Preventing food borne illness, a guide to safe food handling. U.S. Department of Agriculture, Home and Garden Bulletin Number 247.

Shinaburger, A. 1978. Family living topics, spotlight on freezer. Purdue University Cooperative Extension Service, HE 422. West Lafayette, IN.

Shinaburger, A. 1992. Family living topics, spotlight on refrigerator storage. Purdue University Cooperative Extension Service, HE 424. West Lafayette, IN.

Stewart, L.J. 1987. Hazards associated with food preparation and storage. In T.P. Meehan and M.E. Allen, eds., Proceedings of the Third Annual Dr. Scholl Nutrition Conference, Chicago, December 1983, pp. 1-4. Lincoln Park Zoo, Chicago.

Stoskopf, M.K. 1986. Feeding piscivorous birds, a review. Annual Proceedings of the American Association of Zoo Veterinarians, pp. 69-87.

## EAAM Standards and Guidelines

U.S. Navy. 1965. Section IV: Inspection of subsistence items, article no. 1-37, Inspection of fish and shellfish; Section V: Storage and care of subsistence items, article no. 1-47, Fresh and frozen subsistence items; Section VI: Sanitary precautions to be observed when preparing and servicing food, article no. 1-56, Preparing and serving. In Manual of Naval Preventive Medicine. Naval Medical Department, Bethesda, MD.

## EAAM Standards and Guidelines

### **Appendix II: EAAM Fish quality parameters**

#### Fish quality parameters

These tests have to be done every time a new batch arrives. Records have to be kept and need to include: Supplier, batch number, arrival date, analysis date, capture date, date this batch is opened and offered to the animals and type of fish.

#### Microbiology:

- Mesophilic aerobes – max 1.000.000 cfu/gr
- Enterobacteria – max 1.000 cfu/gr
- Salmonella – absence/25gr

#### | Physico-chemical Analysis:

- Fat
  - Humidity
  - Proteins
  - Ashes
  - Carbohydrates
  - Kcal
  - Histamine – max 100 ppm (ideally at 50 ppm max)
  - Peroxides – max 20 mEq O<sub>2</sub>/Kg fat (ideally max at 10 mEq)
  - TBA = Thiobarbituric acid analysis - method to assess lipid oxidation in fish - 1,5-3,5 mg/Kg \*
  - ABVT/TVBA (total volatile basic amines) < 25 mg/100 (Reg. 2074/05)\*
  - Analysis of vitamin concentration is recommended, water-soluble and fat-soluble (B1, B6, B12, Vit E,A,D)
- (\*If peroxides and histamine are not done)