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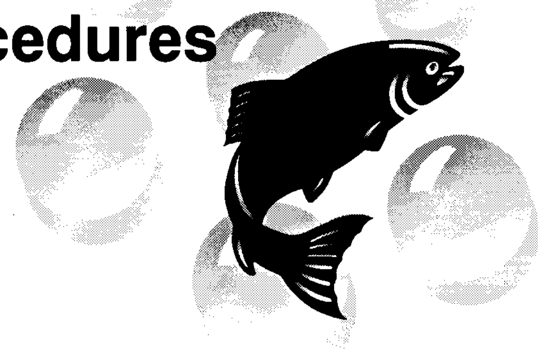
Handling Fish Fed to Fish-Eating Animals

A Manual of Standard Operating Procedures



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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.

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.

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

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Contents

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 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.

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 refreezing (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 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 microorganisms, proper storage temperatures are required (appendix B).

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.

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 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 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 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.

Table 1. Stability and factors affecting loss of selected nutrients

Vitamin	Stability	Sensitivities	Factors Affecting Loss
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, light	exposure to light, oxidation

Source: Kutsky 1981.

- 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.

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.

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.

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).

Factor	Acceptable	Inferior	Unacceptable
General Appearance	shine or luster to skin; no breaks in skin; no bloating or protrusion of viscera; no dehydration	some loss of sheen	luster 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

Form 3

Refrigerator/Freezer Temperature Chart

Record temperatures for each refrigerator and freezer for 3 locations. Record relative humidity.

Circle one: Refrigerator Freezer

Location:

Date	Initials	Temp #1	Temp #2	Temp #3	Relative humidity

Form 4

Feeding Container Temperatures

Record the temperatures once the food is placed in a container ("Start") and before removal ("End"), by placing a thermometer in the container of fish (not touching the sides).

Date	Initials	Container #	Start temperature	End temperature

Form 5

Fish Consumption Chart

Record consumption (in kilograms or pounds) for each animal if fed individually or for each enclosure if fed in groups. Every attempt should be made to feed by kilocalories (gross energy) and record consumption by kilocalories. Thus, quantities from this chart can be converted to kilocalories by using analyzed fish values.

Animal identification:

			Amount fed		
Date	**Time**	**Initial**	**Fish species**	**Fish species**	**Fish species**

Form 6

Temperature Log for Single Box of Fish

Place a minimum/maximum thermometer in a container of fish. Record temperatures at specified points throughout fish handling.

Date	Initials	Box number	Point in handling	High temp	Low temp

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).

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. Microorganisms 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.

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.

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