

Association of Zoos and Aquariums
Nutrition Advisory Group (NAG)



Handling Fish Fed to Fish-Eating Animals

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Abstract

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 for feeding to 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 valuable background and guidance for the handling of fish fed to fish-eating animals in managed care. All points of fish handling are discussed, including ordering, purchasing, and receipt, through storage, thawing, and feeding – including cleaning and sanitation – to validating procedures and sampling. Using these newly updated guidelines along with the appropriate documentation 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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Contents

Definitions	6
Introduction	7
Ordering and purchasing	7
Inspection.....	10
Storage.....	11
Transportation	12
Tempering and Thawing	12
Handling and Feeding Thawed Fish	15
Cleaning and Sanitation	21
Validating Procedures	21
Analyses of Fish Quality.....	22
References.....	25
Appendix A	27
Forms.....	28

Definitions

Thawing – the process of taking frozen product from frozen to a temperature (usually above 0 °C) where there is no residual ice (i.e. “defrosting”). Thawing is often considered as simply the reversal of the freezing process.

Tempering – the process of taking frozen food to a higher temperature, still below the initial freezing point, at which it is still firm but can readily be further processed. Partially thawed. Multiple stages of tempering can be described (see Thawing section).

Freezing – the process by which a substance changes from a liquid to a solid when the temperature is lowered below its freezing point; the solidification phase change of a liquid or the liquid content of a substance due to cooling.

IQF – individually quick frozen; the product is quickly frozen into individual units more easily facilitating individual unit use.

Block frozen – multiple/many units quickly frozen together as a block, maximizing uniformity of the block and minimizing the ability for individual unit use.

Must – imperative need, duty, necessity. For the sake of this document, a requirement either because of legality or long-established best practice.

Should – advice or desirable goal. For the sake of this document, a recommendation, but not a requirement.

Best practice – a procedure that has been shown, by research and experience, to produce optimal results and that is established or proposed as a standard suitable for widespread adoption.

Introduction

This document provides a needed update to the Manual of Standard Operating Procedures titled “Handling Fish Fed to Fish-Eating Animals” (Crissey 1998). The Association of Zoos and Aquariums (AZA) Nutrition Advisory Group (NAG) requested use of the original version of the document as an outline and incorporated guidelines and best practices that have been developed over the previous twenty years to provide this update. Although some of the language remains the same, revisions and additions exist throughout.

Word choice was examined closely as this document was drafted. There are requirements that are listed as “must” and “shall,” and there are best practices that are listed as “should” and “may.” These differences were purposefully chosen and designed to be meaningful in the interpretation of this document and are highlighted at the outset of each section. For the purposes of this document, “must” includes guidelines that the authors feel are critical for food safety/sanitation and appropriate animal welfare. “Should” (also can/may) is used to describe guidelines that are considered examples of best practices for appropriate food safety/sanitation and animal care, but not considered requirements.

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 content of fish varies considerably due to several factors: species differences, individual differences due to season of capture, age, and sex (Stoskopf 1986).

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 managed care 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.

When feeding marine mammals, the USDA marine mammal standards should be used where they differ from these standards.

Ordering and Purchasing

MUST – Identify the species and catch/harvest date and do not maintain fish in inventory older than 18 months from catch date. Fish must be packaged in containers that minimize air exposure (usually with plastic or wax wrappings) and marked with the date of catch on the package.

SHOULD – Ask questions to gain insight into the history of the fish you are purchasing and to learn more about fishery conditions

Choosing the appropriate fish for an institution is based on the needs of the collection, the quantities and types of fish available, the cost of the fish, delivery method and timeline, and the nutrient content of the fish, among other variables. Fish vendors do not always produce or catch the fish themselves. Often, commercial fish vendors work as brokers who purchase fish from fishers, fish farmers, and/or processors that produce or catch fish locally, regionally, and/or globally. These brokers coordinate the sale and transportation of the fish from the primary source to institution-based customers. Visiting production facilities is often not possible, so it is valuable for customers to develop a list of questions to assess the details of how the fish is caught and handled prior to arriving at the institution.

To determine the freshness and wholesomeness of fish, the history of the catch should be ascertained. Knowledge of the harvest/production/catch date allows determination of the previous storage time of the fish (how long was it stored before you purchased it). Some species of fish are harvested in a short period of time, once per year. Others are harvested continuously throughout the year. For fish harvested annually, it is important to know your annual need and, if storage space allows, purchase the amount you need as soon after harvest as possible, to ensure the freshest product and most cost-effective approach. In cases where storage capabilities limit the ability to manage a full year supply of annually harvested fish, some vendors will allow you to purchase the entirety of your needs while they agree to store the product for you, and ship as needed. In other cases, cold storage facilities near your location can be used to provide additional freezer space. In still other cases, you may have to purchase as space and finances allow, while trying to get the freshest and most cost-effective product available. The goal should be to purchase the freshest fish possible, to help ensure the fish is used before 12-18 months post-harvest.

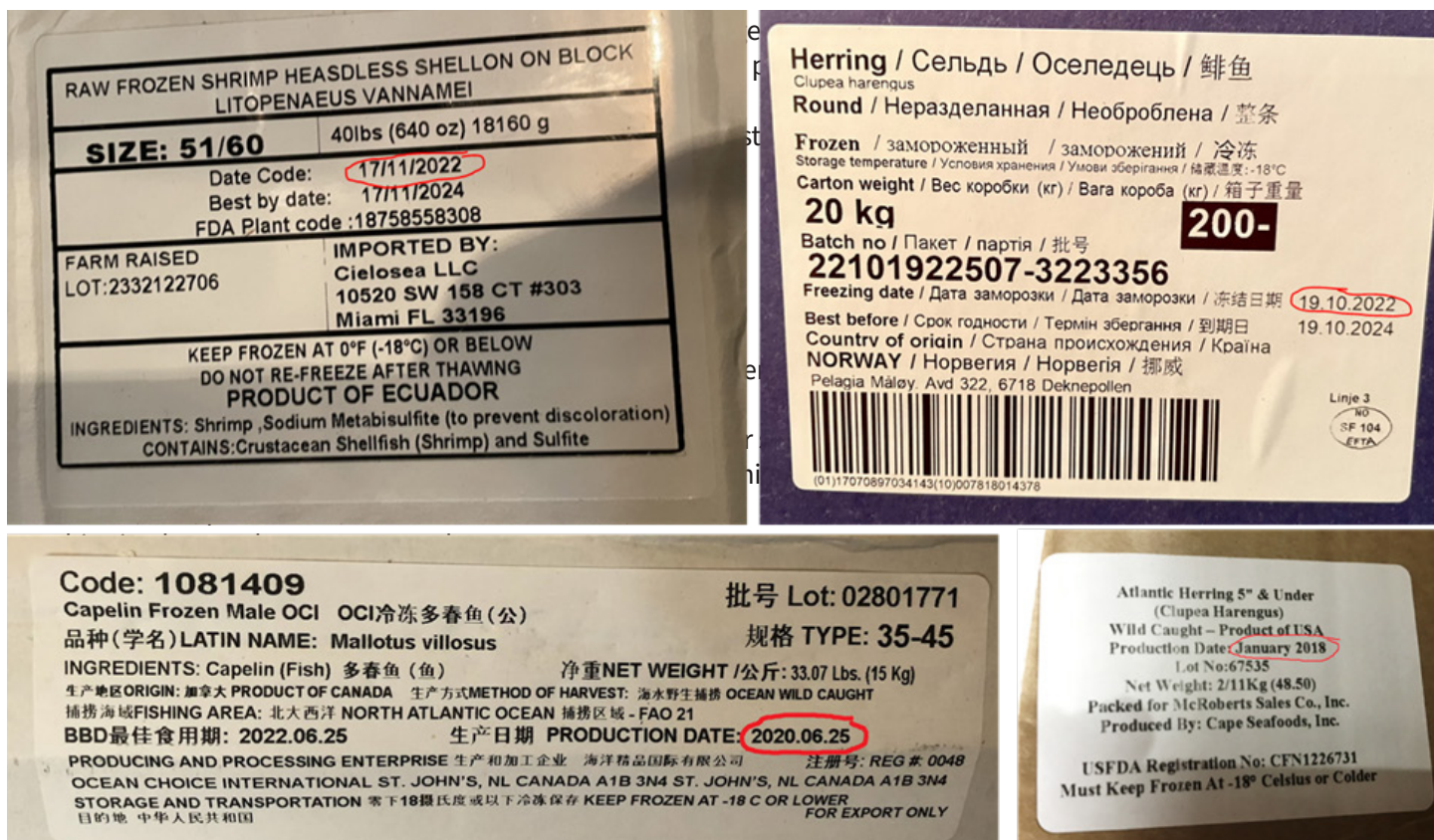


Figure 1. Examples of labels on cases of fish and their production dates. Note that North American producers may use different formats for dates than producers in other parts of the world (i.e. – day.month.year vs month.day.year).

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 fish-eating animals in managed care 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 containers that minimize air exposure (usually with plastic or wax wrappings) and marked with the date of catch on the package. Fish are most often either block frozen or individually quick frozen (IQF). A common commercial size for packages is 10-20 kg to allow for appropriate quick freezing and proper thawing. It is suggested that package size provide 1 day's supply without leftovers (Stoskopf 1986), but this is often difficult to achieve (i.e. – block frozen fish often are processed into smaller amounts by the user while still frozen in an early stage of tempering - see Thawing section). 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.

Size of the individual fish may be important to avoid the necessity of cutting. Cutting fish causes a greater nutrient loss, as well as increased time for preparation (Stoskopf 1986). Cutting fish also increases the risk of contamination by surfaces and equipment. If processed fish are purchased (e.g. fileted), the nutrient content is altered. For example, there may be a substantial decrease in the calcium content of the fish if the heads are removed.

Some questions that may allow an institution to evaluate the vendor, fish species, and fishery include, but aren't limited to, the following list. Keep in mind that an on-site evaluation of the vendor may not be possible or very revealing, and often the best approach is to have an honest discussion with the vendor to get the answers to the applicable questions:

- What are the species, catch/harvest date, catch/harvest location, and harvest method?
- How was the fish handled from live state through frozen storage and how long did it take to get from fresh to frozen?
- In what form is the fish frozen (block, shatter pack, IQF, etc.) and what is the case weight? How is the fish packaged?
- What is the anticipated delivery time?
- What is the identity of the actual catch/harvest company, and do they have a HACCP plan in place?
- What is the status of this fish species and the fishery where it is managed?
- Is this fish species included in the Seafood Watch (or similar) program, and what is its status?
- Do you have a readily available nutrient/energy profile specific to this lot of fish (not the generic, average value, but this specific catch date)?
- How is this fish transported from port of arrival to the institution?
- Do you have references of current customers you can share?

Inspection

MUST – Fish must be visually inspected prior to feeding by staff trained in proper inspection techniques and fish quality, and appropriate actions taken if needed.

SHOULD – Fish shipments should be visually inspected prior to accepting the shipment, ideally using a case from every lot or from different locations within the shipment when there are more than 2 pallets.

Inspection is a process that starts prior to ordering and ends upon feeding the fish to the animal.

Ideally, to ensure that fresh fish are handled appropriately throughout processing by the fisheries, the fisheries (processing plant or location) should be visited during all phases of processing. The fish can be inspected at that time for quality and freezing practices. Since this is impractical for most institutions, they should ask the pertinent questions of the vendor (see previous section) and concentrate on a thorough inspection when the product arrives at the storage facility.

Upon delivery – Often, the first opportunity to examine the fish is at the point of delivery. Since products should be inspected and stored immediately, schedule deliveries during business hours. An inspection should occur at the place of receipt (storage site) before unloading from the delivery vehicle so a representative number of boxes can be examined. Inspection must be performed by one of the institution'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 physical damage or of thawing and refreezing (Crissey et al. 1987). Every lot or shipment of fish must be inspected before the paperwork is signed to officially receive it from the supplier. Form 1 can assist with inspection of the fish shipment.

When inspecting a shipment, it is best practice to consider the following (U.S. Navy 1965):

- Check the supplier's documents to ensure that the fish shipment corresponds to the fish ordered – type, size, quantity, and 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 non-food 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 because 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 to ensure that the entire shipment is suitable. The number of packages to be inspected 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. If there are different lots of fish in the load, attempt to examine one box from each lot in the vehicle.
- Visually inspect the fish to make sure that the product is the correct species and size of fish, as well as the size and type of packaging that was ordered.
- Look for evidence of fish being thawed and refrozen (water or ice buildup on the boxes or floor beneath the boxes; wrappings that are moist, slimy, or discolored; fish with soft flesh, a sour odor, and an "off" color). If any of these indicators are present, do not accept the load.
- Examine individual fish in each selected box. Bodies should be whole (no tears in the skin), healthy looking, and have clear eyes. Inspect the fish on the bottom of the package; there should be no blood present.

If the fish have been found unsatisfactory for any reason, refuse the shipment, 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 vendor. Bad fish are unusable, unpalatable, and a health hazard; they can cause a significant economic loss due to illness or death of the animals.

If the quality is questionable, it is wise to thaw a few fish from several packages for a better determination (Form 2). Do this before officially accepting the shipment. Quick thawing of the fish for quality control purposes can be done in a bucket of warm water on site, if feasible. If the order is acceptable, a sample of fish should be taken for nutrient analyses at this time.

Storage

MUST – The frozen storage facility must be able to ensure and present evidence that the fish remains free of physical, chemical, and biological contaminants and safe by maintaining freezers in proper mechanical working order. Fish must be moved to the destination freezer as quickly as possible. Individual catch lots must be identifiable on each unit (box, case, etc.) by catch/harvest date. A log of freezer temperatures must be maintained, monitored, and recorded. These apply to on- and off-site storage facilities.

SHOULD – Fish should be stored such that a first-in, first-out (FIFO, by catch date) rotation can be employed. Regular monitoring of humidity, air flow, ice buildup, defrost cycles, etc., should occur and storage areas should be regularly inspected by a trained HVAC professional.

Freezer storage must be able to support food that is “wholesome, palatable, and free from contamination” (9 CFR § 3.105 (a)). As such, storage freezers must be in proper mechanical working order and be maintained in a way that minimizes risk of contamination by physical, chemical, or biological contaminants. While various nutrients may decline over time during frozen storage, there are no studies that define a timepoint at which well-stored frozen fish become harmful. In practice, fish frozen and stored under ideal conditions for up to 18 months have been routinely fed without apparent harm to animals. Following sound procedures to monitor storage conditions will help ensure contamination conditions are minimized and that the fish retain their nutritive value and wholesome quality.

Once a shipment has been accepted, all product should be moved to its destination freezer as quickly as possible and should be organized in a way that facilitates first-in, first-out (FIFO, by catch date) rotation. It should be clear when one harvest date has been exhausted and the next has begun circulating at the institution (understanding that there can be several catch dates represented within a single shipment or delivery). Documenting that information can be very helpful if food safety or quality is questioned.

Frozen storage temperatures of -22 to 0 °F (-30 to -18 °C) are recommended (Geraci 1978, Stoskopf 1986). Additional frozen storage details are listed in Appendix A. Refrigeration is used only for thawing of fish and short-term storage until fish are fed (9 CFR 3.105) and is discussed in the section of this document titled “Thawing.”

Storage freezers must be monitored at least once daily for proper temperature and findings should be documented. There are many ways to accomplish this but see Form 3 for an example temperature recording chart. An institution may also choose to schedule more than one temperature check per day or use automated temperature logging systems with alarms that alert when temperatures are out of a set range. Deviations from expected freezer temperature should be documented and potentially investigated, as they may indicate issues with mechanical function. In addition, cold air ducts should be checked regularly to make sure there are at least two feet of clearance between the air ducts and frozen inventory, as this can affect temperature control. Fluctuations in temperature can occur when freezer doors are opened and closed and as part of the regular defrost cycle of a unit. Those times should be considered when developing a schedule for recording temperatures. Thermometers and temperature sensors should be located centrally and not near a door that opens and closes frequently. At least annual inspection of freezer units by a professional is also recommended to maintain proper function.

Transportation within the Facility

MUST – During transport, fish must be maintained at the appropriate temperature for the desired state (frozen, tempered, or thawed).

SHOULD - Ideally, transportation procedures should be verified once per year using temperature/data loggers that are transported with the fish.

Fish that must be transported from a main storage freezer or receiving area to another freezer location within the institution should be done in a way that keeps the fish solidly frozen. If fish is transported in a tempered or thawed state, it should be done so in a manner which maintains appropriate and safe fish temperatures (see Tempering and Thawing Section). Transportation procedures should be verified at least once a year using a temperature logger (or similar device) that travels within the fish and records temperatures.

If fish arrives at its location thawed, it should not be refrozen. Recently thawed product should be used within 24 hours (9 CFR 3.105(d)).

Tempering and Thawing

MUST – Fish must be thawed under refrigeration except in emergent situations. If not fed, fish must be discarded within 48 h* of reaching the thawed stage (Stage 4, Table 1) or from removal from the freezer if thawed by methods other than refrigeration, unless it suffers from time/temperature abuse. (*Note that USDA regulations for marine mammals may differ)

For the purposes of this section “thawing” refers to the overall process from removal from freezer to a temperature where there is no residual ice. “Tempering” or partial thawing refers to the intermediate stages where fish is no longer frozen solid, but still contains residual ice (Table 1). Figure 3 provides a visual representation of thawing stages as a convenient tool for posting in food-handling locations (see also Figure 5 at the end of the document for a printable reference).

Table 1. Descriptions of tempering/thawing stages.

Thaw/temper stage	Description
1	Frozen, rigid, icy (< 0°F, -18°C)
2	Can bend under pressure; feels icy-cold to touch, some crystal ice present (20° - 28 °F, -6° - -2°C)
3	No crystal ice; keeps shape, firm cold (28° - 34 °F, -2°-4°C)
4	Refrigerator temp; no rigidity, no residual ice (34° - 40°F, 0°-4°C)

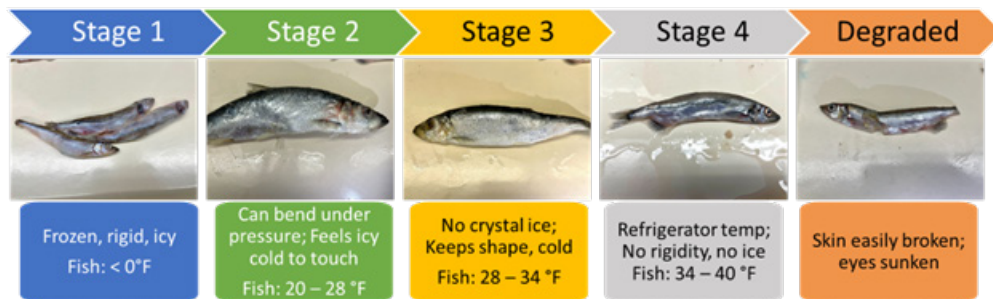


Figure 3. Visual representation of thawing stages of fish. (Figure 5 is provided as a stand-alone printable image on the last page of the document.). Degradation can occur at any point from catch through packing, shipping, tempering, thawing, and handling.

Thawing and Tempering Processes: The tempering and thawing process is critical to the product's final quality. Therefore, it must be carefully controlled. 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).

Incorrect thawing increases the potential for nutrient loss, lipid peroxidation (rancidity), microbial buildup and loss of palatability. The safest and most preferable way to thaw fish is in a refrigerated space (FDA 2019; USDA 2013). Freezing tends to break down tissues, making the food much more susceptible to bacterial invasion after thawing.

Perishable foods should never be thawed on the counter or in hot water and must not be left at room temperature for more than two hours. The USDA (2013) outlined three methods for tempering and thawing frozen seafood: in the refrigerator, in running cold water, and in the microwave.

Refrigerator Thawing: Frozen seafood should be tempered and thawed gradually under refrigeration or in such a manner that the external temperature of the fish does not exceed 40°F (4°C; National Restaurant Association Education Foundation 2006, Pond 1987). Thawing time depends on several factors including the quantity, the method of packaging, and the placement/location in the cooler. Ideally, during refrigerator thawing, fish should be kept in wrapping or in a container which provides insulation, prevents air exposure, and allows the fish to thaw uniformly. The container may include the original shipping box or a covered plastic or metal container. Do not thaw fish above other food items to avoid cross-contamination.

The use of fans to increase airflow in the cooler is supported, but only if fish are covered and protected from direct wind. High speed air flow directly onto fish causes loss of fluid through dehydration and oxidation.

Cold Water Thawing: Although generally not recommended as a standard practice, if seafood must be thawed quickly, it can be thawed under cold running water. The fish should be sealed in a plastic bag and immersed in cold running water. Bacteria from the air or surrounding environment could be introduced into the food if not sealed appropriately. Once thawed (Stage 4, Table 1), fish should be fed to animals within 24 hours (9CFR3.105).

Microwave Thawing: Although generally not recommended as a standard practice, food may be thawed in the microwave on the defrost setting, using caution not to partially cook the item. Fish should be removed when it is at Stage 2 (Table 1). After thawing in the microwave, fish should be fed to animals within 24 hours (9 CFR3.105).

Rinsing: Fish may be quickly rinsed by agitating in cold running water to remove thawing juices and other debris, and to minimize animal contact with dangerous bacteria from the slime coat such as *Erysipelothrix rhusiopathiae* (Palmer and Jones 2005), which have been implicated in the deaths and severe illnesses of Atlantic and Pacific bottlenose dolphins (Kinsel et al 1997; Díaz-Delgado et al 2015), and beluga whales (Choczynski 2007), and which are pathogenic to humans (Brooke and Riley 1999), pinnipeds, birds, and humans (Higgins 2000). The rinsing process should be completed quickly to avoid nutrient losses from soaking the fish.

Refreezing: It is sometimes necessary to temper large blocks of fish in order to repackage and refreeze them in smaller quantities. Purchasing IQF (individually quick-frozen) fish or purchasing smaller package sizes may avoid the need for this. Fish should be tempered as minimally as possible before refreezing. Fish that have reached Stage 4 (completely thawed, refrigerator temperatures) should not be refrozen. Repackaged portions should be labeled with the item name, original catch date, and re-freeze date (or updated expiration date) on either the package itself or on an enclosing container. Repackaged, refrozen portions will have a reduced shelf life. When refrozen fish is later thawed, it should be used immediately and never refrozen a second time.

Handling, Preparing, and Feeding Thawed Fish

MUST – Fish must be processed (weighed, cut) immediately upon removal from the thawing stage and as close as possible to feeding time. Time spent at room temperature must be minimized. All fish handlers must wash their hands both before and after handling fish. Food must be wholesome, palatable, free from contamination, and of sufficient quality and nutritive value.

SHOULD - Fish should be fed cold but not frozen. Feeding procedures should be validated. Fish should be inspected again for quality prior to feeding. Diets of animals fed frozen fish should include supplementation to make up for nutrient losses during frozen storage and thawing. Animal feeding should be overseen by an employee managing the species in question. Employees should be trained to notice changes and issues when feeding.

Of note: Public feeding of fish is allowable if the food is provided by the holding institution, held under proper conditions, and feeding is supervised by a qualified employee.

Quality Control

When thawed, fish have 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 (finger impressions are made easily and remain) (U.S. Navy 1965). Figure 2 provides example images of acceptable and non-acceptable fish.

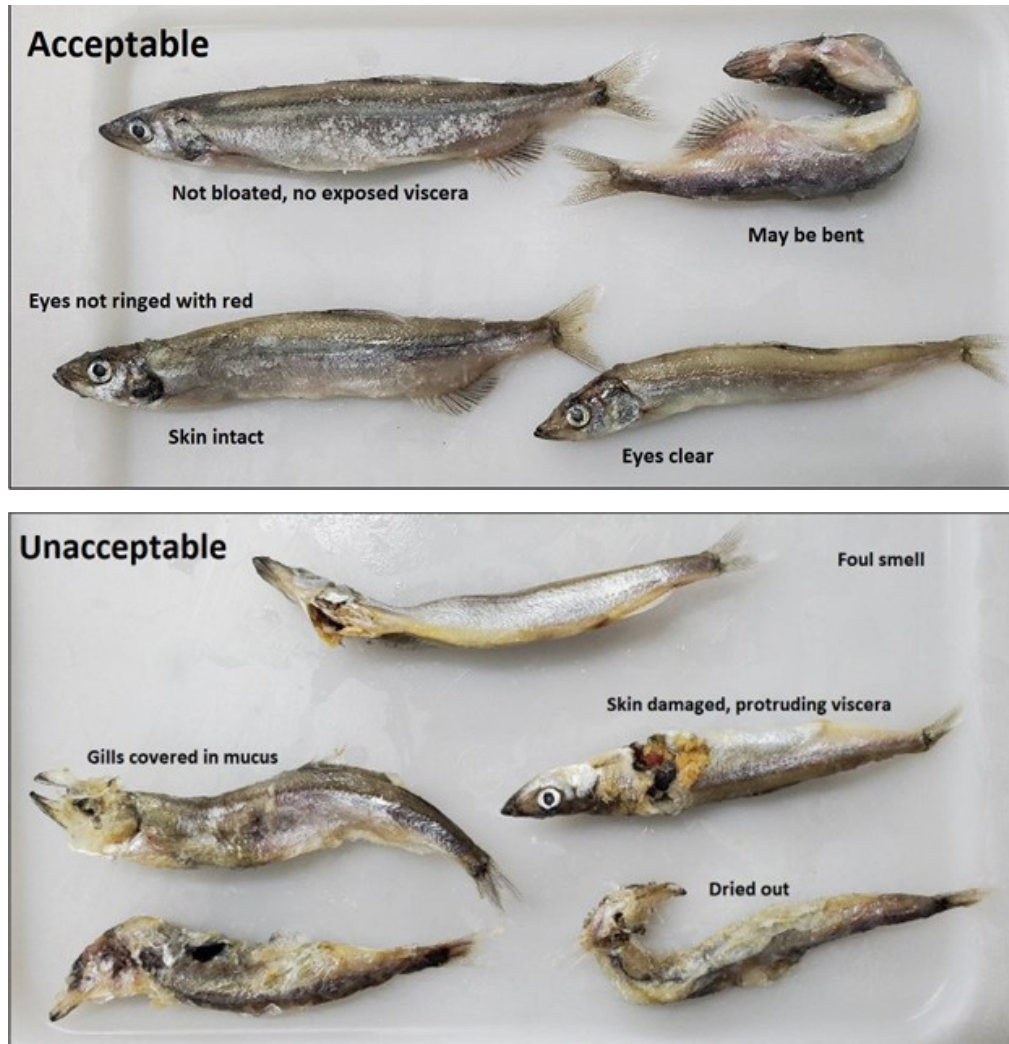


Figure 2. Example images of acceptable (top) and unacceptable (bottom) fish (capelin). Photo courtesy of Ardente, A. and C. Mergogey.

If fish is found unsatisfactory at any step prior to feeding, it should be discarded.

Handling and processing thawed fish (Stage 4)

USDA defines the temperature danger zone as 40°F - 140°F (4°C - 60°C): “Bacteria grow most rapidly in the range of temperatures between 40°F and 140°F, doubling in number in as little as 20 minutes. This range of temperatures is often called the “Danger Zone” (www.foodsafety.gov).

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 management goals.

Processing of thawed fish may consist of weighing fish, removing fins with potentially harmful spines, and cutting whole fish into appropriate sizes for an animal to consume. The goal is to perform these processes while minimizing bacterial contamination, maintaining temperature guidelines (as specified above), minimizing time spent in the “Danger Zone” and assuring wholesomeness and nutritive value.

Sanitation

Even with exact care in handling, most uncooked foods harbor some microorganisms (Frazier and Westhoff 1988). The growth of these organisms can be prevented or slowed 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). Care should be taken to minimize surface contamination while being reasonable about the risks specific to the animals that will be consuming it.

Reasonable precautions should be taken when handling raw fish. Gloves serve to protect the wearer from zoonotic diseases and bacteria that can exist in fish. If the gloves are changed regularly, they can help minimize surface contamination of the fish, thereby protecting the animals as well. Regardless of the use of gloves, all fish handlers must wash their hands both before and after handling fish according to CDC handwashing guidelines.

Time and Temperature Control

There usually is a span of time between processing and feeding when fish are held outside of refrigeration, i.e. above 40°F. Care must be taken to minimize this time while continuing to store fish as close to refrigeration conditions (i.e. not in the “Danger Zone”) as possible. Fish should be fed at stages 3 or 4 (see Table 1) due to the palatability concerns and rigid physical form. If Stage 4 fish is held in the “danger zone” (40°F - 140°F) for more than 2 hours, it should not be re-refrigerated and should be used within 2 hours or discarded (see Figure 4; Australia New Zealand Food Standards Code 3.2.2).

Total time Stage 4 fish can be held between 40°F - 140°F (4°C - 60°C)



Under 2 hours

Fish can be used or go back in the cooler for later use (< 40°F)



2-4 hours

Fish can be used immediately but should not go back in the cooler for later use (< 40°F)



Over 4 hours

Fish should be thrown away

Figure 4. Guidelines for time that Stage 4 fish can be kept out of refrigeration.

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), replace ice as it thaws, or place the fish in a covered insulated container that has a spigot or drain to allow water to run off. This prevents the nutrients in the fish from being leached out into the standing water.

Example B. If the animals are fed inside under relatively unchanging conditions and room temperature is about 65°F (18°C), 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 on ice or 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 feeding, including temperature of the fish at feeding, should be validated before it becomes a standard procedure (see “Validating Procedures” section; see Form 4 for an example). Once the procedure for delivery and feeding has been validated it should be written and added as a standard operating procedure. If a standard procedure changes, it should be re-validated.

Additional Notes

The USDA requires that 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).

The USDA requires that marine mammals be fed at least once daily unless otherwise indicated by veterinary treatment or accepted practices. All feeding receptacles must be cleaned and sanitized after each use (see Cleaning and Sanitation section).

When animals are fed individually, USDA requires an employee or attendant responsible for management to perform or directly oversee the feeding (9 CFR 3.105). The employee must be able to recognize alterations from a normal state of health in order to adjust food intake.

Feeding by members of the public is allowable only if the food is provided by the holding institution (see Purchasing section), held under proper conditions (see Storage section), and feeding is supervised by a trained employee.

Supplementation

Diets should include sufficient vitamin or mineral supplements to make up for nutrient losses during storage and thawing of the food. Table 2 illustrates some of the factors affecting loss of selected nutrients. For example, water-soluble nutrients can be lost from leaching into water or from juices released during thawing. Typically, thiamin and vitamin E are necessary to supplement. Vitamin E is destroyed during fat breakdown (oxidation) during storage. The extent to which this occurs depends on the fat content of the fish and length of storage. Enzymes (thiaminases) naturally present in fish tissue may destroy thiamin during storage. Supplementation should be documented as part of the diet record.

Table 2. Stability and factors affecting loss of selected nutrients.

Vitamin	Stability	Sensitives	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
A	Somewhat unstable	Heat, oxygen, light	Exposure to light
E	Somewhat unstable	Oxygen, light	Oxidation
K	Stable	Oxygen, light	Exposure to light, oxidation

Cleaning and Sanitation

MUST - Sanitize all equipment that contacts food items. Ensure a pest mitigation plan is in place. All chemicals and non-animal food items must be safely stored separately from or below animal food and food prep areas.

Cleaning and sanitation equipment, including all utensils, cutting boards, food containers, and tables, can harbor pathogens and must be properly cleaned and sanitized after each use (Stoskopf 1986). 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 fish-eating animals 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 requires that food-contact surfaces where animal food is prepared must be cleaned and sanitized at least once daily. Surfaces within the preparation area not directly in contact with fish such as floors, doors, and handles should be cleaned and sanitized as frequently as appropriate for their level of contact with contaminants.

The USDA requires food contact surfaces be cleaned and sanitized. Sanitation can be accomplished by one of the following methods using a final sanitizing rinse: (Rutledge, 2016; 9 CFR § 3.105; CDHS 2014. California Code, Health and Safety Code - HSC § 114099.6 | FindLaw, n.d.)

- Contact with a solution of 100 parts per million (ppm) available chlorine for 20 seconds or 50 ppm for at least a minute. In practice this equates to 1 tablespoon bleach (6.0% Sodium hypochlorite solution containing 5.7% available chlorine by weight) per gallon of cool water, soak for at least 1 minute. It is important to check the bleach label for an EPA statement that confirms it sanitizes. Fragranced bleaches cannot be used as sanitizers (Rutledge 2016).
- Contact with a solution of 25 ppm available iodine for 1 minute. Contact with a solution of 200 ppm quaternary ammonium for 1 minute.

- Contact with water of at least 170° to 180°F (77°C to 82°C) for at least 15 minutes (if temperature is increased to 200°F or 93°C, contact time is reduced to at least 5 minutes).
- 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 following the manufacturer’s directions.

A pest management program must be in place to prevent infestations and food adulteration. 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 (1993) states that to prevent possible contamination, such substances are not to be stored above or with any food, food equipment, or preparation utensils.

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.

Validating Procedures

MUST – Procedures and processes used to handle fish must be validated (proven effective) in advance of becoming policy.

SHOULD – Validation should be an ongoing evaluation at periodic intervals, at least annually, or when a procedure is changed.

In order to ensure that the fish nutritional composition, toxin levels, and temperature control are maintained within goal ranges throughout storage and handling, validation of the procedures is needed. Validation must occur before procedures become policy or practice, and they should occur annually or when the procedures are changed.

The nutritional composition of fish can be determined by periodic sampling of the fish at any stage in the handling process. Fish can be analyzed for nutrient content, toxins, or specific microbes, as well as visually inspected for quality. These evaluations can help indicate the effectiveness of the handling procedures. Sampling methods for nutrient, toxin, and microbial analysis are covered in the following section.

Temperature control can be validated by documenting temperatures of the fish and storage compartments at key points in the process. For validation, the temperature of the fish should be recorded at critical control points throughout processing and delivery, including receipt, storage, thawing, preparation, and before feeding (see Forms 2 and 4). One common method is to use a maximum/minimum thermometer or temperature data logger placed in a container of fish. The thermometer travels with the fish from frozen state to feeding (Form 4). A thermometer shows temperatures in the immediate vicinity of the device, so be sure to place it among the fish themselves, not on the outside of the container.

Validation of handling procedures requires careful documentation and should be performed initially and when changes to processing methods occur. The forms provided in this manual are purely examples. The ultimate purpose is that the procedures used at an institution should be checked for consistency and achievement of quality goals.

Analyses of Fish Quality

MUST – Safety and appropriateness of fish must be ensured through either testing or collaboration with vendors.

SHOULD – The processes to assess quality of fish should reflect the institutional needs and risks to animals.

It is recommended to sample fish and perform quality control analysis. While the composition of fish species can vary by catch and season, the frequency of quality checks may depend on budget resources and usage rates. A minimum of an annual nutritional analysis on a representative sample is recommended, with an ideal level of sampling of every new catch used. A commercial nutrition laboratory is commonly used to perform these analyses.

A basic nutritional analysis includes moisture, protein, and fat. These values can be used to estimate caloric content using standard Atwater's values of 4 kcal per gram of protein and carbohydrate, and 9 kcal of metabolizable energy per gram of fat (Clauss et al. 2010, Iske et al. 2016, Kerr et al. 2013), if the direct measurement of energy in the fish via bomb calorimetry is not performed. If using calculated energy values, a conversion factor for protein is needed because most commercial laboratories will use a standard 6.25 conversion factor from nitrogen to protein, but fish protein should use a 5.71 factor (Diniz, et al 2013). Therefore, standard protein values should be multiplied by 0.914 before use in energy estimates.

Additional analyses of interest may include mineral content, which is a factor in water quality and dietary mineral balance. Vitamin A analysis may be indicated in some situations. Apex predator fish species can accumulate high levels of vitamin A, which can be problematic, especially if animals are eating a limited or single-species diet.

It is important when taking samples for nutrient analyses that samples represent what is consumed. For example, if only the filet of a large salmon is fed, sampling the whole fish would not be an accurate representation of the nutrients consumed. Commercial laboratories are often willing to process samples and homogenize on behalf of clients, but this should be communicated and understood prior to sending the samples. Multiple fish should always be used to create a homogenized sample representing the catch date and location of the fish in question. Do not send samples you would not feed to the animals, nor a single item, even if it is large in size.

In addition to nutritional adequacy, seafood may contain toxins or microbial contaminants. The best prevention is working closely with your vendor to ensure that fish are obtained from healthy fisheries and handled well. If you have concerns, you can test for specific toxins and microbes.

Algal toxins may be found in invertebrate seafood taxa as well as some vertebrate taxa. Algal toxins biomagnify in food webs, peaking in higher trophic levels. The blooms of algae are seasonal and usually found in localized areas, and commercial fishers are usually aware and avoid those locations at those times. These blooms can cause significant harm to wildlife and are often a major cause of unusual mortality events (Van Dolah, et al. 2003). These toxins can be found in commercial fish and invertebrate prey used by zoos and aquaria, but the levels that produce clinical toxicity are not known. Toxins commonly of concern include domoic acid (amnesic shellfish poisoning) and brevetoxins (neurotoxic shellfish poisoning). Both of these are found in “red tide” scenarios as well as other types of algal blooms. Few labs test for these toxins, so evaluate the risks to your collection based on where your fish originate, and the sensitivity of your species being fed the fish.

Various state and national government websites can be used to track outbreaks, and reputable fish vendors will be aware of the issues and avoid purchasing fish caught in these areas during outbreaks. Most fish-eating animals in managed care appear able to tolerate the levels of toxins typically found in the fish fed. However, monitoring toxin levels may be warranted, especially as climate change and other factors are increasing the severity and duration of algal blooms. Unfortunately, very few commercial labs can currently test for these toxins. Currently, good communication with your vendors and awareness of outbreaks are the best ways to reduce toxin exposure for animals in managed care. Further research to determine safe limits of various toxins in wildlife is needed.

While measuring microbial load (measurement of colony forming units (CFU)) is possible, fish are not expected to be sterile, and a high number of CFUs does not indicate that fish are compromised or of poor quality. This analysis is best used as a possible way to validate fish handling procedures. For example, a large increase in CFU levels from storage to feeding may indicate contamination or poor handling. Routine measurement of CFUs of fish is not necessary.

While routine bacterial counts are generally not advised, testing for specific microbes may be indicated if there is a specific health concern. In fish, common pathogens to animals include *Erysipelothrix* spp., *Mycobacterium* spp., *Vibrio* spp., and pathogenic *Escherichia coli*.

Several types of harmful bacteria can be found in or transferred by human carriers, including *Salmonella* spp., *Staphylococcus aureus*, *Clostridium perfringens*, *Campylobacter jejuni*, *Clostridium botulinum*, *Listeria monocytogenes*, *Escherichia coli*, and *Yersinia enterocolitica* (Rehe 1990).

Chemical properties of the fish itself 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.

Measuring oxidation (via thiobarbituric acid, TBA) is sometimes tested as a proxy for rancidity or degradation of fish. However, interpreting the results is difficult - there are no standards for acceptable levels. Instead, we recommend following the visual indicators listed in the Inspection and Handling and Feeding Thawed Fish sections and ensuring good handling practices as the best way to ensure fish quality.

Concluding Remarks

Whenever possible and applicable, official United States Department of Agriculture (USDA) or local/state guidelines were referenced to develop these guidelines. The guidelines in this document were designed to meet, or in some cases, when practical, be more stringent than official guidelines from those sources. The use of these guidelines, along with the appropriate documentation as presented in the text, sample forms, and appendix, should allow an institution that feeds fish-eating animals to meet or exceed existing 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. The development and application of a clear Hazard Analysis and Critical Control Points (HACCP) plan to mitigate risks associated with the inclusion of fish as a diet ingredient is strongly recommended (Scott and Stevenson 2006). Evaluation of fish quality is an ongoing process from the time the fish is harvested through the time the fish is ultimately offered to the animal. In situations where the institution is not in direct possession/control of the product, it is best practice to communicate with the vendor to determine the details necessary to satisfy the HACCP provisions in place.

Nutrition and quality must be considered major factors in fish selection. Care must be taken to ensure that food for fish-eating animals in managed care 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 (Ofstedal and Boness 1983) in terms of financial, environmental, and labor resources. 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 dependence on one particular food item, it is prudent to offer a variety of fish to the animal and not rely on a single type or species of fish. It is possible for an animal to become imprinted on a specific food item. If that item becomes unobtainable, the quality becomes poor, or the palatability decreases, it may be 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.

Given the goal of a balanced diet, it is 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 using the freshest fish possible, and to minimize overall quality and nutrient loss during the period of frozen storage.

Ongoing uncertainties with the future availability of fish stocks and increased presence/use of farmed fish make selection of appropriate fish and their handling of utmost importance. Such uncertainties and possibilities require constant awareness and evaluation of the nutritional content and quality of the fish fed.

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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) provided detailed descriptions of the freezing process and is relied upon heavily for the details below, unless other references are noted.

The freezing point of a substance is “that temperature at which the liquid is in equilibrium with the solid.” Many foods, including fish, have a high-water content and freeze between temperatures of 32°F and 37°F (0°C and 3°C); fish freeze, on average, at about 36 °F (2°C). 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 but not all techniques are practical for commercial seafood production.

Changes in flavor and color and losses in nutrients and texture occur rapidly at temperatures above 40 °F (4°C). Because of the physical nature of fish, the method of freezing affects quality and nutrient loss upon thawing. Fish frozen rapidly to 32 °F (0°C) have less “drip” (nutrient loss due to water loss from cells) when thawed. Length of time for fish to freeze depends on temperature of the freezing chamber, temperature of food upon entering the freezing chamber, and type, shape, and size of packaging.

Freezer burn is a condition of discoloration or other damage caused to frozen food by evaporation, typically due to inadequate packaging or storage conditions in the freezer. On fish, it is white in appearance and looks dried out. Fish that are freezer burned are often poking out of the wrapper and are very distinctive looking. Freezer burn 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.

Parasites may be destroyed by freezing temperatures. 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 37 °F (3°C) or above, spores of *Clostridium botulinum* can survive freezing and may grow and produce toxins (Frazier and Westhoff 1988).

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. Freezing only slows enzyme activity, which is usually optimum at higher temperatures.

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 36 °F (2°C) there is a reduction in rancidity of fatty tissue. 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.

Form 1. Fish Delivery Inspection Checklist

Are the documents in order?	YES NO
Type of fish	
Size of entire shipment: number of boxes/containers	
Quantity: total quantity by weight of shipment	
Freezing method: block - IQF - Shatter pack	
Pricing	
Is the packaging size correct?	YES NO
If required, are the boxes dated?	YES NO
If required, is there a history of the catch included?	YES NO
Are there any nonfood items in the shipping vehicle?	YES NO
Does the temperature gauge of the vehicle indicate frozen condition inside?	YES NO
Do the contents appear frozen?	YES NO
Is there any evidence of thawing (and refreezing)?	YES NO
Are there areas of ice under boxes?	
Are any of the boxes stained or distorted?	
Examine three boxes (make appropriate comments).	YES NO
Quality of fish (fish need to be thawed for a thorough inspection)	
Size of fish	
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 for Thawed Fish

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	Ideal	Acceptable	Unacceptable
General Appearance	shine or luster to skin; no bloating or protrusion of viscera; no dehydration	some loss of sheen	luster gone, lumpy; skin loss and major breaks
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, maroon; moist	brownish	grayish-yellow and covered with mucus
Odor	neutral fish odor	mild fishy odor	sour 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

Form 3. Optional Refrigerator/Freezer Temperature Chart for Non-Automated Systems

Record temperatures for each refrigerator and freezer.

Circle One: Refrigerator Freezer

Location:

Date	Initials	Container#	Start Temp/Time	End Temp/Time

Figure 5. Visual representation of tempering and thawing stages for fish.

