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Title 50: Wildlife and Fisheries

PART 16—INJURIOUS WILDLIFE
Subpart B—Importation or Shipment of Injurious Wildlife

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§ 16.13 Importation of live or dead fish, mollusks, and crustaceans, or their eggs.

(a) Upon an exporter filing a written declaration with the District Director of Customs at the port of entry as required under §14.61 of this chapter, live or dead fish, mollusks, and crustaceans, or parts thereof, or their gametes or fertilized eggs, may be imported, transported, and possessed in captivity without a permit except as follows:

(1) No such live fish, mollusks, crustacean, or any progency or eggs thereof may be released into the wild except by the State wildlife conservation agency having jurisdiction over the area of release or by persons having prior written permission from such agency.

(2) The importation, transportation, or acquisition of any of the species listed in this paragraph is prohibited except as provided under the terms and conditions set forth in §16.22:

(i) Live fish or viable eggs of walking catfish, family Clariidae;

(ii) Live mitten crabs, genus *Eriocheir,* or their viable eggs;

(iii) Live mollusks, veligers, or viable eggs of zebra mussels, genus *Dreissena* ;

(iv) Any live fish or viable eggs of snakehead fishes of the genera *Channa* and *Parachanna* (or their generic synonyms of *Bostrychoides, Ophicephalus, Ophiocephalus,* and *Parophiocephalus*) of the Family Channidae, including but not limited to:

(A) *Channa amphibeus* (Chel or Borna snakehead).

(B) *Channa argus* (Northern or Amur snakehead).

(C) *Channa asiatica* (Chinese or Northern Green snakehead).

(D) *Channa aurantimaculata.*

(E) *Channa bankanensis* (Bangka snakehead).

(F) *Channa baramensis* (Baram snakehead).

(G) *Channa barca* (barca or tiger snakehead).

(H) *Channa bleheri* (rainbow or jewel snakehead).

(I) *Channa cyanospilos* (bluespotted snakehead).

(J) *Channa gachua* (dwarf, gaucha, or frog snakehead).

(K) *Channa harcourtbutleri* (Inle snakehead).

(L) *Channa lucius* (shiny or splendid snakehead).

(M) *Channa maculata* (blotched snakehead).

(N) *Channa marulius* (bullseye, murrel, Indian, great, or cobra snakehead).

(O) *Channa maruloides* (emperor snakehead).

(P) *Channa melanoptera.*

(Q) *Channa melasoma* (black snakehead).

(R) *Channa micropeltes* (giant, red, or redline snakehead).

(S) *Channa nox.*

(T) *Channa orientalis* (Ceylon or Ceylonese Green snakehead).

(U) *Channa panaw.*

(V) *Channa pleurophthalmus* (ocellated, spotted, or eyespot snakehead).

(W) *Channa punctata* (dotted or spotted snakehead).

(X) *Channa stewartii* (golden snakehead).

(Y) *Channa striata* (chevron or striped snakehead).

(Z) *Parachanna africana* (Niger or African snakehead).

(AA) *Parachanna insignis* (Congo, square-spotted African or light African snakehead).

(BB) *Parachanna obscura* (dark African, dusky, or square-spotted snakehead);

(v) Any live fish, gametes, viable eggs, or hybrids of the species silver carp, *Hypophthalmichthys molitrix,* and largescale silver carp, *Hypophthalmichthys harmandi* ; and

(vi) Any live fish, gametes, viable eggs, or hybrids of the species black carp, *Mylopharyngodon piceus.*

(3) Notwithstanding §16.32, all Federal agencies shall be subject to the requirements stated within this section. Live or dead uneviscerated salmonid fish (family Salmonidae), live fertilized eggs, or gametes of salmonid fish are prohibited entry into the United States for any purpose except by direct shipment accompanied by a certification that: as defined in paragraph (e)(1) of this section, the fish lots, from which the shipments originated, have been sampled; virus assays have been conducted on the samples according to methods described in paragraphs (e)(2) through (4); of this section; and *Oncorhynchus masou* virus and the viruses causing viral hemorrhagic septicemia, infectious hematopoietic necrosis, and infectious pancreatic necrosis have not been detected in the fish stocks from which the samples were taken. In addition, live salmonid fish can be imported into the United States only upon written approval from the Director of the U.S. Fish and Wildlife Service.

(4) All live fish eggs of salmonid fish must be disinfected within 24 hours prior to shipment to the United States. Disinfection shall be accomplished by immersion for 15 minutes in a 75 part per million (titratable active iodine) non-detergent solution of polyvinylpyrrolidone iodine (iodophor) buffered to a pH of 6.0 to 7.0. Following disinfection, the eggs shall be rinsed and maintained in water free of fish pathogens until

packed and shipped. Any ice or water used for shipping shall be from pathogen-free water.

(b)(1) The certification to accompany importations as required by this section shall consist of a statement in the English language, printed or typewritten, stating that this shipment of dead uneviscerated salmonid fish, live salmonid fish, or live, disinfected fertilized eggs or gametes of salmonid fish has been tested, by the methods outlined in this section, and none of the listed viruses were detected. The certification shall be signed in the country of origin by a qualified fish pathologist designated as a certifying official by the Director.

(2) The certification must contain:

(i) The date and port of export in the country of origin and the anticipated date of arrival in the United States and port of entry;

(ii) Surface vessel name or number or air carrier and flight number;

(iii) Bill of lading number or airway bill number;

(iv) The date and location where fish, tissue, or fluid samples were collected;

(v) The date and location where virus assays were completed; and

(vi) The original handwritten signature, in ink, of the certifying official and his or her address and telephone number.

(3) Certification may be substantially in the following form:

I, ____, designated by the Director of the U.S. Fish and Wildlife Service on ____(date), as a certifying official for ____ (country), as required by Title 50, CFR 16.13, do hereby certify that the fish lot(s) of origin for this shipment of ____ (weight in kilograms) dead uneviscerated salmonid fish, live salmonid fish, live salmonid fish eggs disinfected as described in §16.13, or live salmonid gametes to be shipped under ____(bill of lading number or airway bill number), were sampled at ____(location of fish facility) on ____(sampling date) and the required viral assays were completed on ____(date assays were completed) at ____(location where assays were conducted) using the methodology described in §16.13. I further certify that *Oncorhynchus masou* virus and the viruses causing viral hemorrhagic septicemia, infectious hematopoietic necrosis, and infectious pancreatic necrosis have not been detected in viral assays of the fish lot(s) of origin.

The shipment is scheduled to depart ____(city and country) on ____(date), via ____ (name of carrier) with anticipated arrival at the port of ____ (city), U.S.A., on ____ (date).

_____

(Signature in ink of certifying official)

_____

(Printed name of certifying official)

Date:_____
Organization employing certifying official:_____
Mailing address:_____
City:_____
State/Province:_____
Zip Code/Mail Code:_____
Country:_____
Office telephone number: International code_____
Telephone number_____
Fax number_____

(c) Nothing in this part shall restrict the importation and transportation of dead salmonid fish when such fish have been eviscerated (all internal organs removed, gills may remain) or filleted or when such fish or eggs have been processed by canning, pickling, smoking, or otherwise prepared in a manner whereby the *Oncorhynchus masou* virus and the viruses causing viral hemorrhagic septicemia, infectious hematopoietic necrosis, and infectious pancreatic necrosis have been killed.

(d) Any fish caught in the wild in North America under a valid sport or commercial fishing license shall be exempt from sampling and certification requirements and from filing the Declaration for Importation of Wildlife. The Director may enter into formal agreements allowing the importation of gametes, fertilized eggs, live fish, or dead, uneviscerated fish without inspection and certification of pathogen status, if the exporting Nation has an acceptable program of inspection and pathogen control in operation, can document the occurrence and distribution of fish pathogens within its boundaries, and can demonstrate that importation of salmonid fishes into the United States from that National will not pose a substantial risk to the public and private fish stocks of the United States.

(e) *Fish sampling requirements, sample processing, and methods for virus assays* —(1) *Fish sampling requirements.* (i) Sampling for virus assays required by this section must be conducted within the six (6) months prior to the date of shipment of dead uneviscerated salmonid fish, live salmonid fish, live salmonid eggs, or salmonid gametes to the United States. Sampling shall be on a lot-by-lot basis with the samples from each lot distinctively marked, maintained, and processed for virus assay separately. A fish lot is defined as a group of fish of the same species and age that originated from the same discrete spawning population and that always have shared a common water supply. In the case of adult broodstock, various age groups of the same fish species may be sampled as a single lot, provided they meet the other conditions previously stated and have shared the same container(s) for at least 1 year prior to the sampling date.

(ii) In a sample, or sub-sample of a given lot, collection of 10 or more moribund fish shall be given first preference. The remainder of fish required for collection shall be randomly selected live fish from all containers occupied by the lot being sampled. Moribund fish shall be collected and processed separately from randomly selected fish. In the event the sample is taken from adult broodstock of different ages that share the same container, first preference shall be given to collecting samples from the older fish.

(iii) The minimum sample numbers collected from each lot must be in accordance with a plan that provides 95 percent confidence that at least one fish, with a detectable level of infection, will be collected and will be present in the sample if the assumed minimum prevalence of infection equals or exceeds 2 percent. A total of 150 fish collected proportionately from among all containers shared by the lot usually meets this requirement. A sampling strategy based on a presumed pathogen prevalence of 5 percent (60 fish) may be used to meet sampling requirements for shipments of gametes, fertilized eggs, or uneviscerated dead fish; provided that in the previous 2 years no disease outbreaks caused by a pathogen of concern have occurred at the facility from which the shipment originated and all stocks held at the facility have been inspected at least four times during that period (at intervals of approximately 6 months) and no pathogens of concern detected.

(iv) Fish must be alive when collected and processed within 48 hours after collection. Tissue and fluid samples shall be stored in sealed, aseptic containers and kept at 4 °Celsius (C.) or on ice but not frozen.

(v) Tissue collection shall be as follows:

(A) Sac Fry and fry to 4 centimeter (cm): Assay entire fish. If present, remove the yolk sac.

(B) Fish 4–6 cm: Assay entire visceral mass including kidney.

(C) Fish longer than 6 cm: Assay kidney and spleen in approximately equal weight proportions.

(D) Spawning adult broodstock: Assay kidney and spleen tissues from males and/or females and ovarian fluid from females. Ovarian fluid may comprise up to 50 percent of the samples collected.

(2) *General sample processing requirements.* (i) Ovarian fluid samples shall be collected from each spawning female separately. All samples from individual fish shall be measured to ensure that similar quantities from each fish are combined if samples are pooled. Ovarian fluid samples from no more than five fish may be combined to form a pool.

(ii) Whole fry (less yolk sacs), viscera, and kidney and spleen tissues from no more than five fish may be similarly pooled.

(iii) Antibiotics and antifungal agents may be added to ovarian fluid or tissue samples to control microbial contaminant growth at the time of sample collection. Final concentrations shall not exceed 200–500 micrograms/milliliter (µg/ml) of Gentamycin, 800 international units/milliliter (IU/ml) of penicillin, or 800 µg/ml of streptomycin. Antifungal agent concentrations should not exceed 200 IU/ml of mycostatin (Nystatin) of 20 µg/ml of amphotericin B (Fungizone).

(iv) Sample temperature must be maintained between 4 at 15 °C. during processing. Use separate sets of sterile homogenization and processing equipment to process fluids or tissues from each fish lot sampled. Processing equipment need not be sterilized between samples within a single lot.

(v) Homogenized tissue samples may be diluted 1:10 with buffered cell culture medium (pH 7.4–7.8) containing antibiotics and antifungal agents not exceeding the concentrations described in paragraph (e)(2)(iii) of this section. Centrifuge tissue suspensions and ovarian fluid samples 4 °C. at 2,500 × gravity (g) (relative centrifugal force) for 15 minutes. Resulting supernatant solutions can be stored overnight at 4 °C.

(vi) At the time of inoculation onto cell cultures, total dilution of processed tissue samples must not exceed 1:100 ((volume to volume) (v/v)); total dilution of ovarian fluid samples must not exceed 1:20 (v/v). In samples inoculated onto cell cultures, the final antibiotic concentration shall not exceed 100 µg/ml of Gentamicin, 100 IU/ml of penicillin, or 100 µg/ml of streptomycin and antifungal agent concentrations should not exceed 25 IU/ml of mycostatin (Nystatin) or 2.5 µg/ml of amphotericin B (Fungizone).

(3) *Cell culture procedures.* (i) Both epithelioma papulosum cyprini (EPC) and chinook salmon embryo (CHSE–214) cell lines must be maintained and used in all virus assays. Susceptible, normal appearing, and rapidly dividing cell cultures shall be selected. Penicillin (100 IU/ml), streptomycin (100 µg/ml), and antifungal agents, such as mycostatin/Nystatin (25 IU/ml) or amphotericin B/Fungizone (2.5 µg/ml), are permitted in media used for cell culture and virus assay work.

(ii) Cell cultures shall be seeded and grown, at optimum temperatures, to 80–90 percent confluence in 24-well plates for virus assay work.

(iii) Decant the medium from the required number of 24-well plates of each cell line, and inoculate four replicate wells per cell line with .10 ml per well of each processed sample. When all wells have been inoculated, tilt plates to spread the inocula evenly. Incubate inoculated plates for 1 hour at 15 °C. for sample contact. After the 1 hour contact add cell culture medium. Medium shall be buffered or cells incubated so that a pH between 7.4 and 7.8 is maintained. All cell culture assays shall be incubated, without overlays, at 15 °C. for 21 days.

(4) *Virus identification by serological methods.* All cell cultures showing cytopathic effects (CPE) must be sub-cultured onto fresh cell cultures. If CPE is observed, determine the presence and identity the virus by serum neutralization, dot blot, enzyme-linked immunosorbent assay, or other equivalent serological technique.

(f) Information concerning the importation requirements of this section and application requirements for designation as a certifying official for purposes of this section may be obtained by contacting: U.S. Department of the Interior, U.S. Fish and Wildlife Service, Division of Fish Hatcheries (820 Arlington Square), 1849 C Street, NW., Washington, DC 20240. Telephone 703–358–1878.

(g) The information collection requirements contained in this part have been approved by the Office of Management and Budget under 44 U.S.C. 3501 *et seq.* and assigned clearance number 1018–0078. The information is being collected to inform U.S. Customs and USFWS inspectors of the contents, origin, routing, and destination of fish and eggs shipments and to certify that the fish lots were inspected for listed pathogens. The information will be used to protect the health of the fishery resource. Response is required to obtain a benefit.

[58 FR 58979, Nov. 5, 1993, as amended at 65 FR 37063, June 13, 2000; 67 FR 62203, Oct. 4, 2002; 72 FR 37469, July 10, 2007; 72 FR 59035, Oct. 18, 2007]

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