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Deep-Substrate Incubators

A Field Guide for Atlantic Salmon Enhancement

V.A. Pepper

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Abstract

PEPPER, V. A. 1984. Deep-substrate incubators — a field guide for Atlantic salmon enhancement. Can. Spec. Publ. Fish. Aquat. Sci. 71: 25 p.

This field guide describes procedures for operating an Atlantic salmon (*Salmo salar*) enhancement project in which incubation of eggs is required to assure a juvenile salmon supply for stocking purposes. Much of this manual describes activities and information gathering relating to the egg incubation aspects of an enhancement project. Use of the deep-substrate incubator is advocated as an effective means to secure swim-up fry. Procedures described in this manual include: brood stock collection, holding, stripping and fertilization of eggs, incubator design, preparation, loading and operation; and fry enumeration and evaluation of developmental index. The need for detailed records of project activities is stressed throughout the manual. Methods are given to calculate the number of eggs planted in the incubator, the number of fry produced, egg to fry survival, indicators of incubator performance efficiency, and stage of fry development. Although operating procedures are described as simply as possible to encourage use of the manual by individuals with no formal training in biology, statistical and biological discussions are also presented in greater complexity to encourage critical appraisal and refinement of salmon enhancement methodologies by scientists and technicians.

Résumé

PEPPER, V. A. 1984. Deep-substrate incubators — a field guide for Atlantic salmon enhancement. Can. Spec. Publ. Fish. Aquat. Sci. 71: 25 p.

On trouvera dans le présent guide de terrain une description des méthodes utilisées dans l'exécution d'un projet de revalorisation du saumon atlantique (*Salmo salar*), nécessitant l'incubation des oeufs pour assurer des apports de saumoneaux destinés à des peuplements. Une bonne partie de ce manuel est consacrée aux activités et à la collecte de données relatives aux aspects incubation des oeufs d'un projet de cette nature. Comme moyen efficace de se procurer des alevins au stade de nage vers le haut, nous conseillons d'utiliser des incubateurs enfoncés dans le substrat. Parmi les opérations décrites dans le présent manuel, notons : la collecte des stocks reproducteurs, leur stabulation, l'expulsion et la fécondation des oeufs, la conception, préparation, chargement et fonctionnement des incubateurs, et le dénombrement des alevins et l'évaluation d'un indice de développement. Tout au long du manuel, nous faisons ressortir la nécessité de tenir un journal détaillé des activités relatives au projet. On y décrit des méthodes pour calculer le nombre d'oeufs ensemencés dans les incubateurs, le nombre d'alevins produits, la survie de l'oeuf à l'alevin, les indicateurs de performance des incubateurs et les stades de développement des alevins. Nous décrivons les diverses opérations en termes aussi simples que possible dans le but d'encourager les individus sans formation biologique particulière à se servir du manuel. N'empêche que nous présentons des notions statistiques et biologiques plus complexes afin d'encourager une évaluation critique et un raffinement des méthodes de revalorisation du saumon à l'intention des scientifiques et des techniciens.

Introduction

Atlantic salmon enhancement projects often require a supply of young fish for stocking in river systems where there is potential to support additional salmon production. These juvenile salmon must be obtained by incubating and hatching salmon eggs, most often in artificial incubators. To assure proper operation of these incubators, it is necessary to control the number of eggs placed in the incubators to within limits dictated largely by the size of the incubators and the quality and quantity of the available water supply. Likewise, it is usually necessary to determine the number of fry produced by the particular type of incubator. Enumeration of salmonid eggs and fry is an essential prerequisite to evaluating effectiveness of incubation facilities. With development of reliable electronic counters, time consuming manual egg counting now has an effective alternative. However, where production facilities are small, it is often difficult to justify the expense of automated enumeration devices, especially where the manual process results in a count of sufficient accuracy without incurring undue handling mortality. Since many of our Atlantic coast salmon enhancement projects and project opportunities are of a relatively small scale, one of the immediate project planning considerations is whether it is logistically and economically more desirable to support several projects from a centralized incubation facility (or larger scale hatchery) or to establish site-specific incubation facilities that support a limited production capacity.

The current emphasis on economic viability, as a planning criterion for salmonid enhancement, is restrictive for species with a life cycle in excess of 3 yr. Economic analyses give advantage to projects where the time span between costs incurred by a project and its payoff is minimal. Salmonid enhancement projects having benefit/cost ratios in excess of 1.5:1 are rare. Thus, it is often difficult to justify the expense of high technology devices for application to small-scale enhancement projects. The end result is often an "economy of scale" decision where production targets are high, as are the technological requirements for attaining such high production levels.

In contrast to large-scale production oriented salmonid enhancement is the current interest in community involvement and public participation. Valuable primarily as a vehicle for public education and resource conservation, public participation has the potential to realize enhancement opportunities that are often too small to justify the cost of high technology salmonid hatcheries. However, efficiency of operation of small-scale projects is still essential, especially where brood stock is limited.

In the search for effective production methods for salmonids, the deep-substrate incubator has demonstrated its potential (Bams 1970, 1972; Bailey and Heard 1973; Blackett 1974; Bailey et al. 1975). Requiring very little maintenance and being relatively inexpensive to construct and operate, the deep-substrate incubator has been used successfully on several salmonid species (Bams and Simpson 1977). Such incubators, depending on size, can also cater to a wide range of production requirements, from a few thousand fry for research purposes, to several hundred thousand fry for stocking programs. Regardless of size of incubator used, or species produced, a routine and rigorous evaluation of incubator performance is necessary to assure the greatest possible production of high quality fry, thereby encouraging economic efficiency of adult salmonid production. This manual is intended to contribute to, and indeed, encourage such evaluation.

The purpose of this manual is to provide guidelines for operation of an Atlantic salmon enhancement project wherein salmon eggs must be procured, fertilized, and incubated to provide juvenile salmon for the enhancement project. This manual also encourages maintenance of accurate and detailed records of project data (i.e. egg and fry counts, brood stock, and fry length and weight information, location and numbers of dead eggs left in incubator after fry emergence) to permit objective evaluation of project methods and equipment.

Development of a manual of this sort is based on the premise that considerable variation in operating conditions exists from one location to the next and that comparison of project results among different geological areas requires some standardization of methodologies. With some common operating conditions among projects, there is much greater opportunity to identify possible deficiencies in results of individual projects so that salmon enhancement projects in general can benefit, both from problems experienced in other projects, and from new approaches and methodologies arising from solutions to these problems.

Since this manual is developed around the egg incubation aspect of salmon enhancement, data accumulated in the course of using the manual will be useful primarily as a means of evaluating incubator performance. Unfortunately, there are currently no rules as to what actually constitutes high incubator performance efficiency or quality fry. It is safe to contend that salmonid fry that survive to contribute to the harvest, or to reproduce, are "quality fry." It follows that a fry production facility that effects a large production of adult salmonids relative to the number of eggs incubated, is an efficient installation. Ultimately the two criteria are interwoven in that contribution to the harvest is the justification for project operation. As there are currently no benchmarks by which to judge incubator performance, this manual strives to provide parameters by which such judgments may someday be possible.

In this context, it is important to understand that there is no one formula available that will guarantee success of a salmon enhancement project. It is by an ongoing critical review of project conditions (both biological and engineering) and maintenance of detailed records of project operations, that new knowledge can lead to improvements in enhancement methodologies and concepts. Therefore, this manual represents guidelines for enhancement project operation and is intended to act as a base on which to encourage improvements to present enhancement technologies.

This manual was developed in association with the deep-substrate incubators in use in Newfoundland (Fig. 1). In operation since 1975, these incubators have proven themselves as a viable production option for Atlantic salmon (Porter and Meerburg 1977). As a result of appraisals, such as encouraged in this manual, minor modifications have been incorporated into incubator design with the aim of achieving a more uniform water flow pattern in the incubator. The new design (Fig. 2) is currently being tested under field conditions to determine if it is in fact an improvement in enhancement technology.

Although this manual is written for a three-chambered deep-substrate incubator, many of the statistical methods

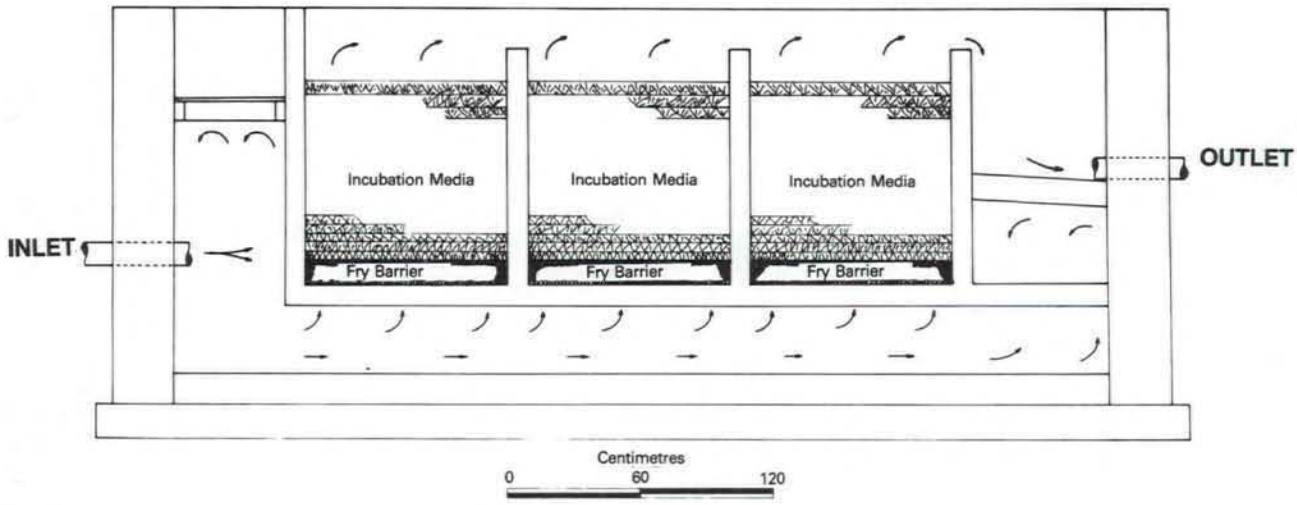

FIG. 1. Serial discharge deep-substrate incubator.

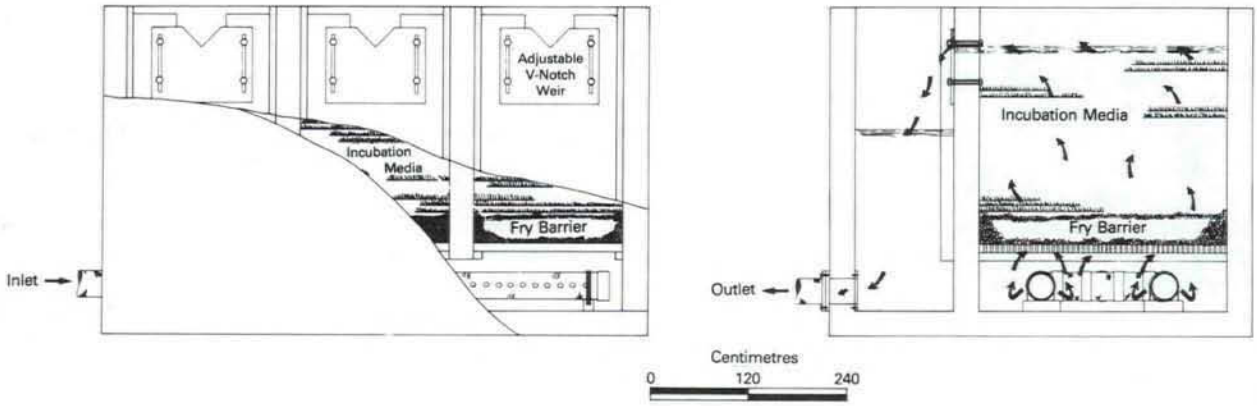

FIG. 2. Parallel discharge deep-substrate incubator.

employed are equally applicable to other incubators such as the Health-Techna, vertical drip incubator. Regardless of the type of incubator used, operation of the incubation facility will require a supply of fertilized eggs, a quality water supply, and considerable effort to load and monitor the incubator and remove and enumerate emergent fry. In performing the various activities associated with operating incubation facilities, information will be accumulated that will be of long-term significance to the goals of the enhancement project. The effort required to maintain these records is small relative to activities such as brood collection and fry distribution and will pay off in the long run, especially with respect to justification of continued funding of the project. Funding support to public groups sponsoring salmonid enhancement projects will usually require preparation of detailed annual progress reports that will present accumulated data in tabular form and provide interpretation of project results for the year.

Performance Assessment

Definition of a technique to facilitate an exact statement of incubator performance would be an unreasonable goal for this manual. With the variability inherent in biological processes, it is more realistic to consider general guidelines of operation and attempt to refine these guidelines as more information becomes available. In assessing incubator performance, archived information is required on average weight per spawner, age distribution of spawners, egg deposition, fry length and weight, number of fry produced, smolt counts, and adult returns, over an extended period. These data will facilitate construction of a management plan for enhancement operations to harmonize enhancement activities with requirements of salmonid genetics. Accordingly, calculations presented in this manual provide descriptive statistics to facilitate derivation of decision criteria regarding incubator performance.

Procedures Required to Operate a Deep-Substrate Incubation Facility

These procedures are described for Atlantic salmon and may have to be modified to cater to the needs of whatever other species are used. Many of the procedures described here have been extracted from Davis and Caines (1977). It is important to realize that these procedures are only general guidelines. Operating requirements of enhancement projects may vary considerably from one location to another. Enhancement projects should always be discussed thorough-

ly with Department of Fisheries and Oceans (DFO) representatives to assure that projects are properly designed and in compliance with the legalities of interference with fish populations. DFO representatives will also be able to advise on the rational design of such apparatus as fish transfer tanks and collection traps.

Brood Stock Collection, Holding, and Preparation for Spawning

At the time of transition from marine to freshwater environments, Atlantic salmon are undergoing physiological changes in adapting to their new surroundings. Fish are sensitive as they enter the river environment and excessive handling at this time could result in mortality. It is recommended that brood stock be accumulated from fish likely to have been in fresh water for several days. Care must also be exercised in the method of capture and some form of trap is suggested. Anderson and McDonald (1978) provide a design for a trapping facility currently in use in Newfoundland. Conlin and Tutty (1979) also provide instruction on trapping salmonids.

Atlantic salmon adults are not ripe when they enter their home rivers and, depending on time of river entry, may be in the home river for 4–5 mo before ripening to reproductive condition. This necessitates that adequate holding facilities be provided. Although the actual cost of incubator construction and operation may cause little financial strain, brood stock holding and security are likely to add significant overhead to a salmonid enhancement project.

In expectation of a limited number of adult salmon that will be available as a brood source to the enhancement project (i.e. if there were many adults available there might not be need for a project) care must be exercised to assure that the enhancement project has access to enough brood to avoid population inbreeding. A project based on too few spawners could lead to anomalies among offspring (such as reduced survival potential) that might ultimately degrade the project's potential for success. It has been suggested (Ryman and Ståhl 1980) that a minimum of 30 of the less numerous sex be used for fish culture. Ideally, considering population genetics, the sex ratio should be 1:1. However, consideration must be given to the sex ratio of the natural river spawning escapement as well. Where there is a disproportionate sex ratio in the natural spawning escapement, arbitrarily evening out the sex ratio of the brood for the enhancement project would further bias the sex ratio of those adults left to spawn naturally in the river. If adult salmon are collected for brood throughout the period of escapement to the river, it is most likely that the sex ratio of the accumulated brood will be similar to that of the spawning escapement of the river. If this sex ratio is significantly greater than three females per male (i.e. 5–7 females per male), DFO should be contacted for advice on an appropriate course of action.

Having collected the fish, facilities will have to be made available for holding adults until the maturation process is complete (1–5 mo). Since it is often not feasible to hold salmon in the same area as they are captured, an adult transfer system may be required. Smith (1978) described requirements of transfer tanks while Haskell (1955), Westers (1970), and Westers and Pratt (1977) have described the carrying capacity of hatcheries based on oxygen consumption and the accumulation of metabolic products.

In general, it is necessary to pay close attention to water temperature at the time of adult transfer and be aware that, the higher the water temperature, the lower the number of salmon that may be transferred per load. With a recirculating water system transfer tank (contact DFO, St. John's, Nfld. for construction details), 15 kg of salmon per m^3 is a reasonable transfer density at a temperature of 15°C. Transfer of adult salmon should not be attempted when water temperature exceeds 20°C. Where transfers are necessary during warm weather, they should be done in the early morning (i.e. 0600–0900). It must also be cautioned that transfer duration can be an important consideration. For any adult salmon transfers that are expected to take longer than 1 h, DFO should be contacted to assure that appropriate safeguards are planned prior to the transfer.

As mentioned above, brood stock may have to be retained for several months before maturity. The general guideline for adult salmon holding is 30 kg of salmon per m^3. Again, temperature is an important consideration for brood holding as is water flow. A water flow of 2.5 L/min for each kilogram of adult salmon held is a rough guideline for water requirements. DFO should be consulted before constructing adult holding facilities so that specific operating characteristics can be defined prior to accumulating any brood fish.

Additional procedures to be followed prior to stripping Atlantic salmon are itemized below:

— accumulate sufficient numbers of male and female salmon (maximum sex ratio of 3 females per male) to load the incubation facility. Expect 1550 eggs per kg female body weight. Plan on loading incubator with 70–75 eggs per 100 cm^2 artificial turf surface area.

EXAMPLE:

For a three-chambered incubator to be loaded with 18 layers of artificial turf (each layer 90 cm × 150 cm), egg capacity would be:

18 layers × 3 chambers × 13 500 cm^2 per layer × 0.72 eggs per cm^2 = 524 880 eggs.

This would require (assuming an average female weight of 2.5 kg)

524 880 eggs ÷ (2.5 kg per female × 1550 eggs per kg) = 136 females + 136 × 4/3 (i.e. 3 females per male) = 181 salmon or 136 females + 45 males.

NOTE: The average weight of salmon available for brood will vary from river to river and from year to year. This value (average weight) should be determined prior to spawning.

Although 181 salmon are required for an incubator of the size described above, at least 200 salmon would be collected to ensure sufficient brood stock considering such factors as uncertainty of sex ratio of spawning escapement (external determination of sex is difficult until some 5 wk before spawning), mortality among brood stock due to predation (bears, weasels, and mink), and egg retention within the female body cavity. At the time of incubator loading, if excess fertilized eggs are available, consideration may be given to increasing egg density. Egg density as high as 80 eggs/100 cm^2 has been used in deep-substrate incubators without increases in egg mortality. However, densities in excess of 75 eggs/100 cm^2 are not recommended due to concern about fry quality. Depending on the depth of the incubator, it may be possible to add another layer of artificial

turf to accommodate excess eggs. However, additional layers will increase resistance to upwelling water flow and may increase egg mortality. Caution is advised.

Adult Gonad Development

In early September, all adults should be seined from their holding area(s) and examined to assure adequate numbers of males and females. Mean weight and fork length by sex should also be determined at this time so that size of fry produced can eventually be compared with parental size thereby evaluating fry characteristics both as a function of incubation environment and genetics.

Beginning in mid-October, adult salmon should be checked for reproductive condition. This is accomplished by seining 20 salmon from the holding area (no more than five at one time). A salmon is then carefully removed from the seine and allowed to relax. The salmon's head is then placed under the operator's arm with the salmon's belly upward. A gloved hand (damp woolen mitt) holds the salmon's tail while the free hand is positioned a few centimetres anterior to the vent. The thumb and index finger are pressed gently against the salmon's abdomen and stroked toward the vent. Do not exert heavy pressure. If eggs or sperm are released, the salmon is ripe. As fish are checked, release them back into their holding area. If 6 of the 20 fish are ripe, begin stripping the following day.

Equipment

Organize all equipment in preparation for spawning on the day before beginning stripping activities. This involves thorough washing (soap and rinse) of the equipment identified below:

- plastic egg pans (small circular wash pans, one pan to three females for each day's stripping)
- plastic colanders (typically used for draining pasta. Make sure colander holes are small enough to retain eggs)
- large feathers for stirring eggs and sperm (one goose feather per 30 000 eggs expected)
- three holding tubs (for holding four or five salmon each immediately prior to stripping)
- two measuring cups (1 L size)
- two graduated cylinders (1 L size)
- precut artificial substrate (plastic grass). Ensure that artificial turf is cut to desired dimensions to fit each incubator chamber and 1.3-cm diameter holes are punched out. Holes should be equally spaced as a grid with rows and columns approximately 5–7 cm apart. Recognizing that artificial substrates vary considerably in the "porosity" of their base grid (the cross hatching to which the plastic tufts are attached), the number of additional holes to be punched may also vary somewhat from one enhancement project to another. It is advisable to contact DFO before attempting to "ventilate" the artificial turf.

At this time, all steps of the stripping process should be practiced using an imaginary salmon.

Stripping Process (Live Method)

All necessary equipment (pans, hand towels, woolen mitts, feathers, etc.) should be clean and waiting immediately before stripping occurs. Pans should be cleaned with warm water and soap, then thoroughly rinsed.

Ripe fish (nine females and three males) are collected and separated into three lots in holding tubs close to the stripping area. No more than five fish should be collected in any one seining operation. Stripping should be done in an area not exposed to direct sunlight.

With a dampened woolen mitt on the operator's left hand, a female fish is gently removed from the container, allowed to relax, and wiped lightly on its lower surface with a hand towel. The head of the female is wrapped in a hand towel and held firmly under the arm while the tail is held with the woolen mitt. During the stripping process, the spawners are held in a steeply inclined position with the head up, the back towards the operator and the whole of the underside held over the pan (no more than 15 cm above the pan). This pan should be placed on a table at a suitable height for the operator. Such a position allows the eggs to flow freely and prevents rupturing of egg wall due to impact against the bottom of the stripping pan. Do not squeeze the fish at any time around the gills and pectoral fin area. The hand without the mitt presses the abdomen with the thumb and index finger several centimetres anterior to the vent and moves along towards the vent. Eggs closest to the vent are the ripest and most easily stripped. With each completed stripping motion, the hand repositions progressively towards the head to strip eggs from farther up in the body cavity. When stripping, eggs should be directed along the sloping side of the pan, being careful not to blast them against the bottom. (Broken eggs will reduce fertilization rate.) Do not press forward of the pectoral fins as this may damage internal organs. In stripping the last eggs from the fish, care must be taken not to exert too much pressure or blood may mix with the eggs, thereby decreasing fertility. If the fish is at the proper "ripeness," eggs should flow continuously, under a slight pressure from the fingers, from the start to finish of the operation. During the stripping process, the salmon (especially the females) may suddenly tense up and go through several periods of body flexing. At such times, the body should be held firmly but not roughly. During such flexing, eggs are being displaced from the anterior to the posterior of the body cavity and will therefore be more easily stripped from the fish. While this flexing is taking place, stop the stripping motions and hold the fish until it is still and the body is again relaxed.

Intermittently, during the female stripping, a stream of sperm (milt) is stripped into the pan and the sperm and eggs mixed with a feather. A second female is stripped into the same pan, then a portion of milt from the second male is added, and the eggs and sperm mixed with a feather. A third female is stripped and more sperm is added from one or both of the two males. Care should be taken that some milt from at least two males is added to each pan of eggs (combined egg production from three females) to maintain genetic diversity within the salmon stock. It is desirable to have two stripping teams working concurrently so that two male salmon are always available for cross fertilization among pans of eggs. Each time a stream of milt is added to the eggs, mixing of

eggs and sperm with the goose feather is repeated. Once the pan is half to two-thirds filled with fertilized salmon eggs (eggs and sperm have been together for 5 min), a volume of river water approximately equal to that of the eggs is added to the pan. The pan is then set aside for 5–10 min.

Males are stripped in a manner similar to that of stripping females, making sure that the underside of the fish has been carefully wiped to prevent water from mixing with the milt. With males, the underside of the fish must be turned downwards and the sides pressed, whereas with the female the abdomen is pressed.

After the 5–10 min period, eggs are rinsed repeatedly to remove excess sperm, bad eggs, or other impurities that may cause fungus problems. The eggs are then poured into colanders (in gently flowing water about 10 cm deep) and then set aside for 2–3 h to allow the eggs to water harden. Care must be taken to protect the eggs from exposure to direct rays of the sun as direct sunlight is lethal to fresh (green) eggs. Spent fish are released gently into the water.

Precautions

- Treat the fish gently. Atlantic salmon may spawn several times.
- Allow no water to drip into the fertilization pans.
- Keep the spawning operation out of direct sunlight.
- Mix the eggs and sperm completely with the feather but be careful not to damage the fertilized eggs (rupture egg capsules).
- After fertilization, assure that all excess sperm is washed out of the stripping pans before the eggs are placed in the colanders for water hardening.
- Do not squeeze the fish or hold them solely by the tail (in tail-up position).

NOTE: If fish are not ripe, place them in an enclosure separate from the main brood stock. These salmon can be retained until the main stripping process is complete at which time the previously rejected salmon should again be checked for ripeness.

Preparation for Egg Planting

Ensure that incubation boxes and artificial turf pieces are scrubbed clean and are free of slime and other foreign material (sawdust, etc.).

Place 7 cm of coarse washed gravel (2.5–5.0 cm size) on the slatted false bottom followed by 7 cm washed pea gravel (0.5–1 cm). Incubators are then flushed several times by turning the valves on and off, hence removing any silt from the gravel layers.

Before planting, water flow through the boxes should be minimal to avoid dislodging eggs.

NOTE: After a 2-h water hardening period in which the fertilized eggs are held in gently flowing water in colanders, all dead eggs (opaque white) must be removed. This is easily done with a pipette and squeeze bulb.

Estimate the number of eggs in each pan by the volumetric displacement method as follows:

- Fill a 1-L graduated cylinder to 900 mL with river water. Add enough drained eggs (using a tea strainer) to bring the volume in the cylinder to 1000 mL.
- Count the number of eggs in the cylinder to determine the number of eggs per 100 mL volumetric displacement.
- Return the counted eggs to the pan and repeat the procedure at least once for each additional pan until all pans have been processed.
- At least 10 counts per day should be made throughout the stripping process. If less than 10 pans of eggs are collected on a particular day, more than one count per pan should be performed until 10 counts have been made.
- After all pans have been assessed, determine the average number of eggs per 100 mL volumetric displacement for the day's egg take, i.e. total number of eggs counted ÷ number of egg counts.
- Determine the total egg volumetric displacement for the day by filling a 5-L jug to the 2-L mark with river water, adding the day's egg take to the jug (water removed by straining through a colander), and recording the water level after all eggs have been added to the jug.
- Estimate the number of eggs fertilized by calculating:
  volumetric displacement × mean number of eggs per 100 mL ÷ 100.

Egg Planting

Place a layer of artificial turf in the bottom of each chamber of the incubator. Each layer of turf will require a small amount of 3-4 cm washed crushed rock to overcome buoyancy of the artificial turf. The bottom layer of turf should not receive any eggs. Start "planting" eggs on the second layer of artificial turf substrate. Determine the number of eggs required to load each layer of turf at a density of 70–75 eggs per 100 cm^2. This number of eggs will be allocated as a volume calculated as:

$$\frac{\text{number of eggs needed per layer}}{\text{mean count of eggs per mL}}$$

As an example:

If a 90 cm × 150 cm layer of turf were used per chamber, at an egg density of 0.73 eggs per cm^2, 9855 eggs would be required per layer.

If a mean egg count of 700 eggs/100 mL displacement (i.e. 7 eggs/mL) had been obtained for the day's egg take, the volume of eggs required per layer of turf would be:

$$\frac{9855}{7} = 1408 \text{ mL}$$

Thus, a 1408 mL volumetric displacement of eggs would be required per layer per chamber. Eggs must be distributed evenly over the artificial turf to assure even oxygen distribution and avoid fungus matting around eggs that die during the incubation period. Eggs should be poured out over the turf at the level of the water surface. Well-distributed eggs should be single or paired in the turf crevices with few clumps larger than five eggs.

Fill all chambers of one incubation box at the same rate as this will simplify control of water flow while planting. As each layer of artificial turf is filled, gently place a new layer of turf on top of the previous layer and repeat the process. When each chamber is filled, a top layer of artificial turf is

added and held in place with several shovelfuls of gravel. Eggs are not placed on this top layer.

Flow through the boxes is adjusted to 0.75 L per min per 1000 eggs.

NOTE: Shortly after the incubators are filled, the eggs become quite sensitive to mechanical shock; DO NOT BANG anything against the incubators.

During egg incubation, care must be taken that incubators are not disturbed. At certain stages in salmonid egg development, embryos become very sensitive to vibrations (Smirnov 1959). At such times incubators should not be touched.

Depending on water temperature, incubators may require periodic treatment with a fungicide. Where mean water temperature, through the incubation period, is less than 4°C, such treatments likely will not be necessary. If water temperature is much above this level, incoming water should be treated periodically with a fungicide. Care must be taken to ensure that this fungicide is zinc free. Treatment procedures are described by Burrows (1949), Johnson et al. (1955), Cline and Post (1972), and Stevenson (1980). Prior to any such treatments, DFO should be contacted for advice on treatment procedure.

Regardless of water temperature, periodic assessment of oxygen content of both incoming and discharge waters is required. At the time eggs hatch, oxygen demand will increase (Mason 1969) thereby greatly decreasing the percent saturation of discharge water. If oxygen concentration drops below 80% saturation, incoming water flow should be increased, either until O_2 is again above 80% or water flow reaches 0.9 L/min/1000 eggs. Too high a flow rate will damage alevins by rupturing the yolk sac.

Inherent in deep-substrate incubator operation is the problem of premature emergence. Ideally, fry should not begin to emigrate from the incubation medium until their yolk supplies are expended and they are ready to seek an external food supply. If alevins with pronounced yolk sacs are observed attempting to leave the incubator, an artificial light source will have to be placed above the incubator until such time as yolk supplies are resorbed. This may require as much as 2–3 wk of continuous artificial illumination.

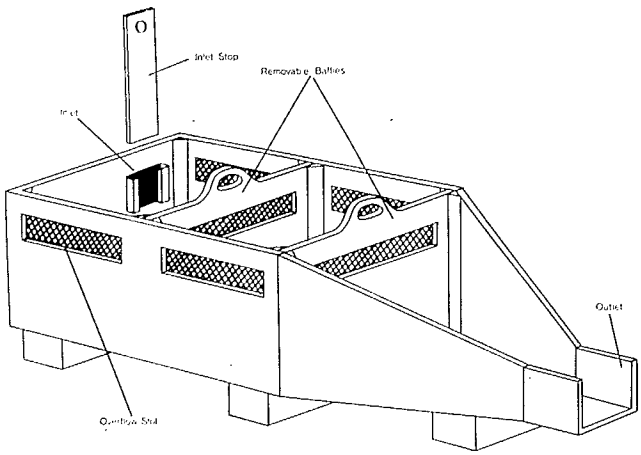

FIG. 3. Salmonid fry trap.

FRY ENUMERATION

Once the yolk sac has been resorbed, artificial illumination should be terminated and fry allowed to escape from the incubator. Ideally, fry traps (Fig. 3) should be installed at the exit of the incubator so that fry can be collected from each incubation chamber. Care must be taken to ensure that each trap has sufficient water flow to provide oxygen to captured fry. However, water flow should not be so fast as to pin fry against overflow screens. In the early and late phases of fry emigrations, when numbers are less than 2000 fry per day, actual counts may be performed. During periods of heavy fry emigration, when an absolute count is not feasible, the volumetric estimation method is used. This method is similar to that described for egg enumeration:

- Fill a 1-L graduated cylinder to the 900-mL mark with water.
- Using a small sieve (wire mesh or tea strainer) dip a quantity of fry from the collection box and place in the cylinder.
- Ensure that as much water as possible is removed from the sieve and repeat the process until the level of water and fry reaches the 1-L mark (be consistent in the procedure used to identify water level in the graduated cylinder).
- Manually count fry from every fifth cylinder and record counts and number of volumes measured plus any additional fry that may not otherwise have been included in the displacement count (Table 1).

NOTE: It must be emphasized at this point that it is the raw data (i.e. volumes measured, counts, and additional fry) that is most important to overall project evaluation and not the rough field calculations. Field calculations will facilitate appropriate fry distribution to rearing options but will not contribute to incubator assessment.

- Fry must not be allowed to accumulate in traps for more than 1 d. At regular intervals, throughout the enumeration process, fry will have to be distributed to suitable nursery areas (i.e. stream habitat, rearing channel, troughs, etc.).
- As an aid to evaluating fry size, samples should be taken each day from each chamber of the incubator. These samples must be preserved immediately in 5% formalin. Care must be taken to preserve each sample (a sample is a group of fry, i.e. 15–20) in a separate vial and label each vial (with waterproof ink and paper) with the date, chamber, incubator number, and incubation facility name.

Sample sizes should be greater than 12 but, for most purposes need not exceed 20. After a period of 80 d in formalin, fry should be measured to the nearest millimetre and weighed to the nearest 0.1 g. Information should be recorded as per Table 2.

Once it appears that all fry have emigrated from the incubator, it will be necessary to remove all the artificial turf and look for residual fry. This is accomplished as follows:

- Turn off water supply to incubator.
- Remove each layer of artificial turf individually, being careful not to dislodge any eggs or fry. This is accomplished by lifting the four corners at the same time and preventing a sag in the center.

TABLE 1. Fry enumeration. Raw data sheet.

Name of production facility _____ Page _____ of _____

Incubator No. _____ Chamber No. _____

Date: Day _____ Month _____ Year _____

Volumes	Count	Volumes	Count	Volumes	Count	Volumes	Count
1		1		1		1	
2		2		2		2	
3		3		3		3	
4		4		4		4	
5		5		5		5	
Additional Fry							

Rough total = Volumes ( ) × Count ( ) + Additional ( )

=	=	=	=
1	1	1	1
2	2	2	2
3	3	3	3
4	4	4	4
5	5	5	5
Additional Fry			

Rough total = Volumes ( ) × Count ( ) + Additional ( )

=	=	=	=

TABLE 2. Fry size data sheet.

Name of production facility _____

Incubator No. _____ Date of fry sample _____

Specimen	Chamber 1		Chamber 2		Chamber 3	
	Fork length	Weight	Fork length	Weight	Fork length	Weight
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						

— Very carefully remove any coarse gravel present on the artificial turf. Normally a small amount of gravel is present to prevent the turf from floating.
— Divide the layer into smaller sections for easier counting (this is best accomplished by using a grid template laid on the surface of the turf as in Fig. 4 for purposes of maintaining consistency of records among chambers).
— Count and record the numbers and location (i.e. the grid reference as in Fig. 4) of dead eggs, and dead fry as per Table 3. Silt conditions and anything relevant should also be recorded.

NOTE: Not all the cells of Table 3 will be required for every production facility. Each cell of the grid template should be about 20 × 20 cm (i.e. for an 80 × 160-cm incubator chamber, there would be 4 × 8 = 32 cells in the grid template).

— Turn the artificial turf over and record anything attached to the reverse side (again recording in Table 3).
— Occasionally turn water supply on in the event live fry are present.
— All live fry found should be counted and distributed to the rearing option being used.
— Once all the artificial turf has been removed from the incubator, both turf and incubator should be thoroughly scrubbed with soap solution and rinsed completely of any residue.
— Incubators (especially wooden ones) should be left wet during the off-season to prevent wood shrinkage and warping.

Incubator Assessments

This section describes methods to calculate the following:

1) number of eggs planted;
2) number of fry produced;
3) egg to fry survival;
4) dead egg distribution (random or clumped); and
5) fry developmental index.

Methods are also described to compare fry morphometrics among incubator chambers.

While numerical manipulations may seem formidable to some, they need not deter anyone from becoming involved in salmon enhancement projects. The important consideration is the information (data) required to attempt the calculations and not the equations as such. In fact, computer programs are available to provide the required numerical indicators of enhancement project performance. To help obtain a basic familiarity with the Statistical Methods section that follows, explanations of specific calculations are also provided (see Calculations, p. 11).

Statistical Methods

This section may be ignored by all but the insatiably curious. Statistical equations used for incubator assessments are defined by Steel and Torrie (1960). Most common are:

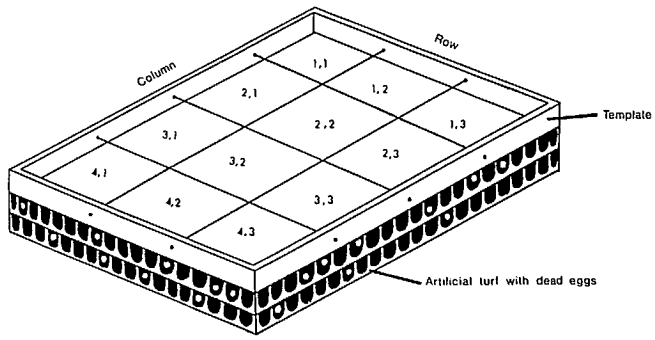

FIG. 4. Grid template for counting dead eggs and identifying their location on the artificial turf.

— sample variance (equation 22.4)

$$S^2 = \frac{\Sigma y^2 - \frac{(\Sigma y)^2}{n}}{n-1}$$

(Note: the standard deviation $S = \sqrt{S^2}$)

— finite population correction factor

$$fpc = \frac{N-n}{N}$$

— confidence interval (equation 22.7)

$$CI = \bar{y} \pm t\sqrt{\frac{S^2}{n} \cdot \frac{N-n}{N}}$$

where

$\bar{y}$ = sample mean
t = Student's-t value (2 tailed), with $n-1$ degrees of freedom (*df*) and a significance level of 0.05 (Appendix 2)
n = number of volumes counted
N = total number of volumes measured.

Moving medians are calculated as per Tukey (1977, p. 210–211). An example of a moving median calculation is given in Appendix 1 (p. 16). Variances for ratio estimators are calculated using the Jackknife method (Smith 1980; equation 11). This variance equation is:

$$\widehat{V(R_J)} = \frac{1}{n(n-1)} \cdot \sum^{n} (R_{-j}' - R_J)^2$$

where

$\widehat{V(R_J)}$ = estimate of variance of ratio
R_J = mean of ratios (i.e. the jackknife estimator)
R_{-j}' = mean of ratios with jth ratio removed.

The mean ratio confidence interval is calculated as:

$$CI = R_J \pm t_{(0.05)} \cdot \sqrt{\widehat{V(R_J)}}$$

TABLE 3. Distribution of dead eggs and residual fry in artificial turf substrate.

Name of production facility _____

Incubator No. _____ Chamber No. _____ Layer No. (top down) _____

Date of incubator cleanings Day _____ Month _____ Year _____

Row	Turf side	1		2		3		4		5		6		7		8		9		10	
		E	F	E	F	E	F	E	F	E	F	E	F	E	F	E	F	E	F	E	F
1	Top																				
	Bottom																				
2	Top																				
	Bottom																				
3	Top																				
	Bottom																				
4	Top																				
	Bottom																				
5	Top																				
	Bottom																				
6	Top																				
	Bottom																				
7	Top																				
	Bottom																				
8	Top																				
	Bottom																				
9	Top																				
	Bottom																				
10	Top																				
	Bottom																				

E = eggs F = fry

Comparison of chamber statistics follow the T3 procedure of Dunnet (1980):

$$\bar{y}_i - \bar{y}_j \pm A_{ij}, \alpha, k \, (s_i^2/n_i + s_j^2/n_j)^{1/2}$$

where

$$A_{ij}, \alpha, k = \mathrm{SMM}\alpha, k^*, \nu_{ij}$$

and

$\mathrm{SMM}\alpha, k^*, \nu_{ij}$ is the α point of the Studentized maximum modulus distribution of k^* uncorrelated normal variates

requiring the Statterthwaite (1946) approximate degrees of freedom:

$$\hat{\nu}_{ij} = \frac{(s_i^2/n_i + s_j^2/n_j)^2}{s_i^4/n_i^2 + s_j^4/n_j^2 V_j}$$

where

ν_{ij} is an estimate of degrees of freedom for pairwise comparison

i and j are indicators of the pairwise comparison (i.e. chambers 1 and 2, 2 and 3, and 1 and 3)

s = $\sqrt{s^2}$ = standard deviation of chamber mean parameter
n = number of fry sampled per chamber
V = $n - 1$.

For computation convenience, studentized maximum modulus critical values, as required in Dunnett's (1980) equation 1.2 (p. 296) are obtained from Stoline and Ury (1979) by linear interpolation.

Calculations

Incubator loading

- mean egg count = sum of counts ÷ number of counts
- eggs per mL = mean count ÷ 100
- volume (mL) of eggs laid down per layer = target # eggs per layer ÷ eggs per mL (as per page 5)
- total volume (mL) eggs planted = total layers planted × mL per layer
- total eggs planted = total volume (mL) × eggs per mL
- expanded "confidence interval" on total eggs = confidence interval for mean count (as per page 9) ÷ 100 × total volume of eggs (mL).

Dead egg distribution

- all calculations as per standard deviation and moving median equations cited above (also see Appendix 1, p. 16).

Fry counts

- estimated fry (per day; per chamber; for incubator) = mean count/100 mL displacement × volumes measured + additional counted
- confidence interval = confidence limits calculated on mean incubator count × volumes measured + additional fry.

Survival estimate

- survival = (point estimate of fry produced ÷ point estimate of eggs incubated) × 100
- intuitive range of survival = (lower limit of fry produced ÷ upper limit of eggs incubated) × 100 to (upper limit of fry produced ÷ lower limit of eggs incubated) × 100

NOTE: Survival estimates are not statistically valid.

Fry morphometrics

- developmental index:

$$k_D = \frac{10 \times \sqrt[3]{\text{weight in mg}}}{\text{length in mm}}$$

- all other calculations as per sample variance and jackknife variance equations cited above (also see Appendix 1, p. 23).

Discussion

During operation of an incubation facility there will be times, such as at the peak of fry emigration, where a tradeoff will have to be made between careful enumeration of fry (where counting is manual) and the release (to whatever rearing option) of healthy fry. The decision as to what percent of the fry run should be counted (i.e. 1 volume in 5, 1 in 7, 1 in 10, etc.) should not be made lightly. If water temperature were to rise during the enumeration process and cause concern about stress among fry, it would certainly be better to relax enumeration procedures rather than to cause increased mortality of fry. However, a partial count is certainly better than no count. The suggested procedure under adverse conditions is to reduce the frequency of the count so that, rather than counting one in five fry volumes, one in 10 or even one in 20, might be undertaken. An attempt should be made to obtain at least 10 accurate counts per incubator chamber per day to maintain some semblance of statistical credibility. The main effect of reducing the frequency of the fry count will be an expansion of the confidence interval. This reduction in count frequency is a practical compromise that is preferable to no appraisal of fry production whatsoever, and may in fact be important to continued funding for the project in question.

In working on projects dealing with renewable natural resources, one is often forced to accept a biological compromise between disciplines. Although engineering, economic, mathematical, and statistical constraints must be recognized in working with biological systems, it is not always possible to satisfy interdisciplinary needs. In this case, assessment of salmonid egg incubators encounters a conflict between economics and statistics. In order to satisfy statistical requirements prerequisite to determining egg survival through incubation, an incubator should have many chambers (or alternately, there would have to be many incubators per facility) so that data would include several estimates (replicates) of eggs incubated and fry produced. This would require small modular incubators so that each incubator could be treated as a separate production unit. In small to medium scale enhancement projects (i.e. 0.5 million to 2 million egg incubation capacity), it has so far proven less costly to construct a few larger incubators than a large number of smaller units. Since salmon enhancement projects must demonstrate potential cost effectiveness in the planning stages, statistical problems arising from the three-chambered incubator design have not yet outweighed the cost factor inherent in replicating smaller incubator units, especially considering desirable production characteristics of the present design.

Egg to Fry Survival

The first problem encountered in evaluating incubator performance is one of establishing a statistically valid estimate of egg to fry survival. This proportion is based on two estimators (i.e. fry produced and eggs incubated) that have been calculated from different data sets. Though Cochran (1977) provides a method for establishing a confidence interval in a case where the numerator and denominator of a ratio are based on equal values for volumes counted and volumes measured (n and N, respectively, Cochran's equation 2.45), egg and fry estimates in the present situation do

not conform to this restriction. An alternative approach is to estimate egg to fry survival by chamber and then, by replication, establish the statistical distribution for the ratio estimator. For a multichambered incubator, this second approach has potential. However, an incubator would have to have approximately 10–12 chambers (i.e. replicates of the incubator survival estimator) to meet the requirements of this statistical approach.

The appeal of a deep-substrate incubator is largely in economy of space, reasonable construction cost, and operating efficiency. In addition, the three-chambered design currently used in Newfoundland is well suited to small-scale public oriented projects where brood stock is limited and projects often require incubation of only 300 000–500 000 eggs. Recognizing an upper limit to incubator egg capacity, incubator partitioning to 10–12 chambers would necessitate abandoning the deep-substrate incubator in favor of the "shallow matrix" design (McNeil 1969; Lannan 1975; Poon 1977). Shallow matrix incubation has not yet been investigated in Newfoundland.

The biological reality of limitations in the number of salmon that may be used for brood in an enhancement project, together with engineering limitations of water supply, economic constraints of production cost per adult salmonid, and experience with different salmonid egg incubation options, all identify the deep-substrate incubator as a desirable means of pursuing salmon production opportunities in Newfoundland. It should be emphasized, however, that statistical limitations, with respect to survival estimates, prevent certainty about quantitative comparison of survival both among production facilities and among year-classes from the same facility.

This manual is developed to encourage recording of incubator related data, and to minimize subjectivity in interpreting incubator performance. The inability to make statistically valid statements about egg to fry survival is simply a challenge to substantiating incubator operating characteristics. With no quantitative means to provide true confidence limits to these survival estimates, the recommended course of action is to provide circumstantial evidence in support of estimates of the number of fry produced. This may be accomplished by inference.

While the ratio estimator (i.e. survival estimate) itself is statistically questionable, its components (fry produced and eggs incubated) taken individually are statistically valid. Therefore, if it is possible to obtain an accurate count of dead eggs remaining in the incubator at the end of the incubation period (such as under conditions of low incubation temperatures), the estimate of fry produced can be refined by subtracting the dead egg count from the upper and lower limits of the egg plant estimate. The resulting figures give biological substantiation to the appraisal provided by the survival estimate described above. This substantiation procedure is most likely sufficient to fulfill most project management assessments until such time as appropriate techniques are proposed to circumvent the survival estimator problem.

Developmental Index

Further concern about statistical comparison of ratio estimators relates to use of developmental index. Although the relation between fry weight and length has definite biological significance in relating stage of development at time of fry emigration (Bams 1970), arguments about statistical comparisons using ratio estimators (Atchley et al. 1976; Atchley 1978; Atchley and Anderson 1978; Hills 1978; Dodson 1978; Albrecht 1978) suggest the wisdom of exercising caution in analyses of ratio data. This manual pursues what is thought to be conservative methodology.

It is obvious that statistical procedures for ratio estimators are desperately needed to facilitate investigations of the nebulous topic of size and shape (Alexander 1971) among organisms. Although multivariate techniques have been proposed for rigorous investigations of shape (Humphries et al. 1981; Hansell et al. 1980) such techniques are beyond the scope of the present manual. In the calculations described above, confidence intervals are assigned to developmental indices in what is thought to be a conservative approach. It has been demonstrated (Durbin 1959) that where there is a linear relation between the numerator and denominator of a ratio estimate, the Jackknife technique reduced both bias and variance. With further evidence that the variance of the Jackknife method has been used successfully when applied to nonlinear estimates (in Smith 1980) and confirmation of Tukey's (1958) postulate of an approximate Student's-*t* distribution for the pivotal quantity $R_J - R/(\widehat{V(R_J)})^{1/2}$ (S. Smith, personal communication), the confidence interval, as calculated for fry developmental index in this manual, should be descriptive of potential difference in fry characteristics among incubator chambers (and/or incubators).

Appraisal Factors

In reaching conclusions pertaining to production efficiency of incubation systems for salmonids, there are at least two broad appraisals to be undertaken. These are:

1) mortality and its occurrence throughout the incubator, and
2) fry morphometrics.

Mortality

An incubator that produces few fry relative to the number of eggs incubated is clearly not a viable production tool. However, rather than asserting a blanket condemnation of the incubation method or particular incubator, an appraisal of the distribution of mortality throughout the incubator may be instrumental in illuminating design or operation modifications that may be implemented with a minimum of cost. Ideally, with a three-chambered incubator using artificial turf, the distribution of dead eggs, assuming a homogeneous planting density, should be homogeneous. Any pronounced trends in the distribution of dead eggs, that persist from year to year, suggests a deficiency in flow characteristics within the incubator. Differences in distribution of dead eggs among chambers may suggest a design fault in plumbing while differences among artificial turf layers could be indicative of insufficient water velocity or local flow impairment due to clogging with silt. This argument supposes insignificant egg decomposition. Where incubation temperatures exceed 5°C, this assumption may not be justified. Since incubation temperatures greater than or equal to 5°C will result in proliferation of *Saprolegnia* sp. (Oláh and Farkas 1978) on dead eggs, biweekly treatment of intake water with zinc-free malachite green and/or formalin (Burrows 1949) will be necessary to prevent increased mortality through fungal pro-

liferation. A further hazard to egg incubation in some areas results from iron bacteria (Bailey and Taylor 1974) precipitating organic compounds, thereby clogging incubator substrates and smothering eggs. Since this material cannot be effectively filtered from inflowing water, there is currently little that can be done to rectify the situation other than future avoidance of the particular water supply. In such situations, periodic backflushing of incubators may be required. Backflushing should not be attempted without close DFO technical supervision.

As a means of identifying trends in dead egg distribution, moving medians are used to smooth fluctuations in counts among substrate layers (Appendix 1, p. 16).

Fry morphometrics

Although departures from homogeneity of distribution of dead eggs throughout an incubator may be obvious, sublethal effects of incubation environment deficiencies are more difficult to detect. The ultimate test of fry condition is survival (Bams 1972). However, it has also been recognized that the larger the fry produced, the greater its survival potential (Bams 1967; Poon 1977; Koski 1975; Mason 1969). Short of stamina testing (Bams 1967), indicators of fry size are volume and measurement of weight and length.

Volumetric displacement data are particularly attractive in assessing fry size (the lower the count of fry per 100 mL volumetric displacement the larger the mean fry size) in that these data are available as part of the enumeration process and therefore require no additional effort. Contrary to ratio estimators, the volumetric displacement variable requires only standard considerations (distribution function, homogeneity of variance) prior to its application in parametric statistics. Thus, ANOVA techniques may be used as the means of testing for discrepancies in fry size among incubator chambers and between units. Should all subdivisions of the incubation environment be functioning equally well, statistical tests should not identify significant departures from homogeneity of mean fry count per 100 mL volumetric displacement among incubator subunits. Rejection of this null hypothesis, assuming the same brood stock has been used to load each incubator chamber (Aulstad and Gjedrem 1973; Larsson and Pickova 1978), suggests the incubator may not be operating as well as it should.

For an efficient incubator, plots of fry volumetric displacement counts per day should follow a similar pattern for each chamber of the incubator. If graphs of mean fry count (per 100 mL volumetric displacement) per day throughout the fry run are parallel among chambers and are of similar magnitude, further concern about fry morphometric discrepancies among chambers is likely unwarranted. Examples of these plots are presented in Appendix 1.

An alternative to volumetric displacement data is fry weight and length measurement. Obtaining these measurements is often undesirable due to the time required to perform such measurements on a sufficient number of specimens. Having once secured the measurements, one is still confronted by the problem of analysis. Statistically, covariance analysis is the desirable approach to this problem but, once again, several restrictions apply to interpretation of results (Steel and Torrie 1960). Failure to meet these restrictions leaves developmental index as the only avenue of inquiry.

While it is not anticipated that developmental index should vary systematically with incubator chamber, differences in mean developmental index throughout the duration of fry emigration are likely. Bams (1970) has identified inferior pink salmon fry quality at developmental indices less than 1.86 and greater than 1.95. To date, no such limits have been determined for Atlantic salmon. Using procedures of this manual, a reasonable starting point for evaluation of survival potential of fry of different development index may be the 95% confidence interval defined for this index over a period of several years.

PROJECT ASSESSMENTS

Critical appraisal of incubator performance, indeed of all aspects of salmon enhancement projects, is the only means of assuring that projects are as effective as they should be to provide the social and economic benefits on which the projects are justified. In consideration of the extensive planning mechanisms prerequisite to implementing salmon enhancement projects, and the accountability required relative to protection of expected benefits to such projects, detailed records relative to biological and financial accounting are required that are both accurate and timely. This requires maintenance of a daily log as well as year-end compilation and tabulation of project operations information. It is on these data that interpretations of the project's merits and weaknesses will be based. Hence, such data will help define an operating plan in support of requests for renewed financial support. The importance of an annual progress report for every enhancement project cannot be overemphasized as this report is the primary vehicle by which the project may be continued. Responsibility for the annual report will rest with the project manager. It is also the responsibility of this individual to maintain day to day project activities and be alert for any indications that project activities may be falling short of their intended goals. In such cases, immediate liaison with DFO technical support personnel will help avoid serious long-term detrimental impacts on the project. Also, by providing information as identified in this manual, a project manager will be able to draw on the technical expertise available from DFO to help identify and address project concerns before they become problems and derive efficient operating plans for continued application to the enhancement project.

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Appendix 1. Example Data Sets for Incubator Performance Assessment.

Contrived Egg Planting Data
Incubator #9, 1981

	Egg counts/100 mL volumetric displacement		
Count #	Day 1	Day 2	Day 3
1	736	814	678
2	842	701	674
3	778	773	669
4	748	592	771
5	749	751	775
6	830	750	754
7		784	781
8		811	622
9		744	777
10			778
Complete layers[a]	12	15	16
Partial layers	1	1	2
Additional eggs in partial layers	6 000	6 000	6 000 10 200

[a]A complete layer takes 12 000 eggs.

EXAMPLE OF CALCULATIONS (as per p. 11)

Using the variable y to represent egg count, the statistics required are:

— average egg count per 100 mL volumetric displacement
$= \bar{y} =$ sum y (i.e. Σy) $\div$ number of observations (n)
$= 18\,682 \div 25 = 747.28$

— variance (from p. 9)

$\Sigma y = 18\,682$

$\Sigma y^2 = 14\,050\,714$ (i.e. each y value squared, then added up)

$(\Sigma y)^2 = 349\,017\,124$ (i.e. $18\,682^2$)

$$S^2 = \frac{14\,050\,714 - \dfrac{349\,017\,124}{25}}{24}$$

$= 3751.21$

— standard deviation $= \sqrt{s^2} = 61.2471$

— t value for 24 df (i.e. 25 – 1 observations) and 0.05 probability level (from Appendix 2) = 2.064

— confidence interval (from p. 9):

s^2 = variance = 3751.21

n = volumes counted = 25

N = volumes that could have been counted
= total volume of eggs $\div$ mean egg count
= 72 732 mL $\div$ 747.28 eggs per 100 mL displacement
= 97.32898 counts

— lower limit;

$$= \frac{747.28 - 2.064\sqrt{(3751.21 \div 25) \times \dfrac{(97.33 - 25)}{97.33}}}{100} \times 72\,732$$

$= 527\,659$

— upper limit

$$= \frac{747.28 + 2.064\sqrt{(3751.21 \div 25) \times \dfrac{(97.33 - 25)}{97.33}}}{100} \times 72\,732$$

$= 559\,363$

These calculations indicate that we are 95% assured that the number of eggs placed in the incubator (since we did not actually count every egg) is in the range of 527 659–559 363.

— point estimate of number of eggs incubated (from p. 11).
$= 72\,732 \times (747.28 \div 100)$
$= 543\,512$ eggs

NOTE: Division by 100 in these equations reduces the average count per 100 ml to average count per mL.

Egg Planting Statistics for Contrived Data Incubator #9, 1981

Mean egg count per 100 mL is	747.2800
(Standard deviation is	61.2471)
Number of volumes counted =	25
Total volume of eggs planted =	72 732 mL

Estimated number of eggs planted = 543 512
95% confidence range for estimate is 527 659–559 363

Contrived Dead Egg Distribution for Incubator #9, 1982

Layer number (top down)	Upper chamber	Middle chamber	Lower chamber
1	8	17	8
2	102	142	125
3	180	163	227
4	160	141	140
5	178	197	178
6	262	285	253
7	187	255	582
8	135	202	209
9	127	177	270
10	102	165	409
11	98	123	396
12	83	114	386
13	77	163	125
14	60	134	59
15	45	70	45

Example of moving median calculation:

A) The INPUT

8, 102, 180, 160, 178, 262, 187, 135, 127, 102, 98, 83, 77, 60, 45

B) Grouping by 3

Three successive values: As given (sequence order)			In order (value order)			Median (mid group value)
8	102	180	8	102	180	102
102	180	160	102	160	180	160
180	160	178	160	178	180	178
160	178	262	160	178	262	178
178	262	187	178	187	262	187
262	187	135	135	187	262	187
187	135	127	127	135	187	135
135	127	102	102	127	135	127
127	102	98	98	102	127	102
102	98	83	83	98	102	98
98	83	77	77	83	98	83
83	77	60	60	77	83	77
77	60	45	45	60	77	60

Analysis of Dead Egg Distribution for Contrived Data 1982 Incubator #9

Substrate layer number	Chamber 1	Chamber 2	Chamber 3	Mean	Standard deviation
1	8	17	8	11.00	5.20
2	102	142	125	123.00	20.07
3	180	163	227	190.00	33.15
4	160	141	140	147.00	11.27
5	178	197	178	184.33	10.97
6	262	285	253	266.67	16.50
7	187	255	582	341.33	211.18
8	135	202	209	182.00	40.85
9	127	177	270	191.33	72.57
10	102	165	409	255.33	162.15
11	98	123	396	205.67	165.31
12	83	114	386	194.33	166.71
13	77	163	125	121.67	43.10
14	60	134	59	84.33	43.02
15	45	70	45	53.33	14.43
MEAN	120.27	156.53	227.47		
SD	65.25	66.19	159.45		

Moving Median of Order 3

Substrate layer number	Chamber 1	Chamber 2	Chamber 3	Total
1				
2	102	142	125	369
3	160	142	140	411
4	178	163	178	553
5	178	197	178	553
6	187	255	253	800
7	187	255	253	800
8	135	202	270	574
9	127	177	270	574
10	102	165	396	617
11	98	123	396	617
12	83	123	386	583
13	77	134	125	365
14	60	134	59	253

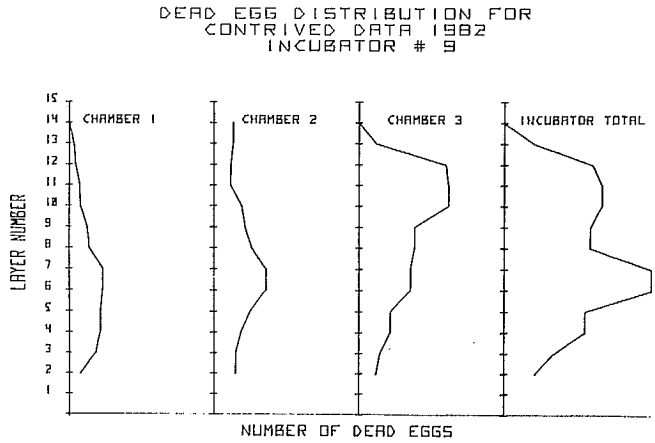

FIG. 5. Moving median plots of dead egg counts.

Contrived fry count (per 100 ml volume displacement)
Data for incubator #9, 1982
(Counts by chamber)

Chamber	Day	Volumes counted	Volumes measured	Counts	Additional fry
1	1	2	9	540,560	110
	2	4	16	620,558,580,580	300
	3	6	29	581,582,637,587,582,591	286
	4	7	54	596,584,592,584,614,597 611	616
	5	4	32	555,562,572,574	280
	6	4	18	580,575,563,578	35
	7	4	27	605,517,554,547	1347
2	1	1	5	631	355
	2	2	9	575,575	220
	3	4	14	574,582,549,582	321
	4	5	40	602,597,560,583,570	339
	5	3	24	565,576,572	284
	6	5	29	595,584,572,590,596	412
	7	3	14	572,615,620	1030
3	1	3	15	625,596,596	85
	2	4	19	620,585,684,684	90
	3	7	29	581,608,585,585,572,626 572	50
	4	6	48	590,597,572,582,563,582	104
	5	4	31	584,561,562,559	193
	6	10	62	585,573,579,583,626,634 614,589,652,583	70
	7	6	39	580,620,574,608,547,568	2975

Salmon Egg Incubator Performance Assessment
Analysis of Fry Count Data for
Contrived Data 1982
Incubator #9

	Chamber 1				
Day	Mean count	Volumes counted	Volumes measured	Additional fry counted	Estimated total
1	550.0000	2	9	110	5 060
2	580.0000	4	16	300	9 580
3	593.3333	6	29	286	17 493
4	596.8571	7	54	616	32 846
5	565.7500	4	32	280	18 384
6	574.0000	4	18	35	10 367
7	555.7500	4	27	1 347	16 352
Chamber parameters	578.7097	31	185	2 974	110 035

	Chamber 2				
Day	Mean count	Volumes counted	Volumes measured	Additional fry counted	Estimated total
1	631.0000	1	5	355	3 510
2	575.0000	2	9	220	5 395
3	571.7500	4	14	321	8 326
4	582.4000	5	40	339	23 635
5	571.0000	3	24	284	13 988
6	587.4000	5	29	412	17 447
7	602.3333	3	14	1 030	9 463
Chamber parameters	584.2174	23	135	2 961	81 830

	Chamber 3				
Day	Mean count	Volumes counted	Volumes measured	Additional fry counted	Estimated total
1	605.6667	3	15	85	9 170
2	643.2500	4	19	90	12 312
3	593.5714	7	29	50	17 264
4	581.0000	6	48	104	27 992
5	566.5000	4	31	193	17 755
6	601.8000	10	62	70	37 382
7	582.8333	6	39	2 975	25 706
Chamber parameters	595.3000	40	243	3 567	148 225

Analysis of Contrived Data 1982
Incubator #9

Day	Incubator total				
	Mean count	Volumes counted	Volumes measured	Additional fry counted	Total
1	591.3333	6	29	550	
2	604.3000	10	44	610	
3	588.3529	17	72	657	
4	587.5556	18	142	1 059	
5	567.4545	11	87	757	
6	592.1579	19	109	517	
7	579.0000	13	80	5 352	
Incubator parameters	587.1170	94	563	9 502	340 049

Estimated total fry production is 340 049
95% confidence interval is 264 823–415 275
(Standard deviation of mean count is 27.4398)

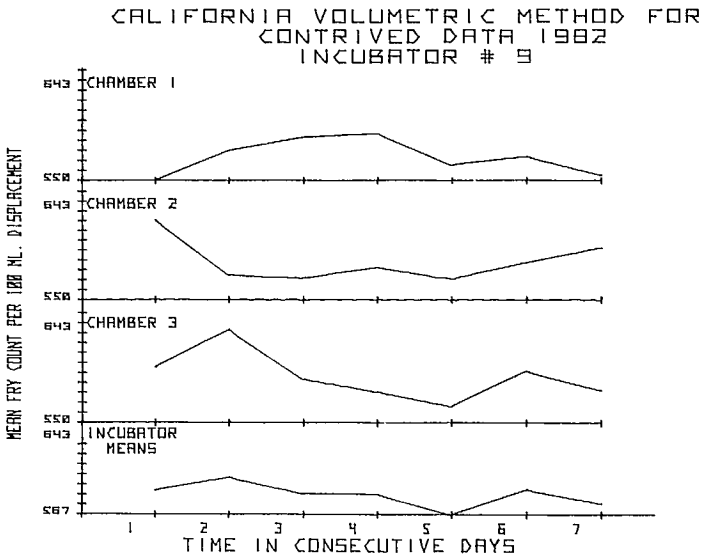

FIG. 6. Fry volumetric displacement counts.

Contrived Fry Count Data for
Incubator #9, 1982

Day	Volumes counted	Volumes measured	Counts	Additional fry
1	6	29	540,560,631,625,596,596	550
2	10	44	602,558,580,580,575,575 620,585,684,684	610
3	17	72	581,582,637,587,582,591 574,582,549,582,581,608 585,585,572,652,572	657
4	18	142	596,584,592,584,614,597 611,602,597,560,583,570 590,597,572,582,563,582	1059
5	11	87	555,562,572,574,565,576 572,584,561,559	757
6	19	109	580,575,563,578,595,584 572,590,596,585,573,579 583,626,634,614,589,652 583	517
7	13	80	605,517,554,547,572,615 620,580,620,574,608,547 568	5352

Salmon Egg Incubator Performance Assessment
Analysis of Fry Count Data for
Contrived Data 1982
Incubator #9

	Incubator statistics				
Day	Mean count	Volumes counted	Volumes measured	Additional fry counted	Estimated total
1	591.3333	6	29	550	
2	604.3000	10	44	610	
3	588.3529	17	72	657	
4	587.5556	18	142	1 059	
5	567.4545	11	87	757	
6	592.1579	19	109	517	
7	579.0000	13	80	5 352	
Summary parameters	587.1170	94	563	9 502	340 049

Estimated total fry production is 340 049
95% confidence interval is 337 155–342 943
(Standard deviation of mean count is 27.4398)

Contrived Fry Count Data for
Incubator #9, 1982

	Volumes counted chambers			Volumes measured chambers			Mean fry count chambers			Additional fry counted chambers		
Day	1	2	3	1	2	3	1	2	3	1	2	3
1	2	1	3	9	5	15	550.00	631.00	605.67	110	355	85
2	4	2	4	16	9	19	580.00	575.00	643.25	300	220	90
3	6	4	7	29	14	29	593.33	571.75	593.57	286	321	50
4	7	5	6	54	40	48	596.86	582.40	581.00	616	339	104
5	4	3	4	32	24	31	565.75	571.00	566.50	280	284	193
6	4	5	10	18	29	62	574.00	587.40	601.80	35	412	70
7	4	3	6	27	14	39	555.75	602.33	582.83	1347	1030	2975

Salmon Egg Incubator Performance Assessment
Analysis of Fry Count Data for
Contrived Data 1982
Incubator #9

	Chamber						Daily statistics	
	1		2		3			
Day	Mean count	Estimated total	Mean count	Estimated total	Mean count	Estimated total	Mean count	Estimated total
1	550.0	5 060	631.0	3 510	605.7	9 170	591.3	17 699
2	580.0	9 580	575.0	5 395	643.3	12 312	604.3	27 199
3	593.3	17 493	571.8	8 326	593.6	17 264	588.4	43 018
4	596.9	32 846	582.4	23 635	581.0	27 992	587.6	84 492
5	565.8	18 384	571.0	13 988	566.5	17 755	567.5	50 126
6	574.0	10 367	587.4	17 447	601.8	37 382	592.2	65 062
7	555.8	16 352	602.3	9 463	582.8	25 705	579.0	51 672
Chamber mean			578.71		584.22		595.30	
Volumes counted			31		23		40	
Volumes measured			185		135		243	
Extra fry			2 974		2 961		3 567	
Estimated total			110 035		81 830		148 225	

Estimated total fry production is 340 049
95% confidence interval is 337 155–342 943
(Standard deviation of mean count is 27.4398)

Fry Production Summary for
Contrived Data
Incubator #9
1982

Estimated number of eggs incubated is 543 512
(95% confidence interval is 527 659–559 363)

Estimated number of fry produced is 340 049
(95% confidence interval is 264 823–415 275)

Egg to fry survival is approximately 62.6%
(Intuitive range is 47.3%–78.7%)

Survival estimates are not statistically valid

Contrived Fry Morphometric Data for
Incubator #9, 1982

Chamber	Specimen No.	Day 1		Day 2		Day 3		Day 4		Day 5	
		[a]Length	Weight	Length	Weight	Length	Weight	Length	Weight	Length	Weight
	1	27.0	210	26.0	180	26.5	170	26.5	180	29.5	180
	2	26.5	200	26.5	190	27.5	190	24.5	170	26.0	175
1	3	26.0	180	27.0	190	26.5	200	27.0	200	25.5	180
	4	26.5	190	27.5	210	27.0	180	27.5	200	28.0	200
	5	27.0	210	25.5	150	26.0	180	26.0	170		
	6			26.5	175	27.0	190				
	1	27.5	200	26.5	180	26.5	200	27.0	190	27.0	200
	2	25.5	160	26.0	170	27.0	190	26.5	170	26.0	180
2	3	26.0	170	26.5	170	25.0	150	25.0	150	26.5	200
	4	27.0	210	25.5	150	26.0	180	27.0	200	25.0	150
	5	27.0	180			25.5	170	28.0	200	26.5	180
	6	25.0	170					25.5	160		
	1	26.0	170	26.5	200	27.0	200	27.0	190	26.0	160
	2	25.5	180	27.0	190	26.0	170	26.0	160	27.0	200
	3	27.0	180	25.0	150	26.0	180	25.5	160	25.5	180
3	4	26.0	190	26.0	180	26.5	160	26.5	180	25.0	170
	5	26.0	180	25.5	170			25.0	200	26.5	210
	6			27.0	200			26.0	180		
	7			26.0	190						

[a]Length in mm; weight in mg.

Fry Morphometric Statistics for
Contrived Data 1982
Incubator #9

Chamber 1

Day	Number of samples	Mean length (mm)	Variance	Mean weight (mg)	Variance	Mean developmental index	Variance
1	5	26.6	0.1750	198.0	170.0000	2.19	0.000065
2	6	26.5	0.5000	182.5	397.5000	2.14	0.000215
3	6	26.8	0.2750	185.0	110.0000	2.13	0.000409
4	5	26.3	1.3250	184.0	230.0000	2.16	0.000653
5	4	27.3	3.4167	183.8	122.9167	2.09	0.004180
Chamber statistics	26	26.7	0.8954	186.5	213.5385	2.14	0.000166

95% confidence interval for chamber statistics

Mean length	26.3–27.0
Mean weight	180.6–192.4
Mean developmental index	2.12–2.17

Chamber 2

Day	Number of samples	Mean length (mm)	Variance	Mean weight (mg)	Variance	Mean developmental index	Variance
1	6	26.8	0.9667	181.7	376.6667	2.15	0.000394
2	4	26.1	0.2292	167.5	158.3333	2.11	0.000160
3	5	26.0	0.6250	178.0	370.0000	2.16	0.000231
4	6	26.5	1.2000	178.3	456.6667	2.12	0.000138
5	5	26.2	0.5750	182.0	420.0000	2.16	0.000221
Chamber statistics	26	26.3	0.6850	178.1	336.1538	2.14	0.000058

95% confidence interval for chamber statistics

Mean length	25.9–26.6
Mean weight	170.7–185.5
Mean developmental index	2.13–2.16

Chamber 3

Day	Number of samples	Mean length (mm)	Variance	Mean weight (mg)	Variance	Mean developmental index	Variance
1	5	26.1	0.3000	180.0	50.0000	2.16	0.000561
2	7	26.1	0.5595	182.9	323.8095	2.17	0.000160
3	4	26.4	0.2292	177.5	291.6667	2.13	0.000804
4	6	26.0	0.5000	178.3	256.6667	2.16	0.001336
5	5	26.0	0.6250	184.0	430.0000	2.19	0.000747
Chamber statistics	27	26.1	0.4103	180.7	237.8917	2.16	0.000128

95% confidence interval for chamber statistics

Mean length	25.9–26.4
Mean weight	174.6–186.8
Mean developmental index	2.14–2.19

Incubator Statistics for Incubator #9, 1982

Number of samples	Mean length (mm)	Variance	Mean weight (mg)	Variance	Mean developmental index	Variance
79	26.3	0.6969	181.8	267.9731	2.15	0.000040

95% confidence interval for chamber statistics

Mean length	26.1–26.5
Mean weight	178.1–185.5
Mean developmental index	2.14–2.16

Parameter Differences Among Incubator Chambers

	Parameters		
Chambers	Mean length	Mean weight	Mean k_D
2–1	0.403846 NS	8.461538 NS	0.002897 NS
3–2	0.138989 NS	–2.663818 NS	–0.023244 *
3–1	–0.542735 NS	–5.797721 NS	0.020347 *

There have been significant departures from homogeneity among chamber parameters.

Appendix 2. Values of Student's *t*.

df	Probability of a larger value of *t*, sign ignored								
	0.5	0.4	0.3	0.2	0.1	0.05	0.02	0.01	0.001
1	1.000	1.376	1.963	3.078	6.314	12.706	31.821	63.657	636.619
2	0.816	1.061	1.386	1.886	2.920	4.303	6.965	9.925	31.598
3	0.765	0.978	1.250	1.638	2.353	3.182	4.541	5.841	12.941
4	0.741	0.941	1.190	1.533	2.132	2.776	3.747	4.604	8.610
5	0.727	0.920	1.156	1.476	2.015	2.571	3.365	4.032	6.859
6	0.718	0.906	1.134	1.440	1.943	2.447	3.143	3.707	5.959
7	0.711	0.896	1.119	1.415	1.895	2.365	2.998	3.499	5.405
8	0.706	0.889	1.108	1.397	1.860	2.306	2.896	3.355	5.041
9	0.703	0.883	1.100	1.383	1.833	2.262	2.821	3.250	4.781
10	0.700	0.879	1.093	1.372	1.812	2.228	2.764	3.169	4.587
11	0.697	0.876	1.088	1.363	1.796	2.201	2.718	3.106	4.437
12	0.695	0.873	1.083	1.356	1.782	2.179	2.681	3.055	4.318
13	0.694	0.870	1.079	1.350	1.771	2.160	2.650	3.012	4.221
14	0.692	0.868	1.076	1.345	1.761	2.145	2.624	2.977	4.140
15	0.691	0.866	1.074	1.341	1.753	2.131	2.602	2.947	4.073
16	0.690	0.865	1.071	1.337	1.746	2.120	2.583	2.921	4.015
17	0.689	0.863	1.069	1.333	1.740	2.110	2.567	2.898	3.965
18	0.688	0.862	1.067	1.330	1.734	2.101	2.552	2.878	3.922
19	0.688	0.861	1.066	1.328	1.729	2.093	2.539	2.861	3.883
20	0.687	0.860	1.064	1.325	1.725	2.086	2.528	2.845	3.850
21	0.686	0.859	1.063	1.323	1.721	2.080	2.518	2.831	3.819
22	0.686	0.858	1.061	1.321	1.717	2.074	2.508	2.819	3.792
23	0.685	0.858	1.060	1.319	1.714	2.069	2.500	2.807	3.767
24	0.685	0.857	1.059	1.318	1.711	2.064	2.492	2.797	3.745
25	0.684	0.856	1.058	1.316	1.708	2.060	2.485	2.787	3.725
26	0.684	0.856	1.058	1.315	1.706	2.056	2.479	2.799	3.707
27	0.684	0.855	1.057	1.314	1.703	2.052	2.473	2.771	3.690
28	0.683	0.855	1.056	1.313	1.701	2.048	2.467	2.763	3.674
29	0.683	0.854	1.055	1.311	1.699	2.045	2.462	2.756	3.659
30	0.683	0.854	1.055	1.310	1.697	2.042	2.457	2.750	3.646
40	0.681	0.851	1.050	1.303	1.684	2.021	2.423	2.704	3.551
60	0.679	0.848	1.046	1.296	1.671	2.000	2.390	2.660	3.460
120	0.677	0.845	1.041	1.289	1.658	1.980	2.358	2.617	3.373
∞	0.674	0.842	1.036	1.282	1.645	1.960	2.326	2.576	3.291
df	0.25	0.2	0.15	0.1	0.05	0.025	0.01	0.005	0.0005
	Probability of a larger value of *t*, sign considered								

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