A Report on a Preliminary Experiment in the Control of *Eubothrium Salvelini (Cestoda: Pseudophyllidea)* in Juvenile Sockeye Salmon *(Oncorhynchus Nerka)* of Babine Lake, B.C., with a Note on the Occurrence of Infectious Haematopoietic Necrosis *(IHN)* in the Fulton River Stock

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March 1982

Canadian Manuscript Report of Fisheries and Aquatic Sciences No. 1645

Government of Canada Fisheries and Oceans
Gouvernement du Canada Pêches et Océans

Canadian Manuscript Report of

Fisheries and Aquatic Sciences 1645

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A REPORT ON A PRELIMINARY EXPERIMENT IN THE CONTROL OF EUBOTHRIUM SALVELINI (CESTODA: PSEUDOPHYLLIDEA) IN JUVENILE SOCKEYE SALMON (ONCORHYNCHUS NERKA) OF BABINE LAKE, B.C., WITH A NOTE ON THE OCCURRENCE OF INFECTIOUS HAEMATOPOIETIC NECROSIS (IHN) IN THE FULTON RIVER STOCK

by

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Cat. No. Fs 97-4/1645 ISSN 0706-6473

ABSTRACT

Boyce, N. P. 1982. A report on a preliminary experiment in the control of Eubothrium salvelini (Cestoda: Pseudophyllidea) in juvenile sockeye salmon (Oncorhynchus nerka) of Babine Lake, B.C., with a note on the occurrence of infectious haematopoietic necrosis (IHN) in the Fulton River stock. Can. MS Rep. Fish. Aquat. Sci. 1645: iv + 19 p.

A study was conducted in 1977 and 1978 to: 1) test in the field (Babine Lake) the laboratory finding that larger sockeye fry were less susceptible to Eubothrium salvelini infection and 2) examine the feasibility of using artificial growth acceleration in fry before lake entry as a means of controlling infection.

Fry were maintained under conditions (temperature, feeding) favouring growth acceleration, then released to the lake. These fish, as well as appropriate controls, were marked differentially for recognition a year later at the time of their migration as smolts, when their respective parasite burdens would be compared.

The recovery of marked smolts was too low for fulfilling the first objective of this study. This low return of marked smolts appears attributable to a high rate of fry mortality, due partly to viral infectious haematopoietic necrosis (IHN).

A supplementary study confirmed the possible role of IHN in fry mortality, and indicated that the causative virus is enzootic in Babine stocks.

Key words: cestode, Eubothrium salvelini, infection control, Babine Lake, sockeye salmon, fluorescent marking, growth acceleration, infectious haematopoietic necrosis (IHN).

RESUME

Boyce, N. P. 1982. A report on a preliminary experiment in the control of *Eubothrium salvelini* (Cestoda: Pseudophyllidea) in juvenile sockeye salmon (*Oncorhynchus nerka*) of Babine Lake, B.C., with a note on the occurrence of infectious haematopoietic necrosis (IHN) in the Fulton River stock. Can. MS Rep. Fish. Aquat. Sci. 1654: iv + 19 p.

En 1977 et 1978, une étude a été entreprise dans le but de: (1) vérifier sur le terrain (lac Babine) la découverte faite en laboratoire que les plus gros alevins de saumon rouge résistent mieux à l'infection par *Eubothrium salvelini* et (2) étudier la faisabilité d'accélération artificielle de la croissance des alevins avant l'ensemensement comme moyen de lutte contre l'inasection.

Les alevins ont été maintenus dans des conditions (température, alimentation) favorables. A l'accélération de la croissance, et relâchés ensuite dans le lac. Ces poissons, ainsi que des poissons témoins appropriés, one été étiquetés de différente façon afin de permettre leur identification un an plus tard au moment de leur migration au stade de saumoneau, et la comparison de leurs parasites respectifs.

A cause du petit nombre de saumoneaux étiquetés récupérés, on n'a pas pu réaliser le premier but de cette étude. Ce faible taux de reprise des saumoneaux étiquetés semble être attribuable à un taux élevé de mortalité des alevins, du en partie à la nécrose hématopoïétique infectieuse (NHI).

Une étude complémentaire a confirmé le rôle possible de la NHI dans la mortalité des alevins, et a démontré que le virus responsable est enzootique dans les stocks du lac Babine.

Mots clés: cestode, *Eubothrium salvelini*, lutte contre l'infection, lac Babine, saumon rouge, marquage fluorescent, accélération de la croissance, nécrose hématopoïétique infectieuse (NHI).

INTRODUCTION

The cestode Eubothrium salvelini occurs in approximately 30% of the sockeye smolts annually leaving Babine Lake, British Columbia (Fig. 1). Infections are acquired by fry through feeding on intermediate host copepods encountered shortly after they enter the lake from their natal streams and, under laboratory conditions, susceptibility to infection is inversely related to fry size at time of exposure (Boyce 1974).

A program was initiated in 1977: (1) to test in the natural environment the laboratory finding of size-dependent susceptibility of fry to Eubothrium infection and (2) to examine the feasibility of artificial acceleration of fry growth prior to lake entry, as a means of reducing infection among juvenile sockeye of Babine Lake.

Three categories of fry were selected for this study: (1) fry captured before lake entry and maintained artificially under conditions favouring growth acceleration, then released to the lake, (2) a subsample from the above catch released to the lake within 24 hours of capture, and (3) fry naturally migrating to the lake at the time of release of the "accelerated" group. The latter category was included to account for the possible effect of timing of lake entry as a factor in the acquisition of infection. It was anticipated that a sufficient number of representatives of each category would be encountered as smolts a year later to enable comparisons regarding prevalence and intensity of E. salvelini infection; it was also anticipated that further information on the timing of lake exit, size, and survival of these three categories of fish would be derived from this study.

As will be reported below, severe losses, partly attributed to viral infectious haematopoietic necrosis (IHN), were incurred among the fry held for growth acceleration. This led to a supplementary study in the following year (1978) to determine the extent of IHN infection in sockeye fry of the Fulton River and spawning channel system, and the potential effect of IHN on fry survival.

MATERIALS AND METHODS

FRY COLLECTION AND TREATMENT

Approximately 400,000 sockeye fry were collected May 4, 1977 from live-boxes at the outlet of spawning channel #1 of the Fulton River Spawning Channel System[1] and transported by vehicle to holding tanks at a site on the south bank of Fulton River, near its outlet to Babine Lake. From this collection, 200,000 fry designated for growth acceleration (Group A) were

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[1]For a detailed description of the spawning channel system see Ginetz 1977.

spray-marked with red fluorescent grit and placed in indoor tanks; the remaining 200,000 fry designated control Group B were marked with fluorescent green and released within 24 hours. At the time of release of Group A 26 days later, an additional 200,000 fry were obtained from channel 1, marked fluorescent orange and released within 24 hours. These fry were designated Group C.

FRY MARKING

Fry were marked with fluorescent grit using slight modifications of the procedures described by Mattson and Bailey (1969) and Healey et al. (1976). The techniques used were tested repeatedly on sockeye fry for 2 years prior to this study. The results of these tests, to be reported separately, indicated that: (1) fluorescent grit of particle size 250-320μ had the best likelihood of penetrating and becoming established in the epidermis of sockeye fry; (2) these particles, while virtually undetectable in incandescent light, would emit a readily visible fluorescence under ultraviolet light; and (3) these marks would be retained by 60-80% of the marked fish for more than 2 years.

Figures 2-4 show some of the steps in the fry marking operation. Fry were "sandwiched" between two layers of Vexlar[2] mesh that were stapled taut onto 28 x 48 cm wooden frames (see Mattson and Bailey 1969). Fluorescent grit screened to 250-350 μ particle size was sprayed onto both sides of the "sandwich" by a modified sandblasting gun[3] at 80 psi (5.6 kg per sq. cm) and nozzle-target distance of 50 cm. After a brief dipping in a rinsing tank to remove extraneous fluorescent material, the "sandwich" was opened over the holding tank to deposit the marked fry. With three operators - one dispensing the fry, one spraying and the third arranging fry between the Vexlar layers, presenting them for spraying, rinsing, and depositing them in holding tanks - up to 50,000 fry were marked per hour.

MAINTENANCE OF FRY FOR GROWTH ACCELERATION

Four circular fibreglass tanks, two of 8,900 L and two of 4,300 L capacity, were housed in a 30 ft x 24 ft insulated building served with heated and ambient temperature river water, electric space heating and fluorescent lighting.

Water was supplied by electric pump from the Fulton River 20 meters away; a portion was heated by oil-fueled boilers. Heated and normal water entered an 800 L mixing tank and was then distributed by electric pump to the fish holding tanks. In order to reduce the anticipated problems of supersaturation, heated water was delivered through 12 household aerator nozzles and directed over a gas-stripping device consisting of an inclined Vexlar-screen-covered corrugated tray, before entering the mixing tank (Fig. 5). Oxygen levels were monitored periodically by the Winkler method. Water was exchanged at 55 L per minute in each 8,900 L tank and at 22 L per minute in each 4,300 L tank. All tanks were supplied with air by an electric compressor located outside the building.

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[2]Vexlar shelf lining material: Raiman Industries, Vancouver, B.C.
[3]Scientific Marking Materials Ltd., Seattle, Washington.

The fry designated for growth acceleration were distributed among holding tanks at similar densities, with approximately 68,000 in each 8,900 L tank and 33,000 per 4,300 L tank. After 48 hours acclimation at ambient (i.e. river) temperature (6°C), water temperature was elevated at the rate of 2°C per 24 hours, until the desired holding temperature of 16°C was achieved. Fish were initially presented frozen plankton to stimulate feeding, then Oregon Moist Pellets (OMP) by hand at 2-hour intervals during the 10-hour daylight hours. This was supplemented with OMP dispensed by timed automatic feeders 2 hours before and after the above period. The outflow system in each tank was designed for continuous removal of wastes; in addition the tanks were cleaned manually by siphon twice per day.

RELEASE OF FRY

Prior to release of accelerated fry, temperature in the tanks was gradually restored to prevailing river temperature (10°C). In order to reduce possible encounter with resident predators reported to congregate at the river outlet, marked fry were transported in tanks by vehicle and released at two lakeside locations 3 km and 5 km south of the Fulton River outlet. Fry of each category were evenly distributed between the two release sites.

The experimental design included transferring live to the Pacific Biological Station (PBS), Nanaimo, a subsample from each category of marked fry for observation of mark retention over 1 year. For reasons given below, this plan had to be abandoned.

EXAMINATION OF SMOLTS FOR FLUORESCENT MARKS - 1978

Smolts caught daily by a fixed fan-trap at the outlet of Babine Lake were examined on-site 6-14 hours after capture. Equipment and procedures for this purpose were designed to ensure effective and rapid examination for fluorescent marks with minimal stress to the smolts.

Equipment (Fig. 6-8) consisted of (1) an 800 L holding tank with running water exchanged at 18 L per hour; (2) a receiving tray 123 x 100 cm with 4 cm sides and a wedge shaped spill bar 2.0 cm high at the bottom edge; (3) an examination table consisting of a chute 280 x 105 cm with 7.5 cm sides, surfaced with corrugated fiberglass roofing panel (with corrugations running lengthwise); (4) a collecting tub fitted with 10 cm inside-diameter flexible drain hose leading to the lake; and (5) a long-wave ultraviolet lamp consisting of eight 40-watt "black-light" tubes fitted in a 4 x 2 configuration in a 35 x 280 cm fluorescent lamp housing suspended over the examination table.

The lower end of the receiving tray nested in the upper end of the examination table, the lower end of which, in turn, was situated over the collecting tub. The lamp housing, receiving tray and examining table were suspended independently so that they could be inclined as required. The components were so arranged that running water directed to the upper end of the receiving tray would spill evenly on to the full width of the examination table and flow along the troughs formed by the corrugated base. Similarly, smolts deposited on the receiving tray would become spread fairly evenly along the spill bar and be distributed among the troughs of the examination table.

With critical adjustment of water flow, rate of smolt deposition on the receiving tray, and inclination of receiving tray and examination table, it was possible to regulate the distribution and rate of procession of smolts past the examiners.

During their travel down the examination table the smolts, in an effort to right themselves, would characteristically flip over, displaying both sides; otherwise examiners could manually turn over individual smolts. Smolts displaying marks were collected. Ultimately, water and unmarked smolts would accumulate in the catch tub and be released at regular intervals, via drain tubing, downstream of the smolt trap.

Electricity for water pumps, aerators and U.V. lamps was provided by a portable gasoline-fuelled generator. Light-proof drapes were closed during the scanning of smolts under U.V. light. U.V. filtering goggles were worn for protection of eyes during examination.

With one operator dispensing smolts from the holding tank, and four observers, up to 100,000 individuals were scanned per 8-hour working day. All smolts caught in the trap during the 1978 smolt migration (May 4-June 6) were examined except 3,000 per day required for smolt enumeration operations. The smolt scanning procedures used had proven effective in preliminary trials with combinations of unmarked and marked smolts from previous fry-marking experiments.

INVESTIGATION OF IHN IN FULTON SOCKEYE FRY - SUPPLEMENTARY STUDY (1978)

Collections of live sockeye fry: 1,053 from spawning channel 1; 345 from channel 2 and 154 from Fulton River, were transported by car and aircraft to PBS, Nanaimo. Due to the suspected presence of IHN virus among these stocks special authority was required for this transfer[4] and approved quarantine measures were applied throughout this study. Shortly after arrival, the fry were assigned to tanks of appropriate size and were acclimated and thereafter maintained at 10°C; food (Oregon Moist Pellets) was presented regularly; dead and moribund individuals were removed at regular intervals; moribund specimens were submitted to the PBS Diagnostics Laboratory for necropsy.

RESULTS

SURVIVAL OF MARKED FRY 1977

Post-marking mortality was observed for the fry maintained in tanks for growth acceleration. The results, summarized in Fig. 9, indicate an initial mortality of approximately 5% over days 1 and 2 post-marking, followed

[4]Interagency Committee on Transplants and Introduction of fish and aquatic invertebrates in B.C.

by relatively low daily mortalities (0.05% to 0.17%) over days 4-9. Daily mortality increased markedly thereafter, to 60% by day 16. Samples submitted to the Diagnostics Laboratory showed signs of severe IHN. A fairly high level of mortality persisted to the date of release, when only 40,000 survivors remained, most of them noticeably listless.

Due to the proscription against the movement of IHN-infected stocks (Anon. 1977), the plan to transfer subsamples of marked fry to PBS for 1-year monitoring was cancelled.

GROWTH OF CAPTIVE MARKED FRY - 1977

Table 1 shows the growth of Group A fry during their captive period. Despite obvious signs of disease these fry at the time of release were significantly larger than the normal migrants of that time (Group C) ($P<.001$).

Table 1. Sizes of Group A fry on capture and release; size of normal migrants (Group C) at time of Group A release.

	$\overline{X}$ Length mm (SD)	$\overline{X}$ Weight gm (SD)
Group A on capture	27.00 (1.77)	.140 (.030)
Group A on release	31.97 (2.40)	.221 (.083)
Group C	28.96 (1.67)	.137 (.022)

RECOVERY OF MARKED SMOLTS - 1978

A total of 1,100,000 smolts was examined, representing 1.3% of the migrant smolt population. Twenty seven marked smolts were recovered: 3 from the growth accelerated group (A), 5 from the earlier control group (B) and 19 from the later control group (C). Virtually no mortalities appeared to result from the smolt scanning process.

The annual outmigration of Babine smolts is usually bimodal with smolts from the North Arm of Babine Lake and Nilkitkwa Lake nursery areas reaching the outlet first, followed by smolts from Morrison Arm and the main lake (Groot 1972). The marked fish were all recovered from the later run of migrants.

SURVIVAL OF CAPTIVE FRY - SUPPLEMENTARY STUDY (1978)

The mortality rates for channel 1, channel 2 and river fry are presented in Fig. 10. Times to 50% mortality (LT 50) for channel 1, channel 2 and river fry were 10 days, 30 days, and 24 days, respectively. By the end of observations (day 44) total mortalities were 95% for channel 1, 59% for channel 2 and 79% for river fry. IHN was diagnosed in virtually all cases.

DISCUSSION

FRY MORTALITY

The losses occurring among the fry held in 1977 for growth acceleration over days 1 and 2 following marking (Fig. 9) are likely attributable directly to stresses associated with marking, since the rate of mortality is in keeping with that observed in previous fluorescent marking trials with cultured sockeye fry. The subsequent decline in mortality (days 4-9) would suggest recovery from the stresses of marking. The substantial increase in mortality rate thereafter is of considerable concern. Necropsy results tend to implicate IHN in this heavy mortality; similarly IHN appears to be associated with the extensive losses observed among the captive fry of the 1978 study.

Since the Fulton River and spawning channel system contribute on the average 75% of the Babine sockeye production (J. McDonald, pers. comm., West 1978), severe survival problems among Fulton fry should be reflected in considerable depression of the Babine smolt production. The levels of smolt output shown in Table 2 would suggest however that, despite the high prevalence of IHN virus among Fulton fry, the virus normally does not seriously affect fry survival, certainly not to the extent observed among the fry held captive in 1977 and 1978. It is highly conceivable, however, that the stresses associated with our treatment of these fry, e.g. capture, transporting, fluorescent marking, temperature elevation, artificial rearing, were sufficient to induce precipitation of the IHN condition in fry that are normally carriers of the causative virus, leading to severe impairment and ultimately mortality. It should be pointed out that similar manipulations have been carried out repeatedly with other stocks of sockeye fry, with minimal resulting mortality.

Table 2. Fry and smolt production in Babine Lake for brood years 1972 to 1979 (in millions) - from C. J. West 1978, and unpublished records, DFO.

Brood year	1972	1973	1974	1975	1976	1977	1978	1979
Fry: Fulton River and spawning channels	126.5	117.5	91.2	126.8	209.7	130.4	100.9	128.4
Smolts: Babine main lake	61.2	27.4	36.1	51.0	71.0	99.2	47.6	181.2

Examples of the exacerbation of latent infections by external stressors have been documented by other workers. Frantsi et al. (1975) reported the development of severe kidney lesions in *Salmo salar* infected with bacterial kidney disease, in response to the stress of hatchery as opposed to wild conditions. Wedemeyer et al. (1976) also identified handling stress among the agents that commonly lead to severe aggravation of otherwise non-virulent infections in fish.

In view of the considerations above it must be concluded that, given the degree of handling required, fry from the Fulton system, infected as they were with IHN virus, were inappropriate subjects for the pilot experiment in Eubothrium control.

GROWTH ACCELERATION IN FRY: 1977

Notwithstanding the high mortality and the obviously impaired health of the survivors, the fry held captive for growth acceleration were noticeably larger at the time of release than their naturally migrating counterparts. It may be presumed that growth in the tanks would have been more pronounced had the fish been healthier.

MARKED SMOLT RECOVERY: 1978

The occurrence of marked smolts exclusively among the second run of migrants confirms further the distinction between the migration times of North Arm stocks and Main Lake (including Fulton) stocks.

The number of marked smolts recovered was much too low to permit fulfilling the primary objective of this study, viz. to assess the effect of fry size at time of lake entry on susceptibility to Eubothrium infection. Some possible explanations of the low return of marked smolts have been considered:

1. Failure on the part of examiners to recognize marked smolts: this appears unlikely in view of the degree of scrutiny provided for in the examination procedures.

2. Poor mark retention: this also appears not very likely, considering the high degree of mark retention displayed in previous trials with captive fry. On the other hand, Healey et al. (1976) speculated that fluorescent mark retention might be substantially lower in wild than in captive fish.

3. Mortality among marked fish: considerable evidence has been presented above of low survival potential among manipulated channel 1 stocks in 1977 and 1978. Since channel 1 was the exclusive source of fry for the study, it seems reasonable to conclude that major losses of marked fry may have occurred shortly after their release into the lake and that this was reflected in the very low return of marked smolts in 1978. There is the further possibility of intense predation on marked fry at their release sites, despite the measures taken to circumvent this.

CONCLUSIONS

1. Due to the high mortality (attributed to IHN coupled with handling stress) among the Fulton stocks, particularly channel 1 fry, a program of artificial growth acceleration (through temperature and diet manipulation) of these stocks of fry as a means of reducing *Eubothrium* infection in Babine sockeye, must be judged unfeasible for the present.

2. The fry marking techniques, along with the procedures for scanning smolts for fluorescent marks, appear to be effective tools potentially useful in other circumstances requiring the marking of large numbers of small fish for identification a year or more later, provided that the health of the target stocks is conducive to the degree of handling required.

3. The IHN problem identified among Fulton stocks merits further investigation since it represents a potential threat to sockeye production in this important system.

ACKNOWLEDGMENTS

I acknowledge with gratitude the assistance of all personnel who participated in this project, particularly J. Martell, N. B. Cousens, D. Whitaker, R. Brouwer and K. Pasmans; and Drs. L. Margolis and T. P. T. Evelyn for their criticisms of the manuscript.

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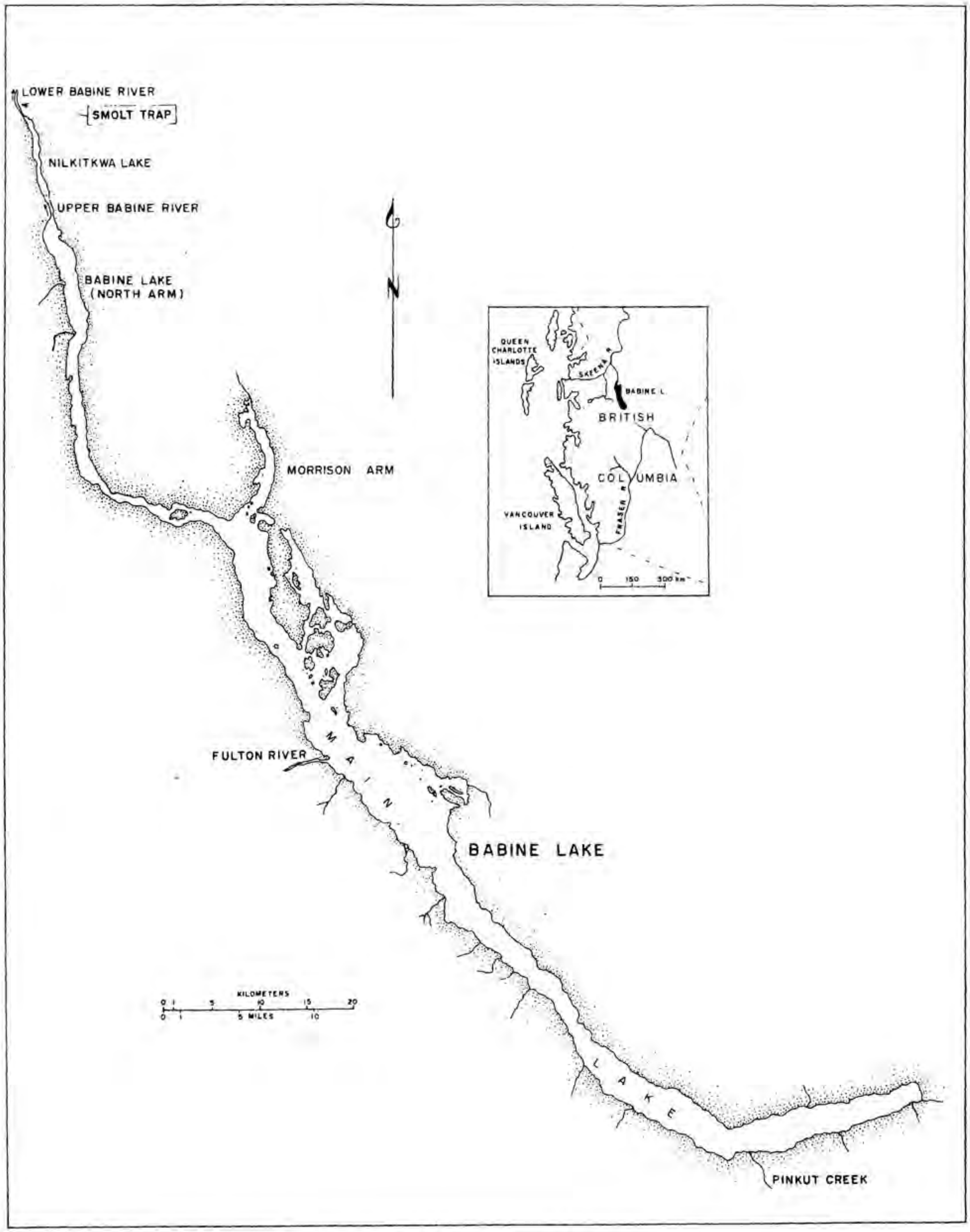

Fig. 1. Babine Lake, British Columbia.

Fig. 2, 3, 4. Procedures for marking fry with fluorescent grit.

Fig. 5. Gas-stripping device for incoming heated water.

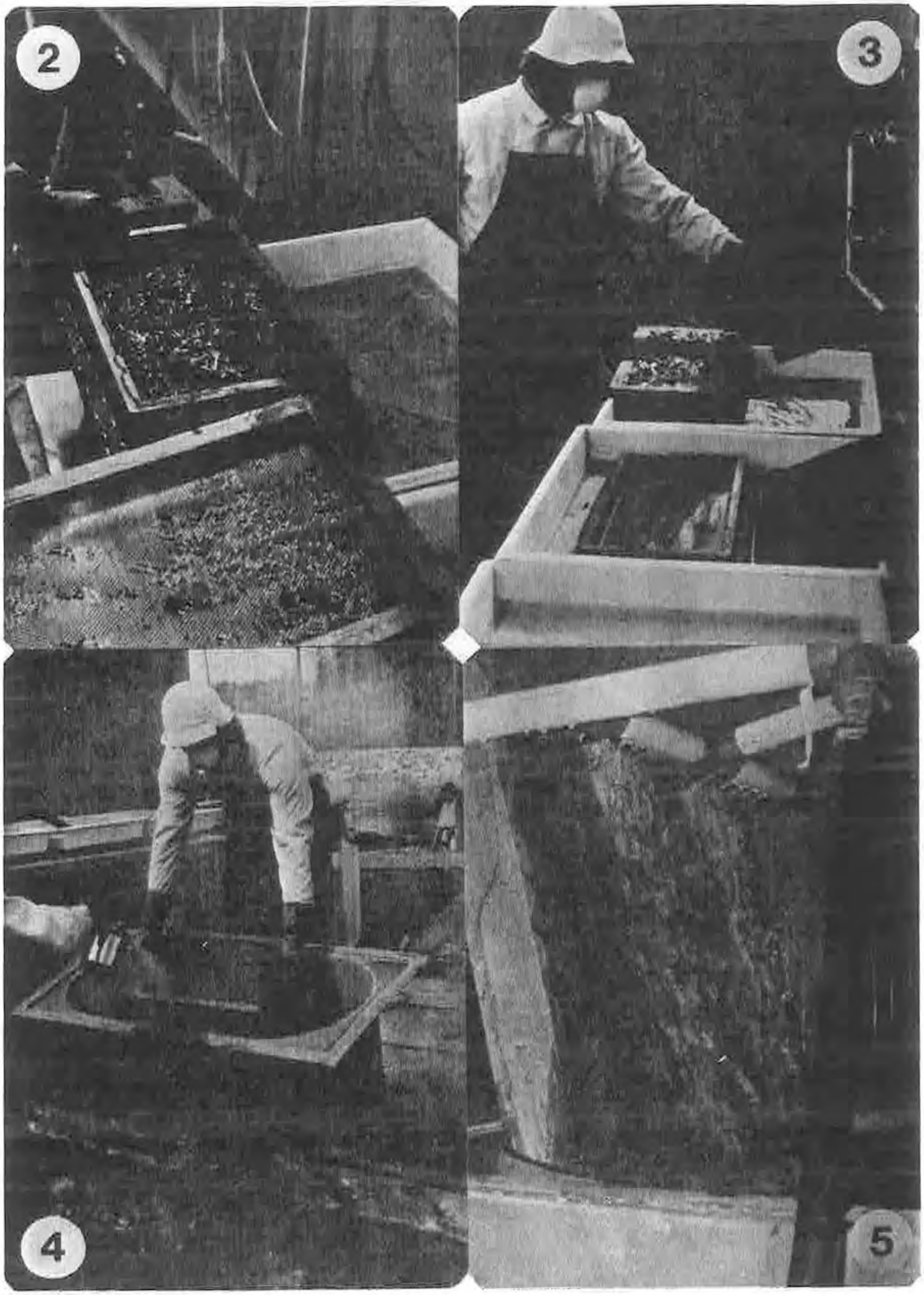
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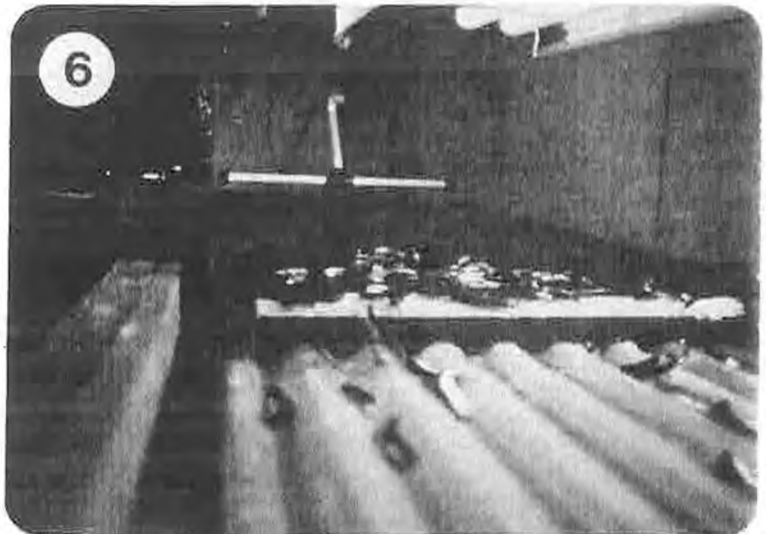
Fig. 6, 7, 8. Equipment for examination of smolts.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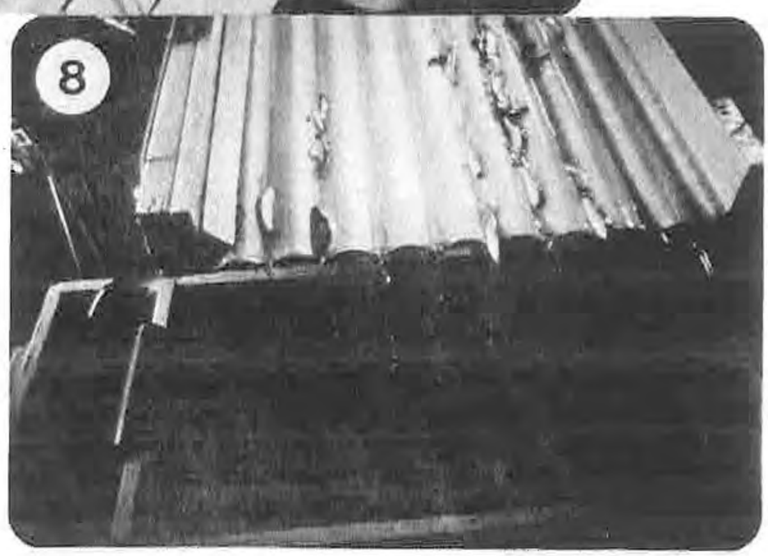
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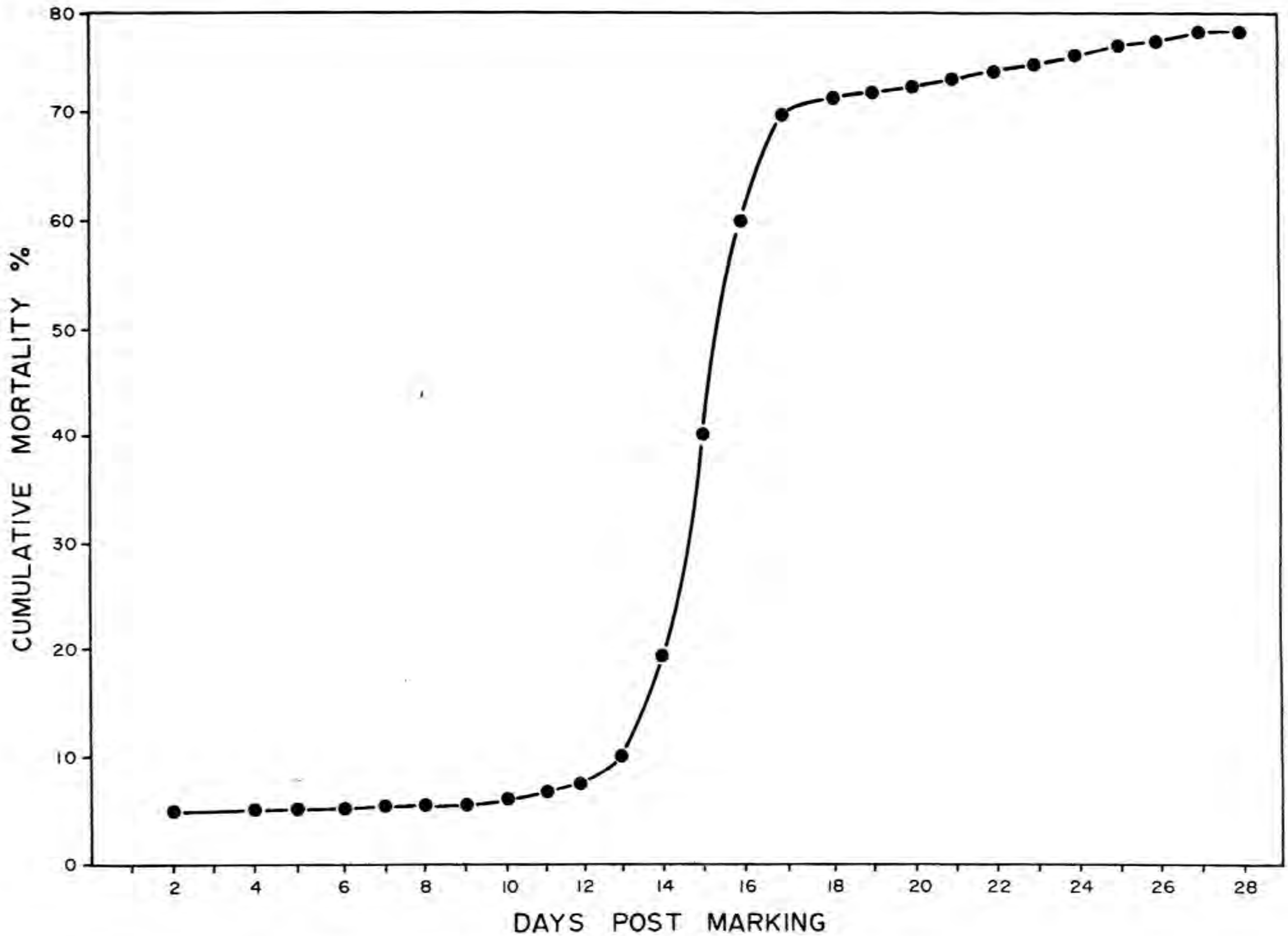

Fig. 9. Post-marking mortalities among sockeye fry held for growth acceleration: 1977.

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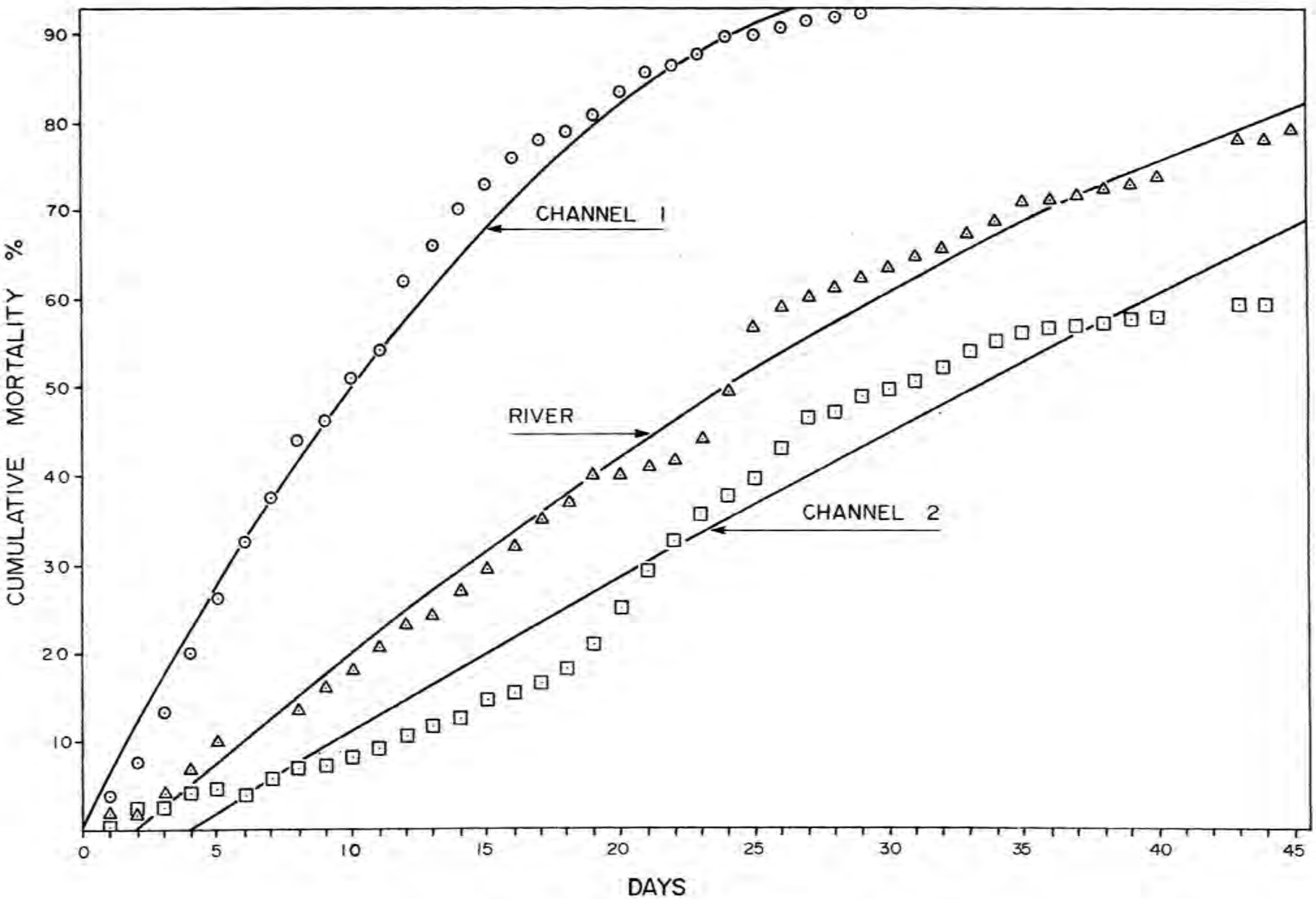

Fig. 10. Mortalities among sockeye fry of Fulton River, Channel 1, and Channel 2 origin: 1978.