Fishery Data Series No. 96-42

Hatching Success of Fertilized Lake Trout Eggs in Artificial Incubation Substrates in Donnelly Lake, Alaska

by

Tim Viavant

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Alaska Department of Fish and Game

Division of Sport Fish

Symbols and Abbreviations

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Weights and measures (metric)

centimeter	cm
deciliter	dL
gram	g
hectare	ha
kilogram	kg
kilometer	km
liter	L
meter	m
metric ton	mt
milliliter	ml
millimeter	mm

Weights and measures (English)

cubic feet per second	ft^3/s
foot	ft
gallon	gal
inch	in
mile	mi
ounce	oz
pound	lb
quart	qt
yard	yd

Spell out acre and ton.

Time and temperature

day	d
degrees Celsius	°C
degrees Fahrenheit	°F
hour (spell out for 24-hour clock)	h
minute	min
second	s

Spell out year, month, and week.

Physics and chemistry

all atomic symbols	
alternating current	AC
ampere	A
calorie	cal
direct current	DC
hertz	Hz
horsepower	hp
hydrogen ion activity	pH
parts per million	ppm
parts per thousand	ppt, ‰
volts	V
watts	W

General

All commonly accepted abbreviations.	e.g., Mr., Mrs., a.m., p.m., etc.
All commonly accepted professional titles.	e.g., Dr., Ph.D., R.N., etc.
and	&
at	@
Compass directions:	
east	E
north	N
south	S
west	W
Copyright	©
Corporate suffixes:	
Company	Co.
Corporation	Corp.
Incorporated	Inc.
Limited	Ltd.
et alii (and other people)	et al.
et cetera (and so forth)	etc.
exempli gratia (for example)	e.g.,
id est (that is)	i.e.,
latitude or longitude	lat. or long.
monetary symbols (U.S.)	$, ¢
months (tables and figures): first three letters	Jan,...,Dec
number (before a number)	# (e.g., #10)
pounds (after a number)	# (e.g., 10#)
registered trademark	®
trademark	™
United States (adjective)	U.S.
United States of America (noun)	USA
U.S. state and District of Columbia abbreviations	use two-letter abbreviations (e.g., AK, DC)

Mathematics, statistics, fisheries

alternate hypothesis	H_A
base of natural logarithm	e
catch per unit effort	CPUE
coefficient of variation	CV
common test statistics	F, t, χ^2, etc.
confidence interval	C.I.
correlation coefficient	R (multiple)
correlation coefficient	r (simple)
covariance	cov
degree (angular or temperature)	°
degrees of freedom	df
divided by	÷ or / (in equations)
equals	=
expected value	E
fork length	FL
greater than	>
greater than or equal to	≥
harvest per unit effort	HPUE
less than	<
less than or equal to	≤
logarithm (natural)	ln
logarithm (base 10)	log
logarithm (specify base)	log_2, etc.
mideye-to-fork	MEF
minute (angular)	'
multiplied by	x
not significant	NS
null hypothesis	H_O
percent	%
probability	P
probability of a type I error (rejection of the null hypothesis when true)	α
probability of a type II error (acceptance of the null hypothesis when false)	β
second (angular)	"
standard deviation	SD
standard error	SE
standard length	SL
total length	TL
variance	Var

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Tim Viavant
Division of Sport Fish, Fairbanks

Alaska Department of Fish and Game
Division of Sport Fish, Research and Technical Services
333 Raspberry Road, Anchorage, Alaska, 99518-1599

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Tim Viavant
Alaska Department of Fish and Game, Division of Sport Fish, Region III,
1300 College Road, Fairbanks, AK 99701-1599, USA

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ABSTRACT

Lake trout *Salvelinus namaycush* eggs and milt were taken from fish in Seven Mile Lake during early September, 1995. After the eggs were fertilized and water hardened for 1.5 h, they were transported to Donnelly Lake and placed into artificial spawning substrates at two different water depths and two different substrate configurations by scuba divers. Artificial substrates were constructed of layers of AstroTurf™ sandwiched within wooden frames. The number of eggs placed into each substrate layer was estimated volumetrically. In early July of 1996, the substrates were retrieved by scuba divers, and the numbers of dead, unhatched lake trout eggs in each substrate were counted. Percent survival to hatching was estimated by subtracting the number of dead eggs in each layer from the estimated number of live eggs originally placed into each layer. Overall hatching success was estimated at 43.4% (SE = 2.2 %), as compared with 72.7% for the same egg lot incubated in a hatchery.

Key words: artificial incubation substrates, artificial turf, lake trout *Salvelinus namaycush*, hatching success.

INTRODUCTION

Lake trout occur naturally in a number of higher altitude, oligotrophic lakes in interior Alaska. These populations tend to be relatively sparse, and lake trout in these lakes relatively slow growing compared with populations in more temperate areas (Martin and Olver 1980). Increases in the harvest of lake trout from several of these lakes during the late 1980s combined with stable or increasing effort during the early 1990s (Mills 1986 - 1994, Howe et al. 1995) lead to concerns over lake trout population abundance. These concerns lead to the implementation of restrictive regulations in several of these lakes over the past several years.

In an effort to diversify fishing opportunity, and to divert effort from wild stocks, the Alaska Department of Fish and Game (ADF&G) stocking program has stocked lake trout annually into many lakes and ponds in the interior which contain no indigenous stocks of lake trout. Many of these lakes do not contain substrate suitable for lake trout spawning, and are not likely to develop naturally-reproducing populations of lake trout. Therefore, annual stockings of fingerlings are required in order to maintain a population that can support a sport fishery. The costs associated with providing these lakes with a yearly cohort of lake trout could be substantially reduced if these lakes could be planted with fertilized, water-hardened eggs (instead of with fingerling lake trout), and if these eggs have a high proportion of successful hatching.

While many factors influence the productivity of lake trout in these lakes, survival of eggs from spawning to hatching may be limited due to lack of suitable spawning habitat (Marsden and Krueger 1991) and/or egg predation (Wagner 1992, Martin and Olver 1980). It is likely that annual recruitment of lake trout could be increased by increasing the survival of eggs to the fry stage. The survival of eyed lake trout eggs planted directly onto spawning reefs has been found to be extremely low (1.8% or less) in Lake Michigan (Wagner 1992), however, lake trout have been successfully hatched in artificial substrates in Lake Superior, with hatching success rates of 70 to 90% (Swanson 1982). This study was undertaken to attempt to determine the hatching success rate of fertilized, water-hardened lake trout eggs placed into artificial substrates at different depths and in different configurations of artificial spawning substrates in Donnelly Lake.

Donnelly Lake does not contain a natural population of lake trout, and therefore, survival from the fry stage to fish of sufficient size to recruit to sampling gear should be quantifiable. Donnelly Lake also appears to contain substrates suitable for lake trout spawning, so it is possible that a naturally-reproducing population of lake trout could be established by having fry emerge from artificial substrates placed over suitable spawning habitat, since several studies have found

considerable evidence that lake trout return to their natal reef to spawn (Eschmeyer 1955, Martin 1957, Loftus 1958, Rahrer 1968, Swanson 1974), and that lake trout imprint on the reef on which they emerged from very early in life (Horrall 1981).

The objectives of this study were:

1. to estimate the proportion of lake trout eggs that successfully hatch from artificial substrates placed into Donnelly Lake at two different depths and with two different numbers of layers per substrate; and,
2. to test the hypothesis (H_o), that the proportion of lake trout eggs that successfully hatch from artificial substrates placed at differing depths and/or from substrates having different numbers of layers per substrate will be equal, versus the alternative hypothesis (H_a), that the proportions of lake trout eggs that successfully hatch from artificial substrates placed at differing depths and/or from substrates having different numbers of layers per substrate are not the equal. This experiment should be able to detect a difference in the proportion of eggs successfully hatching of 15 percentage points 80 percent of the time.

METHODS

Artificial substrates were constructed consisting of four or eight sheets of AstroTurf™ (two or four layers of two sheets each) sandwiched together in a wooden frame (Figure 1). Sheets of AstroTurf™ were 30.5 cm wide by 91.5 cm long, and were positioned such that each layer consisted of two sheets with the turf sides facing each other. Wooden frames were constructed to hold the layers in place with as little as possible movement of the AstroTurf™ sheets and as much possible water flow through and around the layers.

During early September, 1995, lake trout eggs and milt were taken from lake trout captured as part of the hatchery egg-take scheduled for Seven Mile Lake. After fertilization at Seven Mile Lake, the eggs were allowed to water harden for 1.5 h, and were then transported in jars inside coolers filled with lake water to Donnelly Lake. Lake trout eggs were loaded into four-liter jars in the order that the fish were spawned until each jar was three quarters full. Each jar contained the eggs and milt from approximately 12 females and 12 males.

Artificial substrates were loaded with fertilized, water hardened, lake trout eggs at Donnelly Lake, and placed at depth by divers. Artificial substrates were suspended 20 cm above the lake bottom, over substrates suitable for lake trout spawning (mixed cobble/gravel). Substrates were anchored with steel anchors and equipped with floats to maintain the position of the substrates relative to the lake bottom. Three replicates of two and four layers each were placed at depths of 2.5 m and 4.0 m, resulting in a total of 12 substrates containing a total of 36 layers.

A numbers-to-volume ratio for lake trout eggs used in the experiment was developed by counting the number of water-hardened lake trout eggs contained in a volume of 25 ml, measured in a graduated cylinder. For each volume of eggs counted, the number of dead eggs was also recorded. The total number of eggs and the number of dead eggs were counted for each of six different sets of this same volume of water hardened lake trout eggs. After this number-to-volume ratio was determined, 90 ml of eggs were placed in each layer of each substrate. All eggs placed into substrate replicates placed at 2.5 m water depth were taken from egg jar number one.

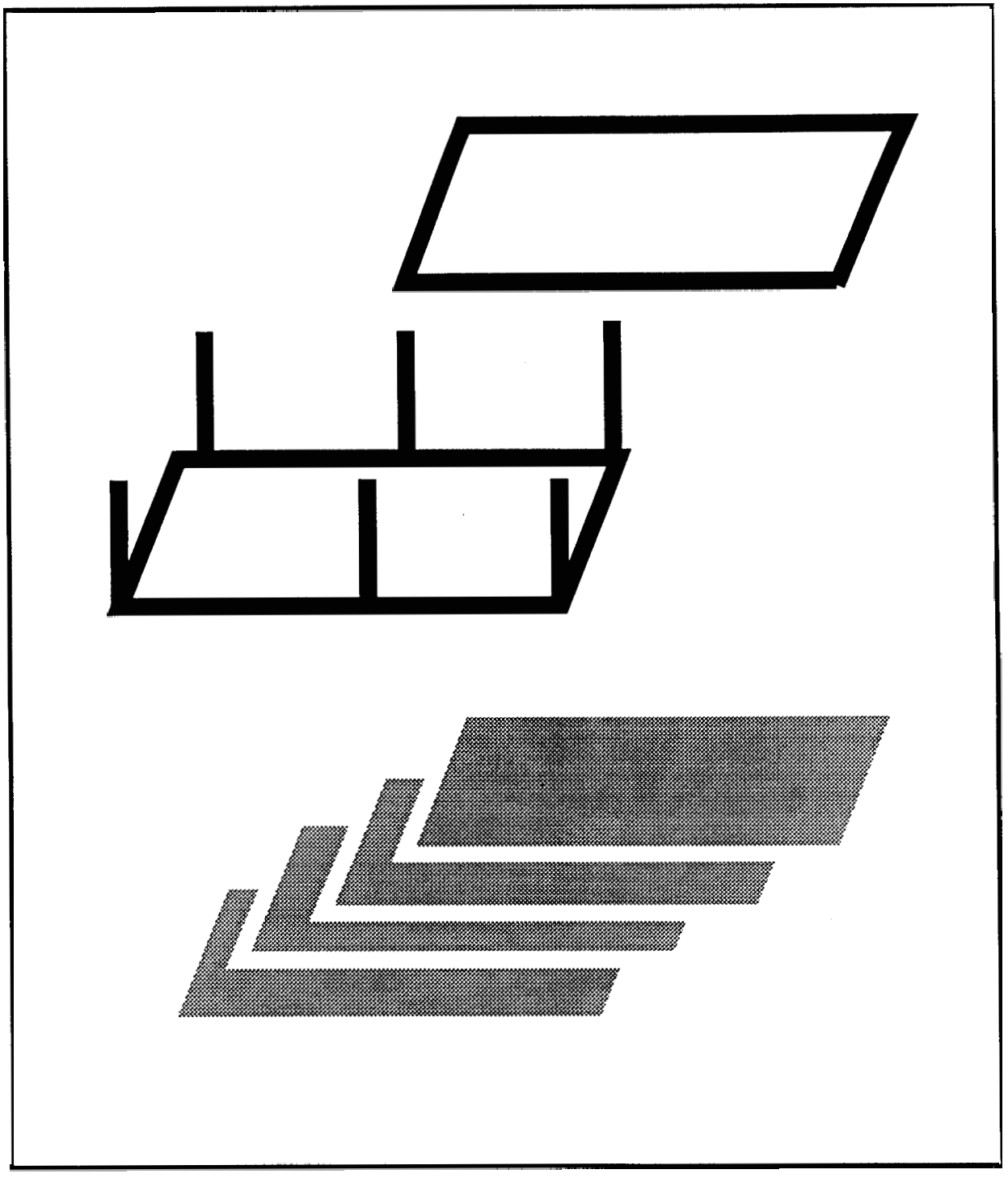

Figure 1.-Diagramatic representation of lake trout egg incubation substrate showing frame (top) and AstroTurf™ layers (bottom).

After these substrates (2.5 m deep) were placed, it became apparent that the lake trout eggs in the jars were still taking on water, and increasing in volume. Because of this, the remaining eggs in jar number one were not used. Before beginning to place eggs into the substrates to be placed at 4.0 m depth, a numbers-to-volume ratio was developed for the eggs in jar number two. The total number of eggs and the number of dead eggs were counted and recorded from six different sets of 25 ml of eggs taken from jar number two. All eggs loaded into substrates to be placed at 4.0 m deep were taken from jar number two. The number of eggs originally placed into each layer was estimated as the average number of live eggs/ml minus the average number of dead eggs/ ml (calculated for each jar) times the number of ml of eggs placed into each layer (90).

Substrates were recovered by divers during early July, 1996. The dead eggs remaining in each layer of each substrate were counted and the number recorded. The difference between the number of live eggs estimated to have been placed in each layer of the substrates in the fall and the number of dead eggs counted the following summer was assumed to be the number of eggs successfully hatched from each layer of each substrate.

Eggs and layers were distributed for a two-way factorial design in the following manner, resulting in a estimated total of 16,596 eggs for the entire experiment.

	Depth 1	Depth 2
2 Layers	3 Replicates (2,904 eggs)	3 Replicates (2,628 eggs)
4 Layers	3 Replicates (5,808 eggs)	3 Replicates (5,256 eggs)

The estimated proportion of successfully hatched eggs from each substrate was calculated as:

$$P_h = \frac{K - M}{K} \qquad (1)$$

where P_h = the estimated proportion of lake trout eggs successfully hatching from a substrate;

K = the estimated number of eggs placed into the artificial substrate; and,

M = the number of unhatched eggs counted after the substrates were recovered.

Analysis of variance was used on the proportions to examine the effects of depth and number of layers on hatching success. This experiment was a completely randomized design with a factorial treatment arrangement, and all treatments are considered fixed. The model used was:

$$Y_{ijk} = \mu + D_i + L_j + DL_{ij} + e_{ijk} \qquad (2)$$

where: Y_{ijk} = the estimated proportion of successfully hatched eggs from the kth replicate substrate, placed at the ith depth level with j^{th} number of layers ;

μ = overall mean hatching success rate of all treatments;

D_i = effect of the ith depth;

L_j = effect of the jth number of layers;

DL_{ij} = effect of the interaction of the ith depth and the jth number of layers;

e_{ijk} = error of the kth replicate substrate, placed at the ith depth level with j^{th} number of layers.

RESULTS AND DISCUSSION

Estimated rates of hatching success from individual substrates ranged from just over 24% to just under 53% (Appendix A). The average rate of hatching success from all substrates was 43.4 % (SE = 2.2%). There were no significant differences in the estimated proportions of eggs hatching from substrates at different depths ($F = 1.63$, $p = 0.21$) or in the estimated proportions of eggs hatching from substrates having differing numbers of layers ($F = 0.17$, $p = 0.68$), so the null hypothesis in objective number one is not rejected. There was a statistically significant interaction between the effects of depth and number of layers ($F = 5.13$, $p = 0.03$), however, there is no reason to believe that this interaction is biologically significant.

The error associated with the estimates of the proportion of successfully hatched eggs from individual layers within each substrate arise from the error in estimating the total number of eggs originally placed in each substrate, and the error in estimating the number of dead eggs originally placed in each substrate. The 95% confidence intervals for the number of live eggs placed into each layer of each substrate was from 441 to 473 for substrates placed at 2.5 m, and from 406 to 432 for substrates placed at 4.0 m (Table 1). This uncertainty translates into 95% confidence intervals for the percentages of successfully hatched eggs (from each layer within each substrate) with a lower bound as low as 0.63 % and an upper bound as high as 1.83 % (Appendix A).

The overall average proportion of successful hatching is substantially less than the 72.7 % hatching success observed from the same eggs that were hatched at Clear Hatchery. It is possible that the substrates were placed in Donnelly Lake at depths that were too deep to allow for wave action to maintain water circulation through the substrates prior to freeze-up. Natural spawning beds at Seven Mile Lake and Paxson Lake are in water generally less than 2 m (J. Burr, Alaska Department of Fish and Game, Fairbanks, personal communication). Other studies outside of Alaska, however, have reported lake trout spawning in depths from 0.2 m to 61 m (Martin and Olver 1980). It is also possible that water quality parameters (such as dissolved oxygen content during ice cover) at Donnelly Lake may only be marginally suitable for lake trout egg incubation.

The hatching success rates observed in this study are also much lower than those achieved by Swanson (1982) in Lake Superior, who observed rates of 70 to 90 %. Artificial substrates used in Lake Superior are placed at a depth of 13.5 m, over a spawning reef that is in an area of very strong currents, which would provide oxygenated water to the eggs during incubation.

Although hatching success rates were not as high in this experiment as in the hatchery or in other studies, the costs of providing lake trout plantings by this method are extremely low in comparison to hatchery costs. The additional potential benefit of stocking fertilized eggs instead of fingerling fish from a hatchery has to do with the homing instinct and fidelity to spawning sites

Table 1.-Results of volumetric estimation from lake trout eggs placed into artificial incubation substrates at Donnelly Lake, Alaska.

Jar Number One (eggs placed into substrates at 2.5 m deep).

ml of eggs	# of eggs	dead eggs			
			Jar # 1 Average Number of Eggs/25 ml		134.33
25	139	5	Jar # 1 S. E. of Average Number of Eggs/25 ml		1.41
25	136	7	Jar # 1 Estimated Number of Eggs/ ml		5.37
25	135	9	Jar # 1 Average Number of Dead Eggs/25 ml		7.33
25	132	7	Jar # 1 S.E. of Average Number of Dead Eggs/25 ml		0.76
25	129	6	Jar # 1 Estimated Number of Dead Eggs/ml		0.29
25	135	10	Jar # 1 Estimated Total Number of Eggs/Layer		483.60
			Jar # 1 Estimated Number of Dead Eggs/Layer		26.40
			Jar # 1 95% CI Dead eggs/Layer		5.51
			Jar # 1 Estimated Number of Live Eggs/Layer		457.20
			Jar # 1 95% CI Live Eggs/layer	+ or -	15.72

Jar Number Two (eggs placed into substrates at 4.0 m deep).

ml of eggs	# of eggs	dead eggs			
			Jar # 2 Average Number of Eggs/ 25 ml		121.67
25	121	3	Jar # 2 S. E. of Average Number of Eggs/25 ml		1.23
25	118	7	Jar # 2 Estimated Number of Eggs/ ml		4.87
25	122	5	Jar # 2 Average Number of Dead Eggs/25 ml		5.33
25	126	6	Jar # 2 S.E. of Average Number of Dead Eggs/25 ml		0.56
25	119	5	Jar # 2 Estimated Number of Dead Eggs/ml		0.21
25	124	6	Jar # 2 Estimated Total Number of Eggs/Layer		438.00
			Jar # 2 Estimated Number of Dead Eggs/Layer		19.20
			Jar # 2 Estimated Number of Dead Eggs/Layer		19.20
			Jar # 2 95% CI Dead eggs/Layer		4.05
			Jar # 2 Estimated Number of Live Eggs/Layer		418.80
			Jar # 2 95% CI Live Eggs/layer	+ or -	12.96

that have been shown in lake trout. If fertilized eggs can be planted over either existing, or artificially created, spawning substrates, there is a much greater likelihood of establishing a spawning population in a lake than simply by stocking fingerling fish into the lake.

Future studies of hatching success from lake trout eggs planted in artificial incubation substrates should examine the estimated hatching success rates from substrates placed in water less than 2 m, and from substrates placed over known spawning substrates. Future studies could also reduce the error associated with the volumetric estimation of the number of eggs originally placed into substrates by either allowing the eggs to absorb water longer prior to conducting volumetric trials, or by the much more time consuming method of counting the live eggs placed into each substrate. Alternatively, a completely randomized block design could be use by allocating groups of eggs to treatments (i.e. volumetrically measure out four units of eggs and randomly assign each to a treatment as a block). Long-term studies should also investigate the success of establishing new spawning sites by placing substrates over suitable natural substrates that are not currently spawning sites in lakes were lake trout already spawn at other locations. These locations could then be monitored five to seven years later for the presence of spawning lake trout.

The long term success of this experiment will be examined by the ongoing periodic evaluation of the stocking program at Donnelly Lake, which involves test netting with fyke traps and gill-nets. Donnelly Lake has never been stocked with lake trout and does not contain natural stocks of lake trout. If lake trout of sufficient size to recruit to the gear used in evaluations are captured, that will confirm that at least some fish survived from beyond hatching.

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APPENDIX A

Appendix A-Counts of dead eggs, estimated number hatched, and estimated proportion hatched from artificial lake trout egg incubation from Donnelly Lake, Alaska.

Depth (m)	Number of Layers	Replicate	Dead Egg Count	Estimated Number Hatched	Estimated Percentage Hatched/ Layer	95 % C.I. of Estimated Percentage Hatched/Layer	Estimated Percentage Hatched/ Substrate
2.5	2	1	251	206	45.1	43.6 - 46.7	47.2
2.5	2	1	232	225	49.2	47.6 -51.0	
2.5	2	2	278	179	39.2	37.9 -40.6	43.0
2.5	2	2	243	214	46.8	45.3 -48.5	
2.5	2	3	267	190	41.6	40.2 -43.1	47.5
2.5	2	3	213	244	53.4	51.6 -55.3	
2.5	4	1	361	96	21.0	20.3 -21.8	24.1
2.5	4	1	349	108	23.7	22.9 -24.5	
2.5	4	1	371	86	18.8	18.2 -19.5	
2.5	4	1	307	150	32.8	31.8 -34.0	
2.5	4	2	286	171	37.4	36.2 -38.8	44.1
2.5	4	2	275	182	39.8	38.5 -41.3	
2.5	4	2	238	219	47.9	46.4 -49.7	
2.5	4	2	223	234	51.2	49.5 -53.0	
2.5	4	3	245	212	46.4	44.9 -48.1	50.0
2.5	4	3	234	223	48.8	47.2 -50.6	
2.5	4	3	226	231	50.6	48.9 -52.4	
2.5	4	3	210	247	54.1	52.3 -56.0	
4	2	1	252	167	39.8	38.6 -41.1	41.1
4	2	1	241	178	42.4	41.2 -43.8	
4	2	2	324	95	22.6	22.0 -23.4	38.5
4	2	2	191	228	54.4	52.8 -56.1	
4	2	3	249	170	40.5	39.3 -41.8	38.9
4	2	3	263	156	37.2	36.1 -38.4	
4	4	1	204	215	51.3	49.7 -52.9	48.4
4	4	1	214	205	48.9	47.4 -50.5	

-continued-

Appendix A.-Page 2 of 2.

Depth (m)	Number of Layers	Replicate	Dead Egg Count	Estimated Number Hatched	Estimated Percentage Hatched/ Layer	95 % C.I. of Estimated Percentage Hatched/Layer	Estimated Percentage Hatched/ Substrate
4	4	1	213	206	49.1	47.7 -50.7	
4	4	1	234	185	44.1	42.8 -45.5	
4	4	2	208	211	50.3	48.8 -51.9	45.5
4	4	2	279	140	33.4	32.4 -34.4	
4	4	2	247	172	41.0	39.8 -42.3	
4	4	2	179	240	57.3	55.5 -59.1	
4	4	3	186	233	55.6	53.9 -57.4	53.0
4	4	3	258	161	38.4	37.2 -39.6	
4	4	3	163	256	61.0	59.2 -63.0	
4	4	3	181	238	56.8	55.1 -58.6	