PRELIMINARY FINDINGS ON THE INCIDENCE AND DISTRIBUTION OF A VIRAL BLOOD

INFECTION (EICDV) IN THE ATLANTIC HERRING, *Clupea harengus harengus*

ALONG THE MAINE COAST.

by

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ABSTRACT

An average of 18.6% (83/446) of the Atlantic herring, *Clupea harengus harengus*, sampled from 7 locations along the Maine coast from Casco Bay to Machias were infected with viral blood lesions. Typical EICDV herring type lesions were found in 13.7% (61/446) of the herring, but 4.9% (22/446) had lesions characteristic of those usually found in EICDV infected Atlantic cod, *Gadus morhua*. Herring were collected in 15 samples from May 27, 1976 to September 29, 1977 and ranged in total length from 11.2-32.5 cm (approximately 1-7 years old). The herring type virus was found at each sampling location; cod type lesions were found in four of the seven locations. Herring with herring type lesions had up to 99.0% of their red cells infected; all herring with cod type lesions were lightly infected (<0.1%). No individual had both cod and herring type lesions. Infected herring were found at temperatures ranging from $3.5^{\circ}C$ to $19.5^{\circ}C$. High incidences were found at Bailey Island (45.7%) and at Mt. Desert Island (44.7%). There may be a relation between length of holding time before transport to the canneries and high incidences of infection. When herring were placed in captivity the incidence increased from 14.3% (4/28) to 95.0% (19/20) after 13 days. Differential cell counts were extremely difficult or impossible in severely infected herring due to the scarcity of distinguishable white cells in the blood smears.

INTRODUCTION

Two erythrocytic icosahedral cytoplasmic deoxyriboviruses (EICDV) have recently been confirmed as agents responsible for blood infections in the Atlantic cod, *Gadus morhua* and Atlantic herring, *Clupea harengus harengus*. Laird and Bullock (1969) observed cytoplasmic inclusions in red blood cells of *Gadus*, *Liparis*, and *Myoxocephalus* and termed the disease piscine erythrocytic necrosis (PEN). Walker (1971) reported electron microscopic evidence that PEN was an EICDV infection in Atlantic cod, *Gadus morhua*. Sherburne (1973) reported cytoplasmic inclusions associated with erythrocytic degeneration in the Atlantic herring from Boothbay Harbor, Maine and Deer Island, New Brunswick, Canada. This disease has recently been confirmed by electron microscopy as an EICDV infection (Philippon, Nicholson and Sherburne 1977; Reno, Philippon-Fried, Nicholson and Sherburne 1978).

This report: 1) shows incidence and distribution data for this viral infection from seven locations along the Maine coast from Casco Bay to Machias; 2) documents the finding of characteristic cod type viral blood lesions in herring from four locations, and 3) suggests that there may be a relation between length of holding time before transport to the canneries and high incidences of viral infection.

MATERIALS AND METHODS

The 446 herring examined in this study from May 27, 1976 to September 29, 1977 were collected in 15 samples from 5 areas (7 individual locations) along the Maine coast from Casco Bay to Machias. The 5 areas were:

Casco Bay (Chebeague Island and Bailey Island), Boothbay (Indiantown Island
and Boothbay Harbor), Mt. Desert Island, Bois Bubert Island and Little
Machias Bay. The herring ranged in total length from 11.2-32.5 cm (approxi-
mately 1-7 years old). Herring were sampled from fishermen's catches and
had been captured in either stop or purse seines. Some herring were sampled
immediately after they were captured, others after being held in confinement
at the catch site for up to 17 days.

The surface water temperature was recorded at the site of capture in
each instance. Approximately 150 herring were held captive in seawater
which was pumped from the ocean through the holding tank and which approxi-
mated the temperature of natural seawater. Groups were sacrificed at intervals
during a period of one month, when the supply of test fish was exhausted, to
monitor changes in infection rates.

Routine morphological examination was made of blood obtained from the
caudal peduncle of live herring. The caudal peduncle was wiped free of
water and mucus with a clean towel, severed, a small drop of blood placed
on a clean slide, and a smear made. Air dried smears were fixed in absolute
methanol for 3 minutes and stained with diluted Giemsa stain for 30 minutes.
Cells were examined under oil immersion at 1000X.

RESULTS

Fig. 1 shows the sampling locations and the incidence of viral
infection at each location. Overall, an average of 18.6% (83/446) of the
herring were infected with viral blood lesions. Typical EICDV herring type
blood lesions (Fig. 2) were found in 13.7% (61/446) of the herring, but 4.9%
(22/446) had blood lesions characteristic of those usually found in EICDV

infected Atlantic cod (Figs. 3,4,5). Infected red cells of both herring and cod usually have cytoplasmic inclusions but infected cod red cells are characterized by nuclear patterns not previously found in herring.

Herring type lesions were found at each sampling location; cod type lesions were found at four of the seven locations (Table 1). Individuals with herring type lesions had up to 99.0% of their red cells infected while all herring with cod type lesions were lightly infected (<0.1%). No individual had both cod and herring type lesions. There was no significant relation of incidence of infection to total length.

Similar incidences were evident from western and eastern Maine. The western area, Casco Bay to Boothbay Harbor, had an average of 17.1% (53/310) of the herring infected; 6.4% (20/310) had cod type lesions. The eastern area, Mt. Desert to Machias, had 22.1% (30/136) infected; 1.5% (2/136) had cod type lesions. High incidences were found in individual samples from both western and eastern Maine - 45.7% (16/35) at Bailey Island; 44.7% (17/38) at Mt. Desert. The Bailey Island sample was unusual in that 40.0% (14/35) of the herring had cod type lesions.

Approximately 150 herring from the May 27, 1976 lot placed in captivity showed a rapid increase in the incidence of infection, i.e., from an initial 14.3% (4/28) to 57.1% (12/21) after 6 days to 95.0% (19/20) after 13 days. Herring had a predominance of immature red cells infected after 6 days in captivity whereas after 13 days up to 90% of both immature and mature red cells were infected. Water temperatures were nearly the same on the initial, 6 day and 13 day samplings - 14°C, 14°C and 15°C, respectively. Approximately a 90% mortality occurred on day 14, but we have no evidence that the viral

infection was associated with the die-off, for we were in the field at the time and the herring had been disposed of before we returned. However, several days later 3 of 5 survivors had >50% of their red cells infected, 1 other had <1%; the remaining individual had no infection.

Differential cell counts were extremely difficult or impossible in severely infected herring due to the scarcity of white cells in the blood smears. Excluding thrombocytes, herring with 90% or more of their red cells infected had less than 100 distinguishable white cells on the slides; most had less than 50. The smears were characterized by more cellular debris and disintegrating cells than are usually found in uninfected or lightly infected herring. Anucleated "balloon" (ghost) cells accounted for approximately 40% of the cells in some smears. White cell differentials of lightly infected herring did not differ markedly from other herring in their respective samples.

DISCUSSION

This disease was first discovered in Atlantic herring, *Clupea harengus harengus*, in 1969 (Sherburne 1973) from Boothbay Harbor, Maine and Deer Island, New Brunswick, Canada. When herring were again sampled in 1976 the incidence from the Boothbay Harbor area was three times that of 1969 - 16.5% (18/109) vs. 5.4% (6/111), and 2.8% of the sample had cod type lesions. Cellular pathological changes were similar to those in 1969 for herring with herring type lesions.

Herring type lesions were previously found in 1969 from 6 of 111 wild herring from the Boothbay Harbor, Maine area, 2 of 50 from Deer Island,

New Brunswick, 0 of 40 from Eastport, Maine, and, in 1972, 0 of 26 from

Georges Bank. A review of these slides showed cod type lesions in 2 of

111 from Boothbay Harbor, 1 of 50 from Deer Island, 0 of 40 from Eastport

and 3 of 26 from Georges Bank. No individual had both cod and herring

type lesions; those with cod type had <0.1% of their red cells infected.

Electron microscopy has not been feasible on herring with cod type lesions

to determine if they are infected with the cod virus per se because of

their light infections.

We have not found any definite external symptoms associated with this

disease. We have observed a few severely infected captive herring swimming

lethargically near the surface. Their backs were darker in color than

normal and some had small hemorrhages at the base of the fins.

Sardine canneries have recently reported poor quality packs from several

areas along the coast. Herring did not transport well and the canneries were

having more trouble with rejected processed lots than in past years. Although

we have no direct correlation between the presence of virus and poor packs

at this time, several catches with a relatively high incidence made poor

packs.

Two of the highest incidences (44.7% and 45.7%) were from herring that

had been held the longest (7 and 17 days, respectively) before transport to

the canneries (Table 1). Whether these incidences were present at the time

of catch is unknown, although one other catch had an incidence of 32.0% when

caught. Catches will have to be monitored in several areas from day one

onward to determine if there is a substantial increase in the disease when

wild herring are held in confinement.

There have been rapid increases in infection rates whenever herring have been placed in captivity. Whether this is a result of the infection passing from one to the other or reflects time for development of a previously latent infection, perhaps induced by the stress of confinement is unknown. Our results in 1976, when the incidence increased from 14.3% (4/28) to 95.0% (19/20) after 13 days paralleled those in 1969 (Sherburne 1973) when the incidence increased from 6.6% (5/76) to 96.0% (24/25) after 14 days. These results reinforce the thought that when herring are held in confinement before transport to canneries they may show increased levels of this disease.

If there is a relation between length of holding time, high viral infection and poor packs we may be able to determine the optimum time herring should be held in confinement before transport to the cannery. If the length of holding time is not a factor but high incidence at the time of catch is, then it would be possible to test sardines at selected catch sites, thus decreasing the number of rejected lots at the cannery.

Literature Cited

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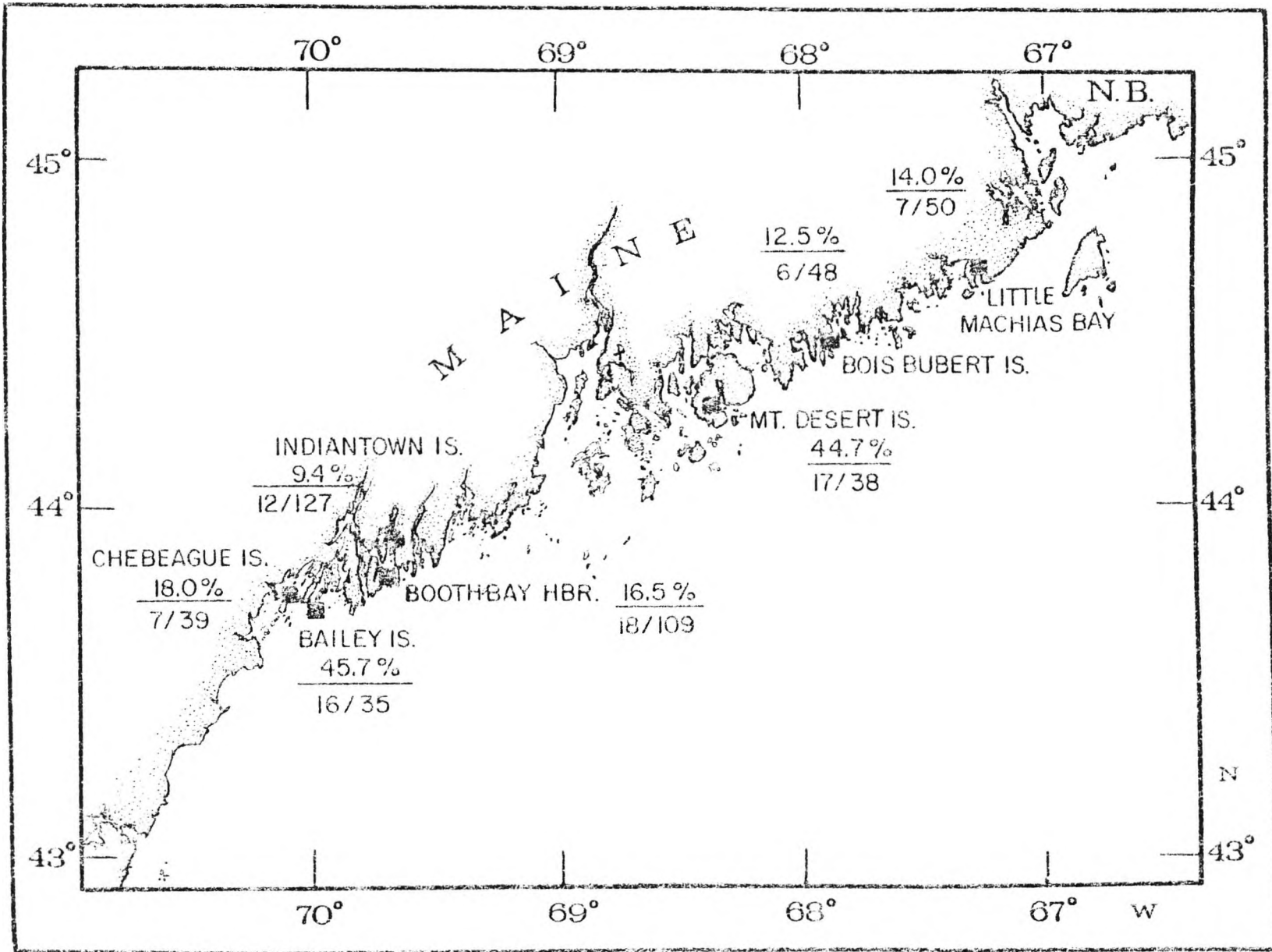Walker, R. 1971. PEN, a viral lesion of fish erythrocytes. Am. Zool.
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Fig. 1 The incidence and distribution of an EICDV blood infection in the Atlantic herring, *Clupea harengus harengus*, along the Maine coast.
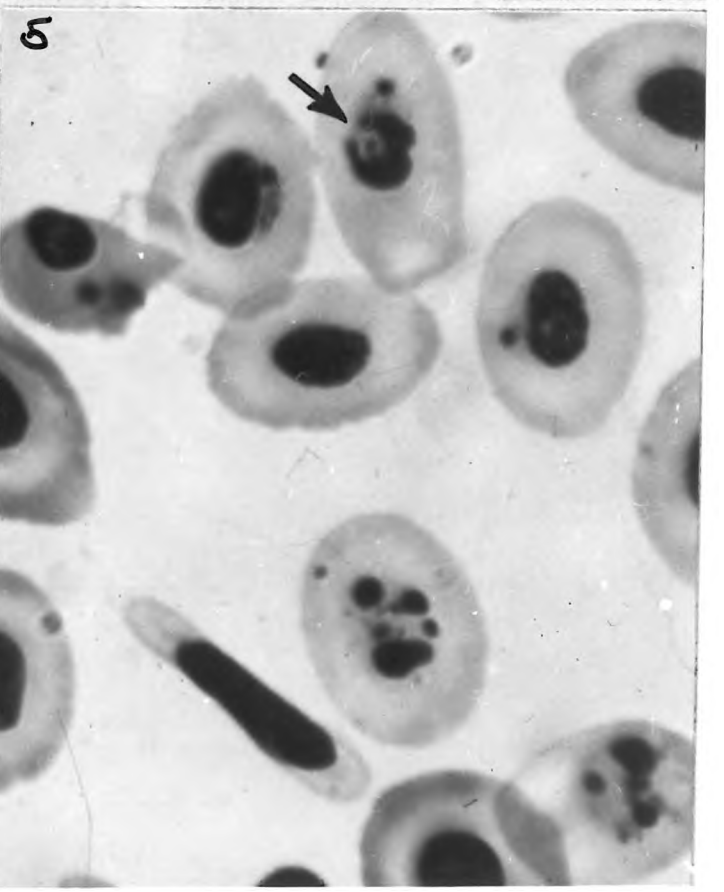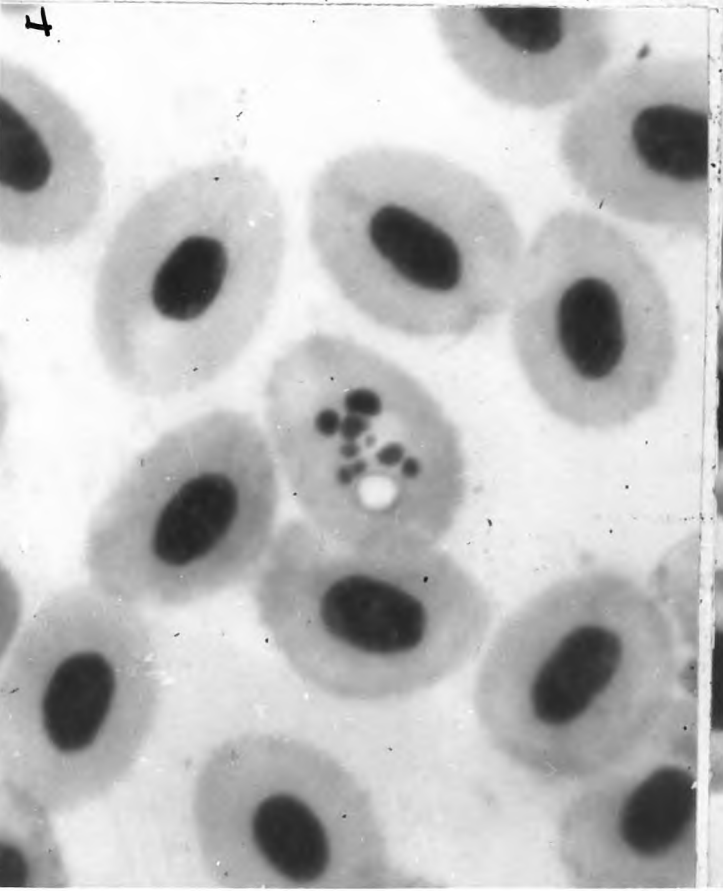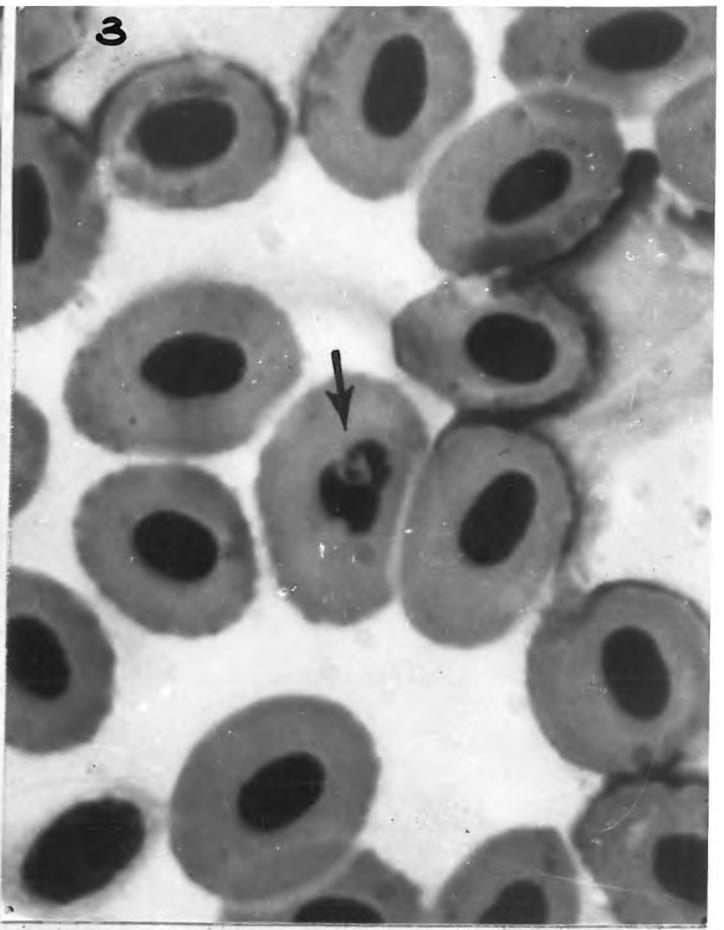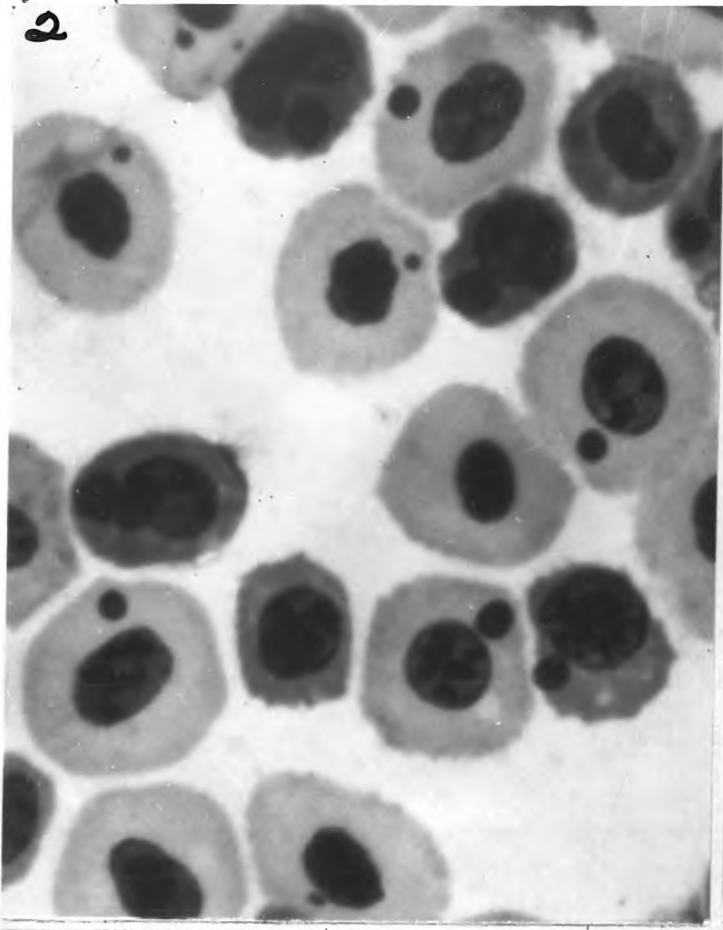
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Table 1. The incidence of an EICDV blood infection in the Atlantic herring, *Clupea harengus harengus*, from Casco Bay to Machias, Maine - May 27, 1976 - September 29, 1977.

Location	Date	Total no. examined	% EICDV Incidence	% of sample with cod type lesions	Holding time (days)	Water temp ($^\circ$C)	Mean length, SD and range (cm)*
Boothbay Hbr.	May 27/76	28	14.3	3.6	1	14.0	14.7±0.6(14.0-16.0)
Boothbay Hbr.	Mar. 8/77	35	8.6	0	0	3.5	12.9±0.7(11.2-13.8)
Boothbay Hbr.	May 22/77	25	32.0	4.0	0	14.0	15.7±1.8(12.7-19.0)
Boothbay Hbr.	July 7/77	2	100.0	0	0	15.0	17.1±3.2(14.9-19.4)
Boothbay Hbr.	Sept. 9/77	14	7.1	7.1	0	16.5	15.5±3.6(12.2-21.2)
Boothbay Hbr.	Sept. 28/77	2	00	0	0	12.0	21.4±1.3(20.5-22.4)
Boothbay Hbr.	Sept. 29/77	3	00	0	0	12.0	24.4±3.5(22.2-28.5)
Boothbay Hbr. Total		109	16.5	2.8			
Indiantown Is.	Sept. 28/76	27	14.8	3.7	1	15.0	26.2±1.4(23.0-27.8)a
Indiantown Is.	Nov. 17/76	75	8.0	2.7	3	7.0	21.2±1.3(17.6-25.0)
Indiantown Is.	Nov. 18/76	25	8.0	0	4	7.0	21.0±1.1(19.7-24.5)
Indiantown Is. Total		127	9.4	2.4			
Bailey Island	July 6/76	35	45.7	40.0	17	19.5	20.1±2.5(18.5-21.5)
Mt. Desert Is.	Aug. 23/76	38	44.7	0	7	14.7	18.3±1.0(16.5-20.3)b
Chebeague Is.	Aug. 31/76	39	18.0	0	2	15.0	20.8±1.4(15.3-23.2)
Bois Bubert Is.	Sept. 2/76	48	12.5	0	2	13.2	no lengths
Little Machias	Sept. 14/76	50	14.0	4.0	7	13.3	28.9±2.4(23.6-32.5)c
Grand Total		446	18.6	4.9			

*Total sample not measured - a. 10 measured, b. 32 measured, c. 24 measured.

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