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**TESTS OF PLASTIC BAGS FOR TRANSPORTATION OF RAINBOW TROUT**

By

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During the past several years, plastic bags have been used as a container by bait and tropical fish dealers in transporting or shipping fish. The tests described here were conducted to determine whether the method might have practical application in transporting trout. Rainbow trout were used in all tests and air in the bags was replaced by oxygen (with two exceptions).

Preliminary tests of plastic bags  
for holding rainbow trout

The results of the first series of 19 tests, which were made from March 13, to 19, 1956, in the aquarium at Grayling, are given in Table 1. Rainbow trout, averaging 7.1 inches in length, were put into plastic bags with 5 or 11 gallons of water; air remaining in the bags was expelled and replaced with oxygen. In all tests, except 13, 14, and 19, the bags were floated on water to maintain a constant temperature of 50° F. In tests 13, 14, and 19, the plastic bags were enclosed in insulated boxes and placed in an electric cooler to maintain a constant temperature.

The preliminary tests were made to learn some of the limitations of holding trout in plastic bags and what type of bag would be best. The

Table 1.--Results of tests of plastic bags for holding rainbow trout, Grayling Hatchery, March 13-19, 1956.

[Water temperature, 50° F]

Test number	Capacity of bag (gallons)	Type of bag ↓	Gallons of water	Pounds of fish per gallon of water	Number of fish ↘	Duration of test (hours:minutes)	Percentage mortality
1	5	E	1	2	not counted	21:30	95+ (est.)
2	5	E	1	4	not counted	2:30	80+ (est.)
3	5	E	1	4	23	8:50	57
4 <sup>3</sup>	5	E	1	4	30	0:35	83
5 <sup>3</sup>	5	E	1	4	30	0:45	87
6	5	E	2	2	22	4:50	27
7	5	E	2	2	30	3:20	27
8	5	E	2	1	15	20:00	87
9	5	E	2	1	19	20:00	84
10	5	E	2	1	?	20:00	100
11	5	E	2	1	19	20:00	84
12	5	E	2	3	? <sup>4</sup>	1:00	0
13	5	E	1	2	16	8:15	19
14	5	E	2	2	27	7:45	33
15	11	S	2	5	75	1:45	27
16	11	S	2	5	83	1:45	16
17	11	S	4	3	87	2:00	29
18	11	S	2	3	? <sup>4</sup>	1:00	0
19	11	S	2	4	56	3:30	71

↓ E = envelope; S = satchel.

↘ Fish used in these tests averaged 7.1 inches in total length. In tests 1, 2, 4 and 15 the fish were measured before the test was begun. In all other tests, measurements were made after the test because it was observed that handling seemed to tire the fish.

↘ No oxygen added to bags in tests 4 and 5. Oxygen added to bags in all other tests.

↘ Fish were in extreme distress and were transferred to fresh water immediately to prevent loss.

satchel (square bottom) type of plastic bag appeared to be most satisfactory in that the fish did not crowd into the corners as they did in the envelope type of bag. With one exception, in tests lasting more than 2 hours, mortality was lowest in bags loaded with 2 gallons of water and 2 pounds of fish per gallon. However, mortality in all tests was much too high for practical application.

In tests 10, 11, 12, and 18, oxygen and carbon dioxide analyses of the water in the bags before and after tests were as follows:

Test number	Oxygen (p.p.m.)		CO <sub>2</sub> (p.p.m.)	
	Beginning	End	Beginning	End
10	7.0	14.0	3.0	133.0
11	7.0	19.0	3.0	155.0
12	4.6	5.6	3.0	30.0
18	4.6	21.2	3.0	29.5

In these tests, the mortality was high, or the fish were extremely distressed, although there was an abundance of oxygen present. In tests 10 and 11, which ran for 20 hours, carbon dioxide was very high. Because of the complex inter-relations between carbon dioxide, oxygen and nitrogenous wastes the possible effects of high carbon dioxide content on fish was not evaluated.

Second series of tests of plastic bags  
for holding rainbow trout

The second series of tests were made at Grayling in February and March, 1957, with rainbow trout from 3 to 6 inches in length. In some tests of this series, a method was used which Mr. George N. Washburn, of the Ozark Fisheries, Inc., Stoutland, Missouri, reported to be successful for carrying fish in sealed containers. The process prescribes

the addition of 1 ounce of granular charcoal per gallon of water to absorb excess ammonia, 300 to 500 p.p.m. di-sodium phosphate ( $\text{Na}_2\text{HPO}_4$ ) to keep the water neutral or slightly alkaline, and 25 p.p.m. each of streptomycin and terramycin to reduce bacterial activity.

All tests in the second series, except Nos. 20, 21 and 22, were made in 0.004-inch-thick polyethylene bags of 15-gallon capacity, measuring 15 by 15 by 24 inches. This thickness is comparatively heavy for plastic bags and was chosen because it would be more serviceable in hatchery operations. Unless otherwise noted, all tests were made with bags floating on water in aquaria to maintain a constant temperature of 50° F. Results of these tests are given in Tables 2 and 3.

Table 2.--Results of further tests of plastic bags for holding rainbow trout, Grayling Hatchery, February 2-March 26, 1957.

Test number	Number of gallons of water	Water temperature (°F.)	Pounds of fish per gallon	Number of fish <sup>1</sup>	Duration of test (hours:minutes)	Percentage mortality	Water analysis at end of tests			Materials added to water <sup>2</sup>				
							O <sub>2</sub> (p.p.m.)	CO <sub>2</sub> (p.p.m.)	pH	Charcoal (ounces per gallon of water)	Di-sodium phosphate (p.p.m.)	Sodium amytal (grains per gal. of water)	Urethane (percentage)	MS222 (grains per gallon of water)
1	3	50	3	204	1:15	8	3.6	65	7.0	1	500	...	.....	.....
2	3	50	4	273	1:15	40	4.0	80	7.0	1	500	...	.....	.....
3	3	50	5	332	1:10	24	4.4	92	6.8	1	500	...	.....	.....
4	2	50	4	181	1:30	8	9.2	105	6.8	1	500	...	.....	.....
5	2	50	4	177	1:15	6	4.6	80	7.2	2	1,000	...	.....	.....
6	2	50	4	171	1:20	13	5.0	68	7.2	2	1,000	...	.....	.....
7	2	50	2	97	5:50	4	4.1	135	7.1	1	500	...	.....	.....
9	2	50	2	105	7:05	0	10.9	59	6.6	..	.....	1/2	.....	.....
10	2	50	2	88	7:05	0	7.5	165	7.1	1	500	1/2	.....	.....
11	2	50	2	94	6:05	0	4.9	100	7.2	4	1,000	...	.....	.....
12	2	40	2	97	5:50	0	3.1	105	7.2	4	1,000	...	.....	.....
13	2	40	4	203	2:05	22 <sup>3</sup>	3.6	71	7.2	4	1,000	...	.....	.....
14	2	40	4	191	1:50	1	4.1	85	7.2	4	1,000	...	.....	.....
15	2	40	4	109	2:15	4	4.5	80	7.4	1	500	...	.....	.....
16	2	40	2	106	15:25	10	18.5	100	6.4	..	.....	1/2	.....	.....
17	2	40	2	100	15:25	9	12.8	175	6.6	1	500	1/2	.....	.....
18	2	40	2	97	15:25	8	16.5	95	6.4	..	.....	...	.....	.....
19 <sup>4</sup>	2	50	3	137	6:00	4	13.0	67	6.4	..	.....	...	.....	.....
20 <sup>5</sup>	1	50	3	90	2:25	23	5.7	48	6.6	..	.....	...	.....	.....
21 <sup>6</sup>	1	50	3	77	4:05	29	6.2	60	6.6	..	.....	...	.....	.....
22 <sup>6</sup>	1	50	2	52	4:00	6	8.4	45	6.6	..	.....	...	.....	.....
23	2	50	2	70	7:05	0	19.0	76	5.7	..	.....	...	0.05	.....
24	2	50	2 1/2	98	7:02	14	10.0	92	5.9	..	.....	...	0.05	.....
25	2	50	2 1/2	90	6:40	36	6.9	68	5.8	..	.....	...	0.1	.....
26	2	50	2	71	6:35	1	13.0	70	5.8	..	.....	...	0.1	.....
27	2	50	2	87	1:30	58	10.2	..	...	..	.....	...	0.15	.....
28	2	50	2	78	1:45	60	7.2	..	...	..	.....	...	0.15	.....
29	2	50	2	73	15:15	56	10.1	109	6.4	..	.....	...	0.1	.....
30	2	50	2	72	15:20	56	10.9	127	6.7	..	.....	...	0.075	.....
31	2	50	2	90	7:25	6	8.7	92	6.3	..	.....	...	.....	0.385
32	2	50	2	100	7:20	4	12.2	87	6.3	..	.....	...	.....	0.385
33	2	50	2	107	7:10	9	8.2	95	6.2	..	.....	...	.....	0.19
34	2	50	2	92	7:00	4	9.6	105	6.0	..	.....	...	.....	0.19
35	2	44	2	70	16:30	21	17.3	127	6.3	..	.....	...	0.075	.....
36	2	44	2	60	16:30	38	21.3	115	6.5	..	.....	...	.....	0.385
37	2	44	2	74	16:30	26	18.1	135	6.4	..	.....	...	.....	.....

<sup>1</sup> Fish in tests 1-22 ranged from 3 to 6 inches in total length; for tests 23-37 this range was 2.9-6.5 inches.

<sup>2</sup> In addition to the materials noted, 25 p.p.m. each of streptomycin and terramycin were added in tests 11-14.

<sup>3</sup> Fish in poor condition at start of test.

<sup>4</sup> Square bottom bag held in aquarium.

<sup>5</sup> Round bottom bag held in aquarium.

<sup>6</sup> Round bottom bag held in cooler.

Table 3.--Results of road tests of plastic bags for transportation of rainbow trout, Grayling Hatchery, March 28, 1957, using 2 gallons of water and 2 pounds of trout per gallon of water per bag

Test number	Water temperature (°F.)	Number ↓ of fish	Duration of test (hours:minutes)	Percentage mortality	Water analysis at end of tests			Materials added to water	
					O <sub>2</sub> (p.p.m.)	CO <sub>2</sub> (p.p.m.)	pH	MS222 (grains per gallon of water)	Urethane (percentage)
38	50-62	89	8:35	7.8	14.1	95	5.8	....	....
39	50-62	78	8:31	2.6	13.6	95	5.9	....	....
40	50-62	87	8:27	1.1	14.5	98	6.0	0.19	....
41	50-62	72	8:23	2.8	12.7	100	6.0	0.38	....
42	50-62	86	8:19	0.0	19.8	100	6.0	....	0.05
43	50-62	89	8:15	0.0	13.2	100	5.9	....	0.05

↓ Fish used in these tests ranged from 2.9 to 6.5 inches in total length.

In the first six tests in 1957, 3, 4 or 5 pounds of fish per gallon of water were used, rather than 2 pounds indicated in the 1956 tests, because some benefit was expected by the addition of certain chemicals. However, the addition of di-sodium phosphate, charcoal and antibiotics did not prevent mortality of fish or permit increase in the length of time fish could be held in the bags, even though the dilutions were doubled in some instances. Di-sodium phosphate kept the water slightly alkaline in most cases, but did not prevent mortality of fish.

#### Transportation of rainbow trout in plastic bags

Practical application of holding fish in plastic bags is in transportation. Since all earlier tests had been conducted with the loaded bags resting quietly in aquaria where the temperature remained constant at 50° F., the last six tests (38 - 43, Table 3) were made in actual transit in a ranch wagon. After approximately 8 1/2 hours in transit, the water temperature raised from 50° F. to 62° F. and the mortality was negligible. The bags to which M.S. 222 (tricaine methanesulfonate) had been added lost only 2.8 and 1.1 percent of the fish and the bags with urethane lost no fish. The loss of fish in the bags without either M.S. 222 or urethane was 7.8 and 2.6 percent. Chi-square tests indicate that only the bag with a mortality of 7.8 percent (test 38) had a loss significantly higher than any other test.

In a further test on April 23, three plastic bags each containing two gallons of water and four pounds of fish were taken to Ann Arbor by station wagon for analysis of the water for ammonia content. The fish were in the plastic bags for six hours and no attempt was made to control temperature. Bag No. 1 contained only oxygen plus the water and fish, bag No. 2 had two ounces of charcoal added to the water, and bag No. 3

had 0.5 percent urethane (3.78 gm. to 2 gallons of water) added to the water.

Upon arrival at Ann Arbor, only four fish were alive. Water temperature had risen from 50° F. to 72° F. This high temperature probably was responsible for the mortality under the circumstances.

Mr. Elmer Bannan made determinations for nitrogen as follows:

No. 1. Control	260 mgm. per liter
No. 2. Charcoal	145 mgm. " "
No. 3. Urethane	99 mgm. " "

Observation of the fish enroute to Ann Arbor was difficult because the bags were packed in cardboard cartons. The few observations made seemed to indicate that fish in the bag with urethane were in distress before fish in the other bags were affected. Possibly the reason for the lower amount of nitrogen in the bag to which urethane was added was because all these fish died first. Since so few fish remained alive in any of the bags this test for nitrogen is considered inconclusive.

#### Recommendations

Fish should be carried in square-bottom bags of 15-gallon capacity measuring 15 by 15 by 24 inches, manufactured from polyethylene with a thickness of 0.004 inch. The bags should contain 4 pounds of fish in 2 gallons of water. All the air should be expressed from the bag, replaced with oxygen and the bag securely closed. During the time the fish are in the bag the water temperature should be kept below 60° F. If the above instructions are followed, trout can be carried safely for about 6 hours. Further tests will be made to improve this method of transporting fish.

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