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BIOLOGICAL BOARD OF CANADA

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Report of Work Done at the Atlantic Biological Station During 1925

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During the summer of 1925, experiments were continued in connection with quantitative measurements on rigor mortis of fish. The first work on this problem was done by Dr. C. C. Benson (1) in 1920, carried on by Dempsey and Panton (2) in 1921, by Benson (3) in 1922, and continued by myself in 1925. Therefore it seems unnecessary to introduce the subject, but sufficient to refer to the reports of earlier years (1), (2), and (3) for the historical background and reasons for attempting this piece of work. It should be mentioned that the definite goal in view in 1925 was to improve the method used in the past, if possible, and to run determinations on different species of fish.

The method used in the earlier experiments in 1925 was the same as that used by Dempsey and Panton (2) and Benson (3). In brief, the apparatus consisted of a horizontal board arranged with pegs in such a way as to hold the fish firmly by the shoulders and at the same time allow the tail to be suspended in the air. By the use of weights and a series of pulleys and a cord which was attached to the fish's tail, the tail was drawn through a distance of 3.4 cm. at regular intervals of time. Records were taken of the temperature simultaneously with the readings of the amount of stiffening of the fish muscle. The first serious difficulty in this method was the supporting of the fish in the apparatus in such a way that the tail would swing free of the horizontal board. There was always a certain amount of sagging of the tail of the fish, which in the extreme case, meant the introduction of considerable friction when measurements were being taken. To overcome this difficulty, the fish was hung tail down, in the vertical position and was fastened securely to stationary crosspieces through the head and in front of the anal fin. A non-stretching strong string was fastened securely around the tail of the fish and passed over a series of small pulleys. To the other end of the string was fastened a scale pan which swang free. A removeable pin was fastened through the string and each time a reading was taken, the pin (and therefore that part of the string) had to pass through a distance of 4.4 cm. (with the new apparatus the 3.4 cm. standard distance was not suitable). This type of apparatus proved to be much more suitable than the earlier one and was therefore used for all further experiments which were done in air.

In Table I are shown results of experiments done by the old and new methods using haddock of comparable weight and size in both cases.

TABLE I.

Fish No.	Species	Condition when experiment began	Weight lbs.	Weight ozs.	Length (cm.)	Girth (cm.)	Greatest* measurement of rigidity (gms.)	Time to reach high point in rigidity (hours)	Measured* rigidity 12 hours after start of experiment (gms.)	Temperature in Centigrade degrees
Old Method										
VII	haddock	muscle irritable	3	11	59	30	420	4½	200	19.0 - 21.5
IV	"	" "	3	8	38	30	320	4	140	19.0 - 21.5
New Method										
XII	haddock	muscle irritable	3	11	60	35	340	4½	160	18.0 - 22.0
XX	"	" "	3	6	59	30	330	5	110	18.0 - 23.5

* Expressed as grams to pull fish's tail through a distance of 4.4 cm.

The results show that the new method gives a rigidity curve which may be compared with that given by the old method.

There were two sets of experiments done by the old method in air: (1) determinations on haddock of various sizes tested at laboratory temperatures; and (2) determinations on fish of different species.

(1) The haddock were tested whenever material was available and therefore necessitated testing on different days so the temperature from day to day was not controlled.

The result of this set of experiments is given in Table II.

(Table II)

On examining this table, it will be noticed that as the size and weight of the fish increase, the attaining of the high point in rigidity is lengthened in terms of hours; and the passing of rigor in 12 hours is more complete with a small haddock than with a large one. This is probably a quantitative effect.

(2). The species of fish tested in air at laboratory temperatures were haddock, eel pout, flounder, hake, and skate.

The results are shown in Table III.

(Table III)

The results show that the hake muscle becomes rigid more quickly than haddock but the height of rigidity is not so great; that is, when considering fish of equal size. Eelpout shows very little rigor and it takes a long time to reach the high

TABLE II.

No. of fish	Condition when experiment began	Weight lbs.	ozs.	Length (cm.)	Girth (cm.)	Greatest measurement of rigidity (gms.)	Time to reach high point in rigidity (hours)	Measured rigidity 12 hours after start of experiment (gms.)	Temperature in Centigrade degrees
XIX	muscle irritable	1	6½	34	23	210	3	50	16.5 - 22.0
XV	dead	1	10	35	21	150	2	65	18.0 - 22.0
XIV	"	1	15½	45	25	200	1½	70	18.0 - 22.0
XVII	muscle irritable	2	12½	50	28	385	3½	155	16.5 - 22.0
XIII	" "	2	12½	50	28	310	3½	115	16.0 - 22.0
XXVI	dead	2	13½	59	30	320	3¼	140	19.9 - 28.0
XVI	muscle irritable	3	4	60	30	280	3½	100	16.5 - 22.0
XX	" "	3	6	59	30	330	5	170	18.0 - 22.5
XII	" "	3	13½	60	35	340	4½	160	18.0 - 22.0

point of rigidity. Flounder is similar to eelpout with the exception of the length of time to reach the high point of rigidity and the passing of rigidity; these are both greater for eelpout muscle. The apparatus used for these experiments did not appear to be useful for skates. It is interesting to read in a report by Anderson (4) that rigor tends to persist longer in fish such as salmon whose musculer tissue is firmer and contains a smaller percentage of water, than in those fish such as haddock whose tissues are not so firm and contain more water.

There was another difficulty which was early realized. The variation in temperature was a great determining factor in the development and passing of rigidity. The determinations done in air necessarily were done at a temperature which varied with the surrounding atmosphere. In the bright sunny laboratory, the temperature was found to vary from 19.0° - 28.0°C. in 12 hours, and this was sufficient to affect the rigor curve. Further difficulty was experienced with the drying of the fish's skin, especially when tests were run as long as 24 hours in the air. The skin in some cases actually became stiff and offered resistance to the weight necessary to pull the fish's muscle through the standard distance. Therefore a third type of apparatus was set up. Big glass-sided, water tight tanks were used. A flat board fitted into the bottom of this tank and was held firmly in place by adjustable flat sticks. Holes were bored in this in such a way that tapes could be slipped over the head and around the body of the fish, passed through the holes and tied securely underneath. The tail could then swing free when the tank was filled with water. Strong string was tied tightly around the tails of the fish to which was attached strong fine copper wire and then more strong string. This was passed over a series of pulleys so the scale pans tied to the end of the string swung free. The weights were manipulated for this apparatus as in former methods (2) and (3). The distance corresponding to the moving of the suspended fish's tail could be as great as 4.4 cm. When the fish were actually placed in the tanks of water, the tails never stuck to the fish boards although the body of the fish was tied flat to the board. The advantages of this method were realized. The temperature of the medium surrounding the fish could be controlled absolutely and there was no error introduced on account of the drying of the skin of the fish as with experiments done in air. The possibility of the skin acting as a semipermeable membrane was discussed and it was feared that if the medium surrounding the fish were of lower salt concentration, that the water might pass through the skin into the muscle tissue; also that the salts dissolved in the muscle juices might pass through the skin of the fish into the surrounding medium. Either of these conditions might affect the oncoming and passing of rigor. Experiments were therefore set up to determine the best medium to use in the tanks. Haddock were used as the experimental fish and those used were of comparable size, weight and girth. The different media tested were tap water, sea water and saturated brine. The temperature at which these experiments were done varied from 14°C. - 16.5°C. The results are shown in Table IV.

TABLE IV.

Fish No.	Condition at start of experiment	Weight lbs.	Weight ozs.	Length (cm.)	Girth (cm.)	Greatest measurement of rigidity (gms.)	Time to reach high point in rigidity (hours)	Measured rigidity after start of experiment (gms.) 12 hours	24 hours	Medium	Temperature in Centigrade degrees.
XLI	muscle irritable	3		51	26	380	5	240	160	tap water	14.7 - 15.0
LIV	" "	5	5½	53	27	470	3¼	200	150	" "	14.5 - 15.7
LIII	dead	3	14½	57	29	400	4¾	160	125	" "	14.5 - 15.7
XLIX	muscle irritable	2	9	50	26	460	4½	180	110	sea "	14.0 - 15.0
L	" "	3		50	27	340	4½	120	80	" "	14.0 - 15.0
XLIV	" "	3		53	29	560	5½	270	260	sat. brine	15.2 - 16.5
XLIII	" "	3	6	49	23	470	9½	400	290	" "	15.2 - 16.5

The results show that rigidity curves for haddock in sea or tap water at a temperature of 14.5° - 16.5°C. are comparable in general outline to those in air at 16.5° - 21.0° (See Table I). The passing of rigor from fish muscle in a medium of saturated brine was very slow, in fact, the values for the height of rigidity after 24 hours were about double those when using sea or tap water. Conclusions were therefore drawn that some factor was introduced to interfere with the normal development and passing of rigidity when salted brine was used. The probability is, that this is due to the fact that the skin acts as a semipermeable membrane. As sea water was more easily run into the tanks used, and as it was the natural habitat for the fish when alive, it was used for all further experiments.

The most outstanding experiments done with fish in the tanks of sea water were:-

(1) Those in which the species of fish was the same throughout (haddock) but in which the temperature varied.

(2) Those in which the temperature was constant and the species of fish varied.

<u>Temperature</u>.

According to Panton and Dempsey (2) and Benson (3) it has been shown that the higher the temperature, the quicker is the coming and passing of rigor. Haddock were used for all tests and the temperatures were from 4.5° - 37°C. The medium in every case was sea water. The results of these experiments are shown in Table V.

TABLE V.

Haddock No.	Temperature (degrees) (Centigrade)	Weight lbs.	ozs.	Length (cm.)	Girth (cm.)	Greatest measurement of rigidity (gms.)	Time to reach high point in rigidity (hours)	Measured rigidity after 12 hours (gms.)
C	4.7 - 7.0	1	12	43	22	380	7	230
LXVII	4.5 - 5.5	2	9	49	26	500	4	400
LXVIII	4.5 - 5.5	2	14	52	27	500	6¼	330
XCIX	4.7 - 7.0	3	2	51	22	460	10¾	430
LX	8.0 -10.0	3	3	50	26	500	4½	300
LXI	8.0 -10.0	3	3½	53	27	520	6¼	420
XC	9.0 -12.0	2	11	47	26	400	4¼	310
XCI	9.0 -12.0	2	14	51	27	340	9¾	300
L	14.0 -15.0	3		50	27	340	4½	130
XXIV	15.0 -15.7	2	11	51	26	200	3½	160
XLV	15.0 -16.5	2	13½	51	25	250	1½	100
LXIV	19.0 -21.0	1	9	42	22	250	3¼	30
LXIII	19.0 -21.0	2	2	48	24	370	1¾	65
LXXV	23.0 -27.0	2	7	47	23	120	1	80
XCIII	24.0 -26.0	3	3	53	28	210	2	45
LXXIV	24.0 -26.0	4	2	54	28	250	1	45
LXXV	27.0 -31.0	2	7	47	23	120	1	20
LXXIV	27.0 -31.0	4	2	53	28	240	1¼	75
LXXXI	33.0 -37.0	3	1	53	27	130	¼	30
LXXX	33.0 -37.0	3	14	57	29	300	¼	30

The results show: That at the lowest temperatures (4.5 - 7.0°C.) the passing of rigor particularly is delayed most and at this temperature the fish muscle is not frozen. Anderson (4) says that by maintaining the temperature at -4 to 5°C., the fish appear to remain in a condition of rigidity indefinitely and if the temperature is kept at -5°C. or lower, that the fish do not appear to pass into rigor. He explains the maintenance or prevention of this state by the fact that putrefactive bacteria are inhibited in their action between 0° to -3°C.

At the higher temperatures, such as 27° - 31° C. and 33° - 37° C., it is interesting to note that the height of rigor curve is not as great for fish of comparable size. The explanation of this condition is at present not known.

<u>Species of fish.</u>

These experiments were done altogether in the water tanks at a temperature of 13.5° - 17° C. With some species, such as hake and eel pout, it was hard to do determinations on the smaller fish because the tail from the anal fin downwards, was narrower and therefore more pliable. In some cases, no readings could be taken until the muscle actually began to stiffen. The results given in Table VI should be compared with those in Table III.

TABLE VI.

Fish No.	Species	Weight lbs.	Weight ozs.	Length (cm.)	Girth (cm.)	Greatest measurement of rigidity (gms.)	Time to reach high point in rigidity (hours)	Measured rigidity after 12 hours (gms.)	Temperature (degrees Centigrade)
XXXI	Haddock	2	2	54	26	157	4½	95	15.0 - 15.5
XXX	"	2	6	54	28	150	6¾	128	15.0 - 15.5
XLV	"	2	11	51	25	250	1	110	15.0 - 16.0
XLVII	Sculpin (longspine)		12½	31	17	320	4¾	175	13.5 - 16.0
LXVI	" "	1	4½	38	21	240	10¾	220	14.2 - 15.5
LXV	" "	1	6½	38	22	340	6¾	220	14.2 - 15.5
LIX	" (shortspine)	1	1½	29	20	450	8½	350	13.5 - 16.0
CIII	Eel pout		15	43	23	55	11½	50	13.5 - 16.0
XCVII	" "	3	13½	63	27	80	6½	60	13.5 - 17.0
XC	" "	4	8	70	24	90	3¾	50	15.0 - 15.5
XCV	Flounder	1	5½	34	31	200	9	180	15.0 - 15.5
CIV	"	2	½	42	39	180	13½	-	15.0 - 15.5
LXXXIV	Hake		12	35	17	80	3	80	14.0 - 14.5
XCII	"	1	1	45	23	90	2½	20	13.5 - 15.0
LXXIX	"	1	4	36	16	90	1½	80	14.0 - 15.5
LXXVIII	"	2	3	49	23	170	2	110	14.0 - 15.5

The most noticeable result is that rigidity varies with the different species of fish. Hake and haddock go into rigidity quickly, eel pout and flounder and sculpin much more slowly. The long-spined sculpin went into rigidity quicker than the short-spined sculpin of slightly smaller size. From these results we have a confirmation of the conclusions on haddock; that the rigidity increases, in regard to time required to develop and pass, as the size of the fish of any one species increases.

Since the advent of the line trawling industry, the question has often been discussed as regards the keeping qualities and general condition of trawl fish and hand line caught fish. As rigor mortis affects the general condition of the muscle, an experiment was done on fish caught on a hand line as compared to those caught on a trawl. One would expect to find that rigor develops in trawl fish more quickly than in hand line fish, because in the former case, they are struggling to get off the line much more intensely and for a longer time.

Only 1 haddock caught on a hand line was available for this experiment. This fish was killed immediately after catching and set up in the apparatus at once and the determination done in air at 19° - 24° C. The height of the rigor curve was at 240 grams in 3 hours. For a fish of this size (length 50 cm. girth 25 cm., weight 1.22 kilograms) this is an average value. The height of the curve in 12 hours was 80 grams. These results show no very great variation from the average rigor curve of fish caught on the trawl (See Table I). However more experi-

TABLE VII.

Fish No.	Species	Weight lbs.ozs.		Length (cm.)	Girth (cm.)	How frozen	How thawed	Greatest measurement of rigidity (gms.)	Time to reach high point in rigidity (hours)	Measured rigidity after 12 hours (gms.)	Temperature (degrees Centigrade)
LXX	Sculpin (long spined)	1	3	37	19	Alive in brine at -12°C.	In fresh H_2O at 15°C.	140	2½	90	15.0 - 15.8 (sea water)
LXIX	"	1	7½	39	22	"	"	120	2	80	" "
CII	haddock	1	15	45	24	"	In fresh H_2O at 12° C. for 2 hours	70	½	50	14.0 - 15.0 (sea water)
XXIX	"	2	4½	52	29	"	In fresh H_2O at 20° in 1½ hrs.	140	½	110	18.5 - 22.0 (in air)
CI	"	3	2	52	29	"	In fresh H_2O at 12° for 2 hrs.	40	7	35	14.0 - 15.0 (sea water

ments should be done to establish this point.

Several attempts were made to measure the coming of rigor on fish that had been frozen alive in brine and then thawed and set up in the apparatus. It was very difficult to thaw the fish after they had been frozen solid. The methods of thawing and the results are shown in Table VII.

(Table VII)

There seems to be a curve quite different from the usual course and the difference is probably due to the extreme mechanical disintegration which occurs when the muscle is frozen. Anderson (4) finds that the thawed fish muscle is very soft and limp and pits deeply on pressure. With such a change in the texture of the muscle, we may look for a change in the stiffening of the muscle over a period of time sufficient to show normal rigor.

It was noticed that with all the fish tested, there was one condition which could not be controlled and which introduced a variable into all the determinations of rigidity of fish muscle. The amount of fatigue of the muscle varied so in one fish from another, that even though fish were of the same species, size, weight, girth and freshness, they would show great differences, especially in the rate of becoming rigid. This was found to be a serious handicap because it was not possible to start an experiment with any idea of the activity of that fish for some hours before death. Dr. A. H. Leim thought of putting the live haddock from the trawl into the fish car to

TABLE VIII.

Fish No.	Condition before being tested	Weight lbs.	Weight ozs.	Length (cm.)	Girth (cm.)	Medium	Greatest measurement of rigidity (gms.)	Time to reach high point of rigidity (hours)	Measured rigidity after 12 hours (gms.)	Temperature (degrees Centigrade)
CX	from trawl	2	6	50	25	Sea water	330	2½	120	12.0 - 15.0
CIX*	in fish car 7 days	2	8	50	26	" "	260	12	260	12.0 - 15.0
CVIII	from trawl	2	12	48	18	" "	370	2½	130	14.0 - 15.0
CVII	in fish car 4 days	3	15	57	30	" "	430	6½	360	14.0 - 15.0

*Fish had been punctured to treat swim bladder.

see if they would live there comfortably, and they did, one of them for a week. Several experiments were run by Dr. Leim after I had left the Station and the results are shown in Table VIII.

(Table VIII)

The results of these experiments are very interesting. Although there should be further tests done before the results are confirmed, it goes to show that the high point of rigidity can be delayed extensively by resting the fish before its tests are being taken. It suggests a method of preparation of the fish before starting rigor experiments which would give much more uniform material as far as the state of the muscle is concerned.

Conclusions.

A. 1. The larger the fish of one species, the slower is the coming and passing of rigor. This is true of tests done in air or sea water. This agrees with work done by Dempsey and Panton (2) and Benson (3).

2. Rigor curves differ according to the species of fish tested. This agrees with work done by Benson (3).

B. 1. Two new methods for the quantitative determination of rigidity of fish muscle have been developed.

2. Haddock tested in tanks of sea water at 4.7° - 37.0° C. showed the development of rigidity more quickly as the tempera-

ture increased; at temperatures of 24° - 37°C. the maximum rigidity was definitely lower than that at lower temperatures, using the same sized fish.

3. The temperature being constant, the different species of fish went into rigidity in the following order: hake, haddock, eel pout, sculpin, flounder.

4. Haddock caught on a handline developed rigidity at the same rate as one caught on a trawl.

5. Fish frozen alive and thawed at 12° - 20° C. and tested for rigidity, appeared to give an abnormal rigor curve.

6. A method of controlling the extent of fatigue of fish muscle is suggested. This could be used as a preliminary to a rigidity experiment.

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