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ARTICLE I.—PLANKTON STUDIES. I. METHODS AND APPARATUS IN USE IN PLANKTON INVESTIGATIONS AT THE BIOLOGICAL EXPERIMENT STATION OF THE UNIVERSITY OF ILLINOIS.

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URBANA, ILLINOIS.
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ERRATA.

Page 136, line 2, and page 182, line 17 from bottom, for '*95a* read '*95*.

Page 226, line 2, page 263, line 17 from bottom, and page 267, lines 2 and 15, for '*98*, read '*96*.

Page 233, line 15 from bottom, for '*82* read '*82a*.

Page 355, line 2 from bottom, for C. *F*. Hudson read C. *T*. Hudson.

Page 389, foot-note, for Vol. *V*. read Vol. *IV*.

Page 457, line 5, for *Genera* read *Genus*.

Article I.—*Plankton Studies. I. Methods and Apparatus in Use in Plankton Investigations at the Biological Experiment Station of the University of Illinois.* By C. A. Kofoid.

Less than ten years ago a new field of biological science was opened by the German investigator Hensen, namely, the quantitative examination of the "Plankton." This term was applied to all plants and animals floating free in the water and incapable by their own efforts of materially changing their position. Thus adult fish which brave the waves and stem the current would not be included in the plankton, while the passive eggs or the helpless fry would fall within the limits of the definition. Practically, the content of the term plankton as applied to fresh water is the sum total of its minute life, both plant and animal.

The scope of our plankton work upon the Illinois River and its adjacent waters includes a continuous, systematic, and exhaustive examination of the plant and animal life suspended in the waters of a river system, with a view to determining its amount and seasonal changes, its local and vertical distribution, its movement and relation to the current, the effect upon it of floods and of drouth, of light and of temperature, the organisms which compose it, their seasonal and cyclic changes, and their mutual interrelations. Added interest arises from the fact that this is the first application of this method of biological investigation to a river system and its related waters.

It is the purpose of the present paper to describe the methods and apparatus employed in the plankton work at the Biological Station at Havana, Illinois, during the years 1894–1896. Both are, as a rule, the result of mutual conference of the various members of its staff. During the first fifteen months of the existence of the Station the plankton work was in the hands of Professor Frank Smith, and when, on July 1, 1895, the writer assumed charge of this work at the Station he found the oblique haul, described on a subsequent page, already inaugurated and in successful operation. It now devolves upon him to prepare for publication a description of this method, but the credit for devising it and putting it in operation belongs to those from whose hands he received it. The apparatus was used without modification until August 23, 1895, when the detachable bucket was added to the net, and in October of the same year the separable carriage was introduced. In May, 1896, the pumping method was substituted for the oblique haul in making plankton collections.

Upon the opening of the Station in April, 1894, the examination of the water by the plankton method was decided upon, and in the early part of June the first regular collections were made. The method of plankton collection ordinarily employed,—as, for example, by Hensen ('87 and '95) in the Baltic and North Seas and in the Atlantic Ocean, by Apstein ('92 and '96) and Zacharias ('93–'96) in the lakes of northern Germany, by Reighard ('94) in Lake St. Clair, and by Ward ('96a) in Lake Michigan,—has been without exception the vertical haul, in which the net is lowered to the bottom of the body of water and then raised in a vertical line to the surface, thus filtering a vertical column of water. Difficulties beset the application of this method to the waters at Havana. In the first place all the bodies of water examined at the Station are quite shallow, the majority of the plankton collections being made in less than three meters of water. The river itself is the deepest water in the locality, but at its lowest stage there are only three meters of water in the main channel, where collections are made. This depth is increased at times of flood, the maximum reached in the past three years being 6.1 meters. The shallowness of the water thus practically precludes examination by means of the vertical haul.

A second difficulty exists in the unstable nature of the bottom generally found throughout the locality to which the operations of the Station are confined. This consists of a soft black mud, composed largely of the detritus of decaying vegetation and alluvial soil deposited from the silt-charged waters at times of flood. It is extremely unstable and upon the least disturbance mingles with the water, rendering it impossible to take a clean plankton collection. The soiling of the net and the fouling of the water consequent upon dropping a large plankton net upon the bottom further preclude the vertical haul in the plankton work at Havana.

I. THE OBLIQUE-HAUL METHOD. (PLATES I.–III.)

The oblique haul was at first adopted as the method best suited to the situation. This is accomplished by suspending the net to a carriage which runs upon a rope stretched obliquely from the bottom to the surface of the water. By this means the column of water traversed by the net is increased to an adequate length. It also permits the employment of a net small enough to be easily operated from a rowboat. A short description of this method of plankton collection has been given by Professor Forbes ('94). The parts of the apparatus used in making the oblique haul will now be described.

1. *The Quantitative Net* (Plates I. and II.).—The net used by us is the modification of the Hensen net suggested by Apstein ('91 and '92) for fresh-water work, and more fully described by him ('96) as the smaller model quantitative net. It consists of three parts: (*a*) the filtering net proper, (*b*) the detachable bucket, and (*c*) the head-piece of the net.

a. The *filtering net* has the form of an inverted truncated cone, whose base has a diameter of 25 cm. and whose side is 40 cm. The truncated apex, to which the bucket is attached, is 4 cm. in outside diameter. This net is made of No. 20 silk bolting cloth, which can be obtained from wholesale dealers in supplies for flouring mills. That used by us bears the brand, "Especially for milling purposes. Dufour & Company." This is the same brand of cloth as that which was used by Reighard ('94) and Ward ('96a) in their plankton work upon the Great Lakes. It is stated by the manufacturers to contain 29,929 meshes to the English square inch. Examination under the microscope shows that this cloth contains 32477 ($= 172.75 \times 188$) meshes to the square inch after sponging and pressing with a hot iron four times.

The average area of the openings is reported by Reighard ('94, p. 57) to be:

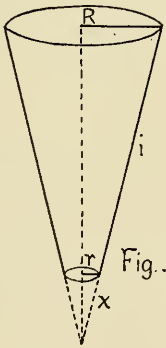
.00003596 sq. cm. in the new cloth,

.00002808 sq. cm. in cloth that had been wetted and then dried,

.00002336 sq. cm. in cloth that had been used for 40 hauls of a net.

As the new silk shrinks considerably after its first wetting, we have followed the practice of thoroughly and repeatedly sponging it and pressing it with a hot iron before cutting out the net. Otherwise in a single-seam net there is sufficient shrinkage to cause the filtering cone to take a position oblique to the true axis of the net (See Apstein, '96, p. 34, Fig. 3).

The following directions for making a pattern for the net are here inserted, as they may be of use to those to whom the original descriptions given by Apstein ('91, '92, and '96) are inaccessible.



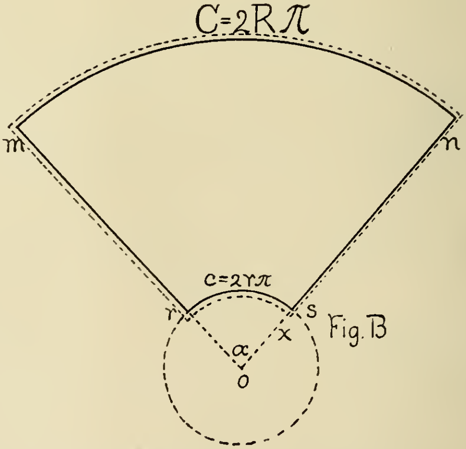


Fig. A.—Completed cone. Fig. B.—Completed pattern for the silk net. (Both after Apstein.)

The net has the form of a truncated cone (Fig. A), whose side ($i = 40$ cm.) and radii of the ends ($R = 12.5$ cm., and $r = 2$ cm.) are known. The silk exposed in the net has the area and dimensions of the surface of this truncated cone.

If, now, we complete the truncated cone and denote the side of the small cone, added for completion, by x, it follows that

$$x : x+i :: r : R;$$

$$x = \frac{ri}{R-r} = 7.619 \text{ cm.}$$

If, now, we imagine the surface of the completed cone unrolled and spread in one plane (Fig. B), the circumferences $C\ (= 2R\pi)$ and $c\ (= 2r\pi)$ will form arcs subtending an unknown angle α, and it follows that the circle described by the radius $x\ (= 2x\pi)$ is to $2r\pi$ as 360° is to α, or

$$\frac{2x\pi}{2r\pi} = \frac{360}{\alpha};$$

$$\alpha = \frac{360\,r}{x};$$

$$\alpha = 94.5°.$$

This angle α being known, it is a simple matter to lay off the pattern for the net. The pattern for the linen head-piece is constructed in a similar manner. Allowance must be made along the edges *mr* and *ns* (Fig. B) for the longitudinal seam. It has been our custom to allow 4 mm. upon one margin and 8 mm. upon the other (Fig. 2, Plate I.). The two edges are brought together and backstitched at *a* (Fig. 2), and the wider margin is then turned under the other and felled at *b* upon the outside of the net. Thus only a single seam 4 mm. in width traverses the length of the net. A very fine needle is used in the sewing, and the line of stitches can be effectually closed with a light coat of rubber cement. Allowance must also be made along the edge *mn* for attachment of the silk between the linen cone (Plate I., Fig. 1, *c. b. l.*) and the cover-strip (*c. s.*) at the seam *sm*, and for the fastening of the silk, along the margin *rs*, between the net clamp (*n. c.*) and the head-piece (*h. p.*) of the bucket. In order to get the net into the clamp it is necessary to slit the silk at several points to within a short distance of the line *rs* (Fig. B).

b. The *detachable bucket* (Plate II., Fig. 3–5) used by us differs in several particulars from that described by Apstein ('91, '92, and '96) and also from the one devised by Reighard ('94, p. 26, Plate II.). It copies the Reighard bucket in substituting a removable plug for the turncock in the Apstein bucket, and differs from both in the base and in the manner of attachment of the silk in the windows.

In form it is a cylinder 7 cm. in height and 4 cm. in outside diameter. (Owing to the material available for construction our bucket measures 4.4 cm. in outside diameter.) It consists of three pieces: the head-piece, the bucket proper, and the band clamp.

The *head-piece of the bucket* (Plate II., Fig. 3, *h. p.*) is a cylinder 2 cm. in height, whose upper edge is rounded upon the inner face. The lower edge is threaded at *m* (Fig. 3) so as to screw into the top of the bucket proper, which is correspondingly threaded at *n* (Fig. 4). The screw is so constructed that the inner surface is uninterrupted when the bucket proper is fully screwed on. The head-piece also bears three equidistant eyes (Fig. 3, *e.*), in which are tied the three stay-lines (*s. l.*) which pass up to the large, and then to the small, rings of the net, and finally unite with the draw-line (Plate III., Fig. 6, *d. l.*). The position and manner of attachment of these stay-lines can be seen in Plate II., Fig. 3, and in Plate III., Fig. 6. The net is clamped on the head-piece by the circular net clamp (Plate II., Fig. 3, *n. c.*), which is a band of brass 1 cm. in width. To the ends of the band are attached wings (*w. n. c.*), which are approximated by means of a thumb-screw (*t. s.*). By this operation the silk beyond the line *rs* (Fig. B.) is firmly clamped between the brass band of the net clamp and the head-piece of the bucket.

The *bucket proper* (Plate II., Fig. 4 and 5) is a brass cylinder 5 cm. in height, and of the same diameter inside and out as the head-piece. In the side of the bucket at a height of 2 cm. are cut three windows 2.5×3.5 cm. These windows are closed by a band of No. 20 silk held in place by a band clamp, in which are windows similar to those in the wall of the bucket. The bucket was constructed from a heavy piece of brass tubing, the bottom (Fig. 4, *b.*) being inserted in the following manner. The tube is turned out to the shoulder (Fig. 3, *sh.*) and heated in a jet of steam, and while still hot the piece of brass from which the bottom (*b.*) is finished, is inserted upon the shoulder. The shrinking of the tube as it cools holds the bottom firmly in place, and it can then be completed as shown in Fig. 3. At the center of the bottom is the outlet of the bucket, which is reamed to hold the tapering plug (*p.*). Both the plug and the bottom are finished obliquely to a drip-point (*d. p.*) at one side. This facilitates the removal of the last few drops of the catch from the bucket. The bucket below the bottom of the windows holds about 7 cu. cm. The base (*ba.*) retains the original thickness (.5 cm.) and its weight adds to the stability of the bucket. Since the drip-point does not project below the base the bucket can be set down, and, owing to the fact that its center of gravity is low, it is not easily overturned. This is a distinct advantage where the work must be done in a small rowboat at the mercy of the waves.

The *band clamp* (Plate II., Fig. 4 and 5, *b. c.*) is a sheet of brass 4.5 × 15.3 cm., and about .1 cm. in thickness. At a distance of .75 cm. from the ends the sheet is bent out at right angles, and a brass bar 4.5 × .75 × .25 cm. is soldered in the angle thus formed (Plate II., Fig. 4 and 5, *pl.* and *pl.'*). The band is now bent around the bucket and the ends brought together by means of two screws (*sc.*) which pass through the one bar and screw into the other (Plate II., Fig. 5, *pl.* and *pl.'*). The windows (Fig. 4, *w.*) can now be cut through both the band and the walls of the bucket. The pillar (Fig. 5, *pi.*) between the windows adjacent to the ends of the clamp is .5 cm. wider than the other pillars, whose width is about 1 cm. The inner edges of the windows are carefully rounded so as to afford no lodgment for the plankton. It is also a convenience to attach the band of silk (Fig. 4, *s.*) which closes the windows to the side of the bucket by means of a thin coat of King's waterproof cement. The band clamp can then be slipped over the bucket and the silk bound firmly in place by tightening the two screws (Fig. 4 and 5, *sc.*). The base also bears an eye (*e.*) for the line which fastens the bucket to the carriage at *z* (Plate III., Fig. 6).

The above-described detachable bucket, devised by the writer, has certain obvious advantages over the Apstein and

Reighard buckets after which it is very largely modeled. Its advantages are its stability, the drip-point, and the band clamp, the latter permitting readily the renewal of the silk in the bucket.

Previous to the adoption of the bucket just described, the net in which the collections at Havana were made was closed by a circular piece of silk clamped on the end of a brass cylinder screwing into the head-piece (Plate I., Fig. 1, *h. p.*). The clamp used for this purpose is similar to that employed to fasten the net to the head-piece. (See Plate I., Fig. 1, and Plate II., Fig. 3, *n. c.*) When a collection had been made the silk circle was removed and, with the plankton condensed upon it, transferred to the killing fluid. This form of plankton bucket was used by Apstein ('92) on his qualitative plankton net, and was afterwards described by Borgert ('96) for a net to be towed behind steam-vessels.

c. The *head of the net* (Plate I., Fig. 1) is a truncated cone, at whose upper and lower ends are rings 10 and 25 cm. in diameter respectively (*u. r.* and *l. r.*). These rings are made of No. 5 (American Standard Gauge) brass wire. The side of the cone (*c. b. l.*) is made of heavy linen, known in the trade as "butchers' linen." Its lower edge is joined to the silk net at the seam (Plate I., Fig. 1, *sm.*), where it also meets the cover-strip (*c. s.*)—a narrow band, made of the same cloth—which extends over the lower ring (*l. r.*). In the upper edge of the cone is bound a heavy cord (*cd.*) which, in turn, is fastened to the upper ring (*u. r.*) by a series of loops of strong thread (*th.*). The upper ring and the cone are supported by three equidistant wire stays (*w. s.*). These are made of No. 8 brass wire and are provided with an eye at each end. The lower eye (*l. e.*) embraces the lower ring and is held in place by small hips soldered upon each side. The upper eye (*u. e.*) is attached to the cord (*cd.*) by a small cloth strap (*st.*) and also serves as a point of attachment for a stay-line (*s. l.*) which runs from the bucket to the lower end of the wire stay (*w. s.*) to which it is fastened, passing from this to the upper eye (*u. e.*) and then to the draw-line (Plate III., Fig. 6, *d. l.*).

2. *The Carriage* (Plate III., Fig. 6 and 7).—The carriage for the plankton net is a wooden bar, 100×5×2 cm. It bears upon one edge two ceiling pulleys (Fig. 6, *p.* and *p.'*), through which passes the carriage rope (*c. r.*). In order that there may be no tendency on the part of the carriage to float, two bars of lead (*l.*) are inserted in its lower edge, so that it naturally assumes a position upon the lower side of the rope. The carriage is so constructed that it may be separated along the line *a b c*, thus freeing the net from the fixed carriage rope. Its two parts are held together by two flat brass hooks (*h.*) which enter the staples (*s.*). The net and the lines pertaining thereto are attached to the removable part of the carriage at four points (*w*, *x*, *y*, *z*) at which screw-eyes are inserted, the attachment being made by means of small metal snaps (*sn.*). At the anterior end of the carriage a stay-line (*s. l. 4*), coming from the end of the draw line (*d. l.*), is fastened to the screw-eye *w*. Upon this line comes the main stress when the carriage is drawn along the rope. The uppermost of the three stay-lines of the net (*s. l. 1*) bears a snap which is fastened to the screw-eye *x* at the lower anterior corner of the carriage. This makes it certain that the plane of the mouth of the net will be kept perpendicular to the line of progress of the carriage. A snap at *y* binds the net to the carriage and another at *z* supports the bucket.

3. *Accessory Apparatus* (Plate III.).—The stay- and draw-lines are of braided linen. The latter is about thirty-five meters in length and is carried on a hand-reel. It is knotted at intervals of one meter to a point thirty meters from the opening of the plankton net. The carriage rope is a five-sixteenths inch braided rope known in the trade as "sash cord." It does not kink in handling, and if once thoroughly seasoned is subject to little change when wet and cannot easily be stretched. This rope is about forty meters in length and is marked by colored threads at a point exactly thirty meters (Plate III., Fig. 7, *m.*) from the opening of the net. The location of the end knot (Fig. 6, *k.*) is marked in a similar manner.

Three sharpened stakes (Fig. 7, *b. s.*, *m. s.*, and *e. s.*), the brace-, main-, and end-stakes, respectively three, four and one half, and five meters in length, complete the apparatus necessary for making an oblique haul.

4. *Operation of the Oblique Haul* (Plate III.).—In this operation two persons are required, an oarsman to handle the skiff and an operator for the apparatus. The method of procedure is as follows. The brace rope (Fig. 7, *b. r.*) is fastened near the lower end of the brace stake (*b. s.*) and the latter is then set as firmly as desired. The main stake (*m. s.*) is then put in place and the brace rope is tied to it. Next, the carriage rope (*c. r.*) is fastened to the main stake (*m. s.*) at a point 5 to 8 meters distant from the thirty-meter knot (*m*). The boat is then rowed away in line with the two stakes and the carriage rope is unreeled until the end is reached, when it is run through the pulleys (Fig. 6, *p.* and *p.'*) of the upper part of the carriage. The end knot (*k.*) is then tied and fastened to the rear pulley (*p.'*) by the release thread (*th.*). The carriage rope is next tied to the lower part of the end stake (Fig. 7, *e. s.*), and at a point just in front of the anterior pulley (*p.*) it is bound to the end stake by the release line (*r. l.*). The lower part of the carriage with the suspended plankton net can now be attached to the upper part, and the end stake is ready to be placed. When the end stake is set, the carriage line (Fig. 7, *c. r.*) runs obliquely from the release line to the surface of the water at the main stake. The net occupies the position *n.* (Fig. 7), and having been lowered vertically does not strain any water in its descent. After placing the end stake the skiff is rowed to the main stake as the draw-line (Fig. 7, *d. l.*) of the carriage is unreeled. A quick jerk upon the carriage rope snaps the release line (*r. l.*) on the end stake, and the slack in the carriage rope can be taken up at the main stake. The carriage rope and net now take the position *c. r.'* and *n.'* (Fig. 7), and the thirty-meter knot *m* is at the surface of the water. Everything is now in readiness for making the catch. At the signal the release thread (*th.*) which binds the rear pulley to the end knot is broken by a quick pull upon the draw-line. The carriage bearing the plankton net is drawn up the oblique rope the distance of thirty meters in one minute

by the operator, who regulates the speed by the meter knots on the draw-line and the counting of the oarsman, one count being given every other second by the watch. By this method a *uniform* velocity for the plankton net at all parts of its ascent and in different hauls is assured, and a very important source of error in the vertical haul, as it is usually made, is avoided. The oblique haul is not so complicated as it may perhaps seem to the reader. With a little practice the whole operation may be completed in less than twenty minutes.

Whenever a current is present in the body of water examined, it has been our custom to make the oblique haul across the current, thus eliminating, so far as may be, its effect upon the coefficient or straining capacity of the net. In water in which there is little or no current it is of advantage, in working in a skiff, to set the apparatus "with the wind." In waters abounding in vegetation, channels of the length and width requisite for making the oblique haul were opened from time to time by cutting out and removing the rank growth of aquatic plants.

5. *Difficulties encountered.*—Certain difficulties attended the operation of the oblique haul. Owing to the turbidity of the water, in many situations it was practically impossible to place the apparatus so as to avoid vegetation. Whenever the plankton net or the ropes strike a submerged plant, a cloud of flocculent debris is set free in the water and the collection is fouled. It was only with the greatest labor that channels could be kept open in the vegetation, for its rapid growth and its shifting by the wind soon closed any opening that had been made. Again, the manipulation of the apparatus in rough weather is somewhat difficult, the waves at times tearing loose the stakes before the completion of the collection. At periods of high water the strong current and the increased depth made it impossible to set the apparatus or keep it in place. This necessitated the substitution of a series of vertical hauls from a floating boat for the customary oblique method of collection.

The plankton at Havana is subject to extreme local and seasonal variations, not only in volume but also in composition. For example, at one time *Cladocera* predominate and at another diatoms are present in vast numbers, and at still another rotifers constitute practically the whole of the plankton. Observations upon the operation of the net in the midst of these fluctuations awakened the suspicion that the amount of water *actually strained* was subject to considerable variation, dependent, among other causes, upon the amount and, more especially, the composition of the plankton. If the plankton were constant in quantity, kind, and distribution, the error arising from the progressive clogging of the net as it traverses the thirty meters would be distributed alike in all of the catches, and they would still be comparable; but the existence of the fluctuations in the plankton just noted and the consequent variation in the amount of water strained, constitute an important source of error in any deductions based upon comparisons of catches made under these variable conditions. This source of error is present in the vertical as well as in the oblique haul. Furthermore, change in the *silk itself* consequent upon use adds to the errors due to the fact that the collection is made by *drawing the net through the water.* A series of field experiments (to be described in a later paper) upon the progressive clogging of the net and the coefficient of various plankton nets, in a wide range of season and situation, have abundantly justified our abandonment of the system of collection in which the *net is drawn through the water* for one in which a known quantity of *water is put through the net.*

II. THE PUMPING METHOD.

For many years the biological examination of potable water has been conducted by straining or filtering water delivered through service pipes at the faucet by pressure due to the use of a pump.

Giesbrecht ('96) describes the collection of *Copepoda* in the Red Sea by Krämer, who strained the water delivered by the ship's pump to the bath-tub of an ocean steamer.

Cleve ('96), at the suggestion of Dr. John Murray, collected plankton on board a steamer in the North Sea by attaching a silk net to the pump when the deck was washed.

Previous to this, Hensen ('87) used the steam pump for putting known amounts of surface water through the filtering net. Hensen's quantitative work was, however, based upon collections made by vertical hauls of the plankton net. Peck ('96), in his work upon the marine plankton of Buzzard's Bay, obtained water for examination by the Sedgwick-Rafter method (see Rafter, '92, and Twenty-third Annual Report of the State Board of Health of Massachusetts for 1891, pp. 395–421) by means of a steam-pump connected with a two-inch hose which was lowered to the desired depth. Beyond these instances no other applications of the pump to the collection of plankton have come to my notice, and there appears to be no record of its use in quantitative work by the Hensen method.

The impossibility of using the vertical haul in shallow waters, the difficulties in the operation of the oblique haul, and especially the error involved in the variable coefficient of the net, have led to the adoption of the pumping method in the plankton work at Havana.

1. *The Pump* (Plates V. and VI.).—The pump we use is a double acting force-pump, known in the trade as a "Thresher Tank Pump." It is worked by an upright handle, and has two cylinders, each 6 × 9 inches, and throws an almost continuous stream. Its capacity is one cubic meter of water per six hundred strokes, provided that the water is delivered to the net without elevation. The stroke is of definite length and its action is regular, the rate employed being one stroke per second. The pump is provided with 20 feet of 2-inch spiral-wound suction hose, terminating in a funnel 20 cm. in diameter. The mouth of the funnel is covered with a linen net of ½-inch mesh to prevent the entrance of stray bits of vegetation, and the end of the hose is weighted to insure its sinking readily. It was found necessary to paint all exposed iron in the water chambers of the pump with a thin coat of asphaltum to prevent the formation of rust scales.

Before the pump was put in use for regular plankton collections, tests were made of the straining capacity of the silk under the impact of the current from the discharge hose. It was found that when the water was delivered from a 1-inch hose into the plankton net and the filtrate refiltered in a second net of the same silk (No. 20), a considerable quantity of the more minute forms, *Rotifera* and *Protozoa*, were forced through the meshes of the first net. This led to the adoption of the $1\frac{1}{2}$-inch discharge hose, and of a net devised by the writer to reduce the force of the discharge, to protect the silk from direct contact with the current, and to equalize the pressure upon the filtering surface.

2. *The Net* (Plate IV., Fig. 8–10).—The net consists of the cover with its accessories and the net proper, the two being so constructed as to be readily separable. From the under side of the conical copper top to which the hose is attached hangs the silk net. When in use the net is supported in a wooden frame, which also serves as a float. The rim fits into the circular central opening and rests upon a projecting ledge of the frame (Fig. 8, *fl.*) in such a manner that the silk does not come in contact with the wood. The frame is so proportioned that the net projects about 8 cm. above the level of the water. Experience has demonstrated that even when the water is full of silt or the plankton is very abundant this elevation is sufficient to provide for filtration without forcing the water into the net by the pump. In ordinary circumstances the water does not rise more than 2–4 cm. above the level of the water in which the net is submerged. Thus practically the whole straining surface is under water. Two turn buttons (*t. b.*) hold the net firmly in place so that it cannot be dislodged by the action of the waves when the water is rough.

The cover (Plate IV., Fig. 8, *cov.*) is an obtuse cone of sheet copper, 33 cm. in diameter and 20 cm. on the side. The apex bears a curved connector (*con.*) upon which the $1\frac{1}{2}$-inch hose can be slipped. The cover is beaded for rigidity, and carries two handles (*h.*) for lifting the net from the frame. After the water enters the net two devices are employed to check the force of the discharge. The first is an inner copper cone (*i. c.*), with diameter of 13 cm. and side of 10, placed in the axis of the net immediately below the orifice of the connector. The cone is suspended from the top by means of three stays (*st.*), and sheds the water centrifugally against an inner net (*i. n.*) of No. 12 silk. This net is hung from a ring (*r. i.*) fixed to the under surface of the cover by three supports, one of which (*t. s.*) swings upon the pivot (*p.*) and permits the removal of the net. The inner net conforms to the proportions of the outer net but is only 27 cm. in diameter at the top. At its apex is an opening 8 cm. in diameter, through which plankton caught on the sides can be washed into the lower part of the outer net.

To secure rigidity the margin of the cover is provided with a projecting horizontal wing (*w.*), to which is attached the foot (Plate IV., Fig. 8–10, *f.*). This in cross-section is L-shaped, extending obliquely downward and outward, the oblique and horizontal arms being respectively 2.5 and .75 cm. in length. The foot fits into a circular trough (*tr.*) 1–1.25 cm. in width and 2.75 in height. The inner wall (Fig. 9, 10, *i. w.*) of the trough is parallel to the oblique face of the foot, against which it rests when the cover is in place. The cover is held in the trough by means of four turn clamps (Fig. 9, *c.*), which are fastened by straps (*str.*) upon the outer wall (*o. w.*) of the trough. When the clamps are released and swing to the position *c′*, the cover can be removed, and the upper margin of the outer net (Fig. 9, *o. n.*) can be folded over the inner wall of the trough. When, now, the cover is replaced, the net is firmly clamped between the oblique face of the foot and the inner wall of the trough. (Cf. Fig. 9 and 10.) This method of attachment permits the ready removal of the net for the purpose of drying the silk, and at the same time insures a tight joint.

The net is made of the customary No. 20 silk and measures 92 cm. on the side. The upper border is faced upon the outer surface for 6 cm. and upon the inner for 2 cm. with butchers' linen, so that the wear in the fastening of the net falls upon the linen, while the silk only is exposed to the water to be filtered (Fig. 10). To insure the uniform placing of the margin of the net in the fastening, a heavy cord (*cd.*) is sewed in the border, against which the angle of the foot rests when placed in position. The plankton bucket, with its method of attachment to the net, is similar to that described for the vertical net.

3. *The Method of Operating the Pump.*—The pump is carried in a suitable row-boat, and the suction hose is operated from the stern by one person while a second attends to the pump and the net (Plate VI.). In the choice of a location and in the position of the boat, due regard must be had to the direction of the wind and the current, if any, so that no filtered water may reënter the pump. In our work in the lakes it has been our custom to tie the boat to poles set for this purpose; but in the river the boat has been allowed to drift with the current in order to make the collection, so far as may be, from the same body of water. After the depth is ascertained the suction hose is lowered to within a foot of the bottom, the pump is thoroughly rinsed, and while still filled with bottom water the discharge hose is connected with the net. As the pumping progresses the funnel is raised at regular intervals; for example, every tenth stroke, the interval and the distance raised varying, however, with the total depth of the water to be traversed. Since the pump is filled with bottom water when pumping begins, it is necessary to shorten the first interval by the number of strokes required to fill the pump and to correspondingly lengthen the last one. In this manner a vertical column of water of the desired volume may be pumped through the net. In addition to the vertical catch we have followed the custom of making one from bottom water and another from surface water. After the requisite number of strokes of the pump have been made the hose is disconnected and the net removed from the frame and thoroughly rinsed down. The catch is concentrated in the bucket and transferred to the bottle of alcohol or formalin.

The pumping method has been successfully employed in freezing weather by attaching a foot-warmer to the side of the pump and encasing the whole in a wrapping of felt paper. The foot-warmer burns a cake of specially prepared coal, and will keep the pump warm during a day's work of ten hours. A special drain-cock (not shown in Plate V.) provides for the removal of all water from the cylinders when the pump is not in use.

4. *Advantages of the Pumping Method.*—As compared with methods dependent upon hauling the plankton net through the water, several points of advantage are to be found in the pumping method. It is more accurate, since the actual volume of water strained can be determined, and the changes in the coefficient of the net due to seasonal and local variations in the quantity and composition of the plankton and to alterations in the silk of the net with use are to a very large degree eliminated. The method is also widely applicable: as water may be drawn from any desired level, it may be applied to the problem of vertical distribution; and it may be used in very shallow water, in the midst of vegetation, in creeks, in strong currents, under the ice—in fact, in a wide variety of situations from which the vertical or oblique hauls are wholly excluded or to which they are with difficulty applied. Again, no matter how poor the water may be in plankton, it is always possible to strain an amount sufficient to furnish enough plankton for measurement. The method is also a comparatively rapid one, requiring for a plankton collection only about one third of the time consumed in making the oblique haul.

The pumping method is thus admirably adapted to the situation with which we deal at Havana, *i. e.*, shallow water and an abundance of vegetation. It is not, however, limited in its applicability to such situations, but with the help of a steam-vessel and a steam-pump it is capable of application to larger and deeper bodies of water.

III. PRESERVATION AND EXAMINATION OF THE PLANKTON.

1. *Preservation.*—The living plankton is transferred directly from the bucket of the net to a wide-mouth two-ounce bottle, and the sides of the bucket are rinsed down thoroughly with a spray of 1% formalin to insure the complete removal of all of the catch. Enough strong alcohol is then added to the bottle to make a grade of about 75%. Surface and bottom collections are usually preserved in 1% formalin, or in 75% alcohol after killing in picro-sulphuric acid. The bottles are all labeled with a gummed slip bearing the accessions'

catalogue number, designation of the catch (whether surface, bottom, vertical, or qualitative), station, killing agent, and date. For convenience in handling they are then arranged chronologically in racks, each holding six bottles. Data blanks bearing the catalogue number are filled out for each station examination. The locality, date, time of day, the condition of the sky, the direction and force of the wind, the stage of the river and the amount and direction of its change in the twenty-four hours preceding, the depth of the water, its turbidity (measured by means of a porcelain disk), the disturbance of the surface, the temperature of the air and that of the water at surface and bottom, the current, the kind of vegetation and distance from it, the manner of collection and means of preservation of the catches made,—all are matters of regular record, together with any other data peculiar to the collection which could possibly interest the student of the plankton.

2. *Quantitative Examination.*—The quantitative examination of all of the plankton collections made at Havana has been undertaken by the writer. Determination of the quantity of the plankton by both the volumetric and enumerative methods is necessary, owing to the presence, especially in flood waters, of a large amount of silt. The gravimetric or weighing method suggested by Zacharias ('95) is, as Ward ('96) has suggested, objectionable on account of the unknown and presumably variable amount of water or alcohol present in the still moist plankton. Many of the planktons at Havana taken in silt-laden waters contain a considerable amount of mineral and earthy matter. This constitutes a further objection to the application of the gravimetric method to our collections. A combination of the gravimetric and volumetric method has been suggested by Ward ('96), in which plankton of known volume is dried to a constant weight, burned, the ash weighed and afterwards digested in concentrated HCl, and the residue then washed, dried, and weighed. The amounts of organic matter, of soluble salts (calcareous), and of silicious matter can then be determined, and thus corrections for sand-laden planktons can be approximately computed. It is evident that this method cannot be applied to

planktons rich in diatoms nor to the silt-laden planktons from Havana, for a large part of the silt is debris of organic origin, and the method above described does not differentiate the organic material of the plankton from that of the silt. For the quantitative investigation of these silt-laden planktons we are thus practically limited to the enumerative method with such incidental help as may be derived from volumetric determination.

The *volumetric determination* has taken two forms, the settling and the centrifugal methods. The former as used by us is the same as that employed by Reighard ('94) and Ward ('96a). The plankton is transferred to graduated tubes and is allowed to stand twenty-four hours, when the amount of the plankton settled at the bottom of the fluid, is read by the graduations upon the tube. The tubes used are the carbon tubes employed by chemists in the Eggert color test for the estimation of carbon in steel. Our tubes in most frequent use contain 25 and 50 cubic centimeters respectively, are about twelve millimeters in inside diameter, and are graduated to tenths of a cubic centimeter. For very small planktons another tube, containing only ten cubic centimeters and measuring six millimeters in inside diameter, was used. After a series of measurements in the tubes above described it became evident that a considerable error was involved in the method. Repeated measurements of the same plankton in the same tube, after standing twenty-four hours, revealed a considerable variation in the volume, as high as 30% in some instances. Furthermore, planktons do not settle to an equal density. Those composed of *Rotifera* or small *Cladocera* (as *Chydorus*) pack closely, while others containing filamentous forms, as *Oscillaria* or *Fragillaria*, and those in which the larger *Entomostraca* are predominant, settle very loosely. Thus the determination of the volume of the plankton by the settling method does not give a uniform test of the amount of plankton present. Furthermore, the process is a tedious one, especially when large numbers of catches are to be handled.

The centrifugal machine (Plate VII.) was finally hit upon as affording the best solution of the difficulties presented in the settling method. In our machine we have utilized the double arms, aluminum shields, and percentage tubes of the Purdy Electric Centrifuge. The tubes contain 15 cubic cm., are graduated to tenths of a cubic cm., and the conical tips permit the measurement of small planktons with accuracy. The arms are borne upon an upright shaft which is driven by a system of gears turned by means of a crank handle, one turn of the crank giving 24 revolutions of the vertical shaft. The direction and the speed are thus easily controlled by the operator. The machine is clamped firmly to a table when in operation. All parts of the machine, except those from the Purdy Centrifuge, were devised by Professor W. H. VanDervoort, of the College of Engineering, and were constructed in the University shops. Planktons are subjected to 2,000 revolutions in two minutes, the motion at first being slow and frequently reversed. The practical limit of compression by this machine is thus reached, and successive measurements of the same planktons show that its action is quite uniform. The average amount of compression in a wide range of planktons is about 50%, the volume by the centrifuge method ranging from 30% to 70% of that obtained by the settling method. No discussion is needed to prove that the more perfect the compression the more accurate are the volumetric determinations of the plankton. In this lies the main argument for the use of the centrifuge in quantitative plankton work. It also permits rapid work and is easily manipulated. Our machine was completed in January, 1896, and this is, I believe, the first application of the centrifugal machine to quantitative plankton work. Cori ('96) has devised a simple centrifugal machine for precipitation purposes in zoölogical work, but it does not seem to be fitted for quantitative determination of plankton.

The machine employed by us was also in use for the precipitation of living plankton from the water when Dolley's paper ('96) was received describing a large and powerful centrifuge, "the planktonokrit," devised for the same work. It is only by means of some such machine as this that a complete examination of the contents of the water is possible.

The *enumerative or counting method* involves a recognition of all of the different organisms composing the plankton and the enumeration of the individuals of each species present in a part or the whole of the catch. The number present under a square meter of surface or in a cubic meter of water can then be computed. This work is the basis of the discussion of the seasonal range, local distribution, and interrelations of the components of the plankton. The method of counting at present employed by us is that described by Rafter ('92) as a part of the Sedgwick-Rafter method of microscopical examination of potable waters. This method was employed by Professor J. I. Peck ('96), and I am indebted to him for many kind suggestions on its use. The apparatus consists of a brass cell, 20×50 mm. and 1 mm. in depth, cemented upon a glass slide, a 1-cubic-cm. pipette, a mechanical stage, and an area-stop for the eyepiece. After the plankton to be examined is diluted to the desired degree and thoroughly stirred, one cubic centimeter is transferred with the pipette to the cell, in which one cubic millimeter underlies each square millimeter of the cover-glass. By means of the mechanical stage any desired cubic millimeter of the cell can be placed in the center of the field. The area-stop is a circle of black paper to be placed in the eyepiece, which cuts off all the field except that visible through a square opening at its center. This opening should be of such a size that with the objective employed for the counting work exactly one square millimeter of the cell is subject to inspection.

Ordinarily the counting of from ten to twenty squares suffices for a fair test of the occurrence of organisms in plankton; but in the work upon the richly diversified plankton at Havana we have found it necessary to increase the number to fifty or even one hundred for the commoner and smaller species, while for the larger and the rarer forms a great part or even the whole of the catch must be examined.

URBANA, ILLINOIS, November 23, 1896.

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*EXPLANATION OF PLATES.

Plate I.

Fig. 1. Longitudinal section of plankton net. × ¼.

c. b. l.	Cone of butchers' linen.	*s. l.*	Stay-line.
cd.	Cord.	*sm.*	Seam.
cs.	Cover-strip.	*s. n.*	Silk net.
e.	Eye.	*st.*	Strip fastening cone to stay
h. p.	Head-piece of bucket.	*th.*	Thread.
l. e.	Lower eye of wire stay.	*u. e.*	Upper eye of wire stay.
l. r.	Lower wire ring.	*u. r.*	Upper ring.
n. c.	Net clamp.	*w. s.*	Wire stay.

Fig. 2. Seam in silk net. × 2.

a.	Backstitch.	*b.*	Fell.

Plate II.

Fig. 3.—Longitudinal section of head-piece of plankton bucket. × 1.

e.	Eye.	*s.*	Silk.
h.p.	Head-piece.	*s. l.*	Stay-line.
n. c.	Net clamp.	*t. s.*	Thumb-screw.
m.	Thread, screwing into *n* of Fig. 4.	*w. n. c.*	Wing of net clamp.

*Plates I., II., III., and IV. were drawn by C. A. Kofoid and inked by Miss L. M. Hart.

FIG. 4.—Longitudinal section of plankton bucket. × 1.

b.	Bottom.	*pl.*	Plate of clamp.
ba.	Base.	*pi.*	Pillar.
b. c.	Band clamp.	*s.*	Silk.
d. p.	Drip-point.	*sc.*	Screw.
e.	Eye.	*sh.*	Shoulder.
n.	Thread, screwing on *m*, Fig. 3.	*w.*	Window.
p.	Plug.	*x–y.*	Line of section shown in Fig. 5.

FIG. 5.—Cross-section of plankton bucket at *x–y*, Fig. 4. × 1. Lettering as in Fig. 4.

PLATE III.

FIG. 6.—Carriage. with suspended plankton net. × ⅛.

a b c.	Line of separation of carriage.	*s.*	Staple.
bu.	Bucket.	*s.l.1, s.l.2, s.l.3.*	Stay-lines from the draw-line to net and bucket.
c. r.	Carriage rope.	*s. l. 4.*	Stay-line to carriage.
d. l.	Draw-line.	*sn.*	Snap.
h.	Hook.	*th.*	Thread.
k.	End knot.	*w. s.*	Wire stay.
l.	Lead.	*w x y z.*	Screw-eyes for attachment of net.
p.	Front pulley.		
p.'	Rear pulley.		

FIG. 7.—Operation of the oblique haul. × $\frac{1}{200}$.

b. r.	Brace rope.	*e. s.*	End stake.
b. s.	Brace stake.	*m.*	30-meter knot.
bt.	Boat.	*m. s.*	Main stake.
c. r.	Carriage rope before release.	*n.*	Net before release.
		n.'	Net after release.
c. r.'	Carriage rope after release.	*r. l.*	Release line.
		sur.	Surface of water.
d. l.	Draw-line.		

PLATE IV.

FIG. 8. Plankton net used with the pump, shown in longitudinal section. × ¼.

b. l.	Butchers' linen.	*o. n.*	Outer net.
con.	Connector.	*p.*	Pivot for support of ring.
cov.	Cover.	*ri.*	Ring for inner net.
f.	Foot.	*st.*	Stay for inner cone.
fl.	Float.	*t. b.*	Turn button.
h.	Handle.	*t. s.*	Turn support.
i. c.	Inner cone or spreader.	*tr.*	Trough.
i. n.	Inner net.	*w.*	Wing.

Fig. 9.—Rim of cover of net, showing clamp for holding cover in place. × 1.

c. Clamp, in position.
c'. Clamp, released.
cov. Cover.
f. Foot of cover.
i. w. Inner wall of trough.
o. w Outer wall of trough.
str. Strap of clamp.
w. Wing of cover.

Fig. 10.—Same, showing method of fastening outer silk net; cover partially removed. × 1.

b. l. Butchers' linen.
cd. Cord.
o. n. Outer net.

Other lettering as in Figure 9.

Plate V.

The plankton pump. × $\frac{1}{10}$.

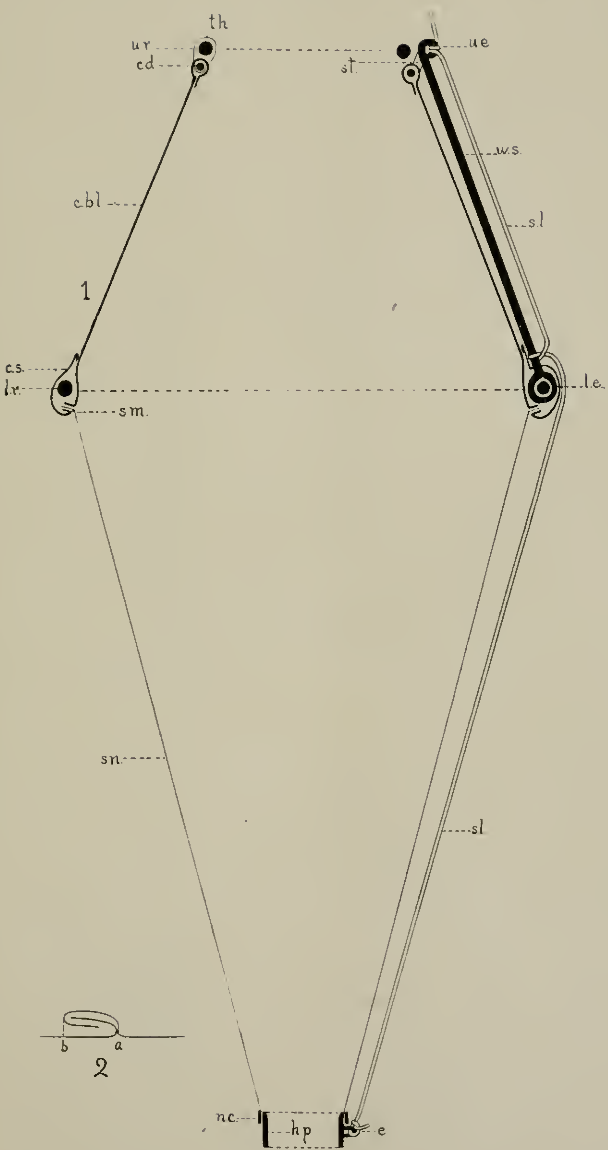
Plate VI.

The plankton pump in operation.

Plate VII.

The centrifugal machine. × ¼.

PLATE I.



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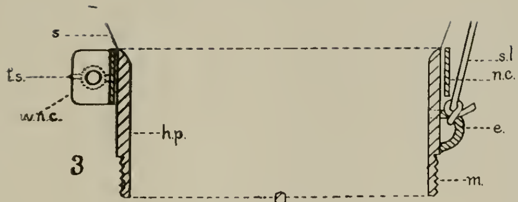
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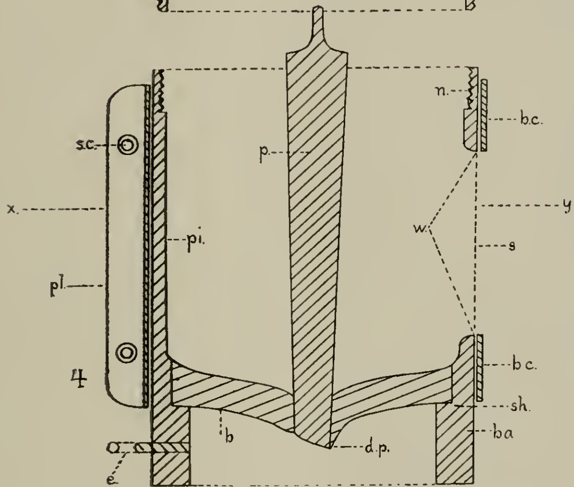
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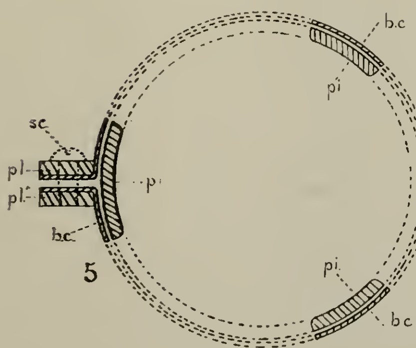
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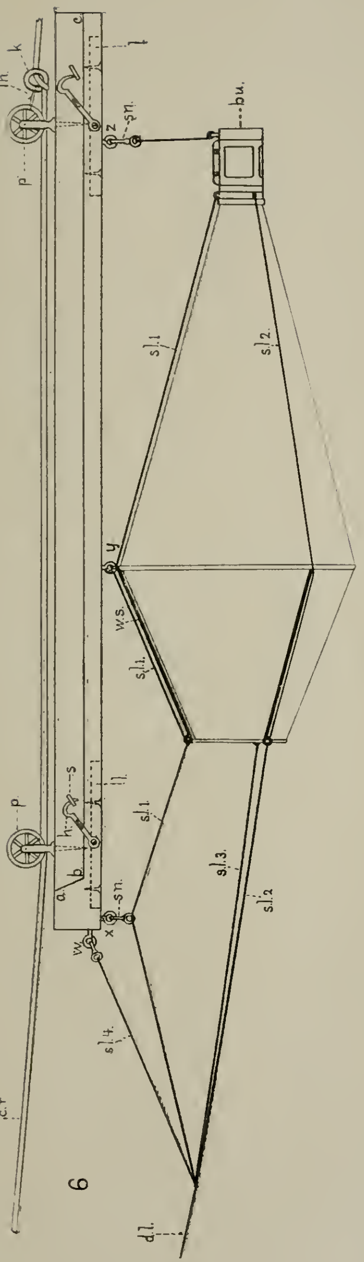

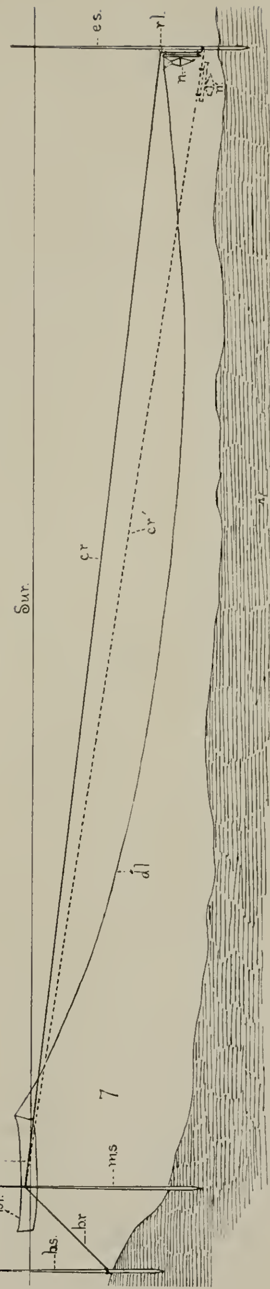

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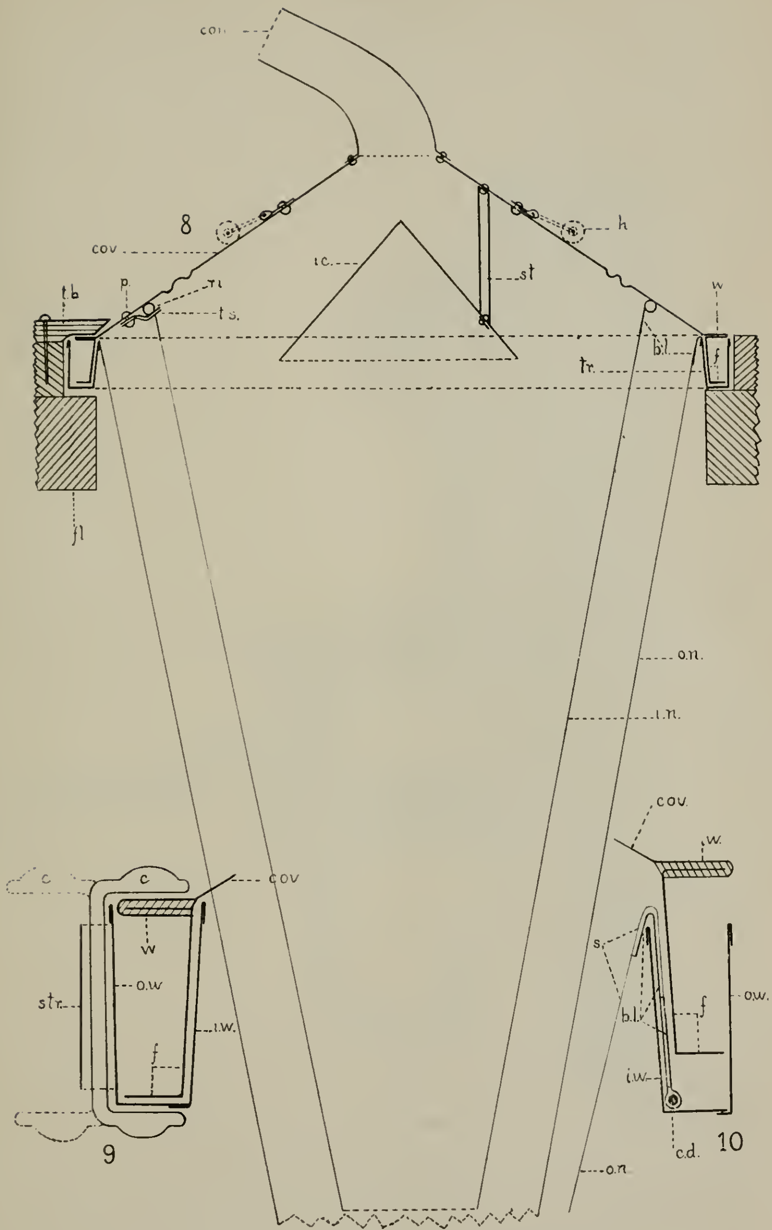

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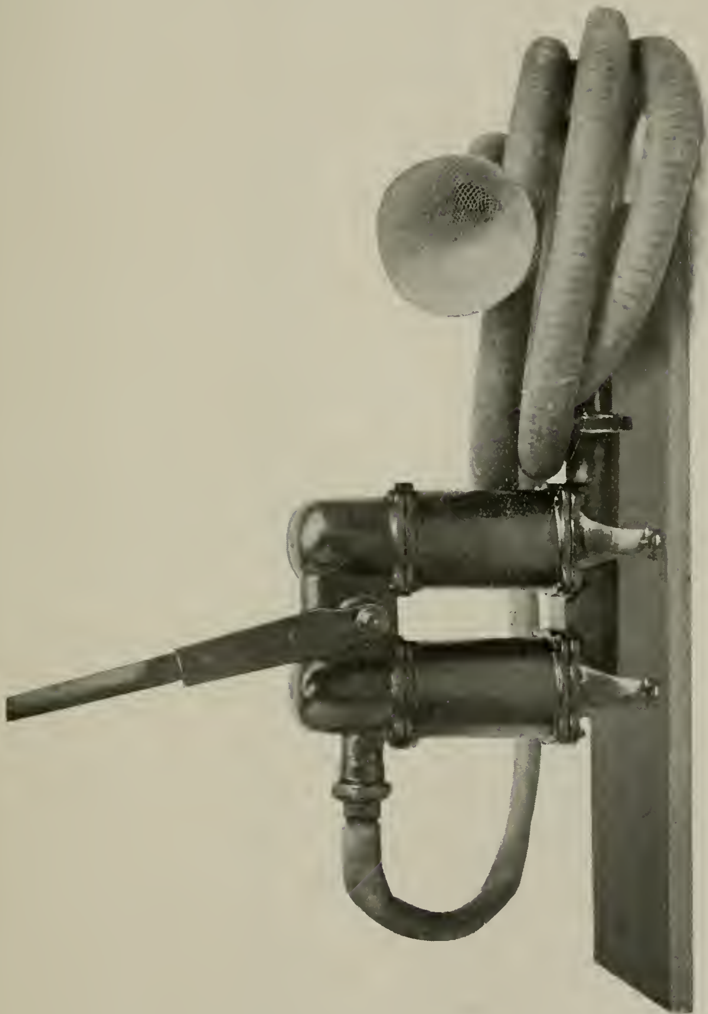
Plate V.



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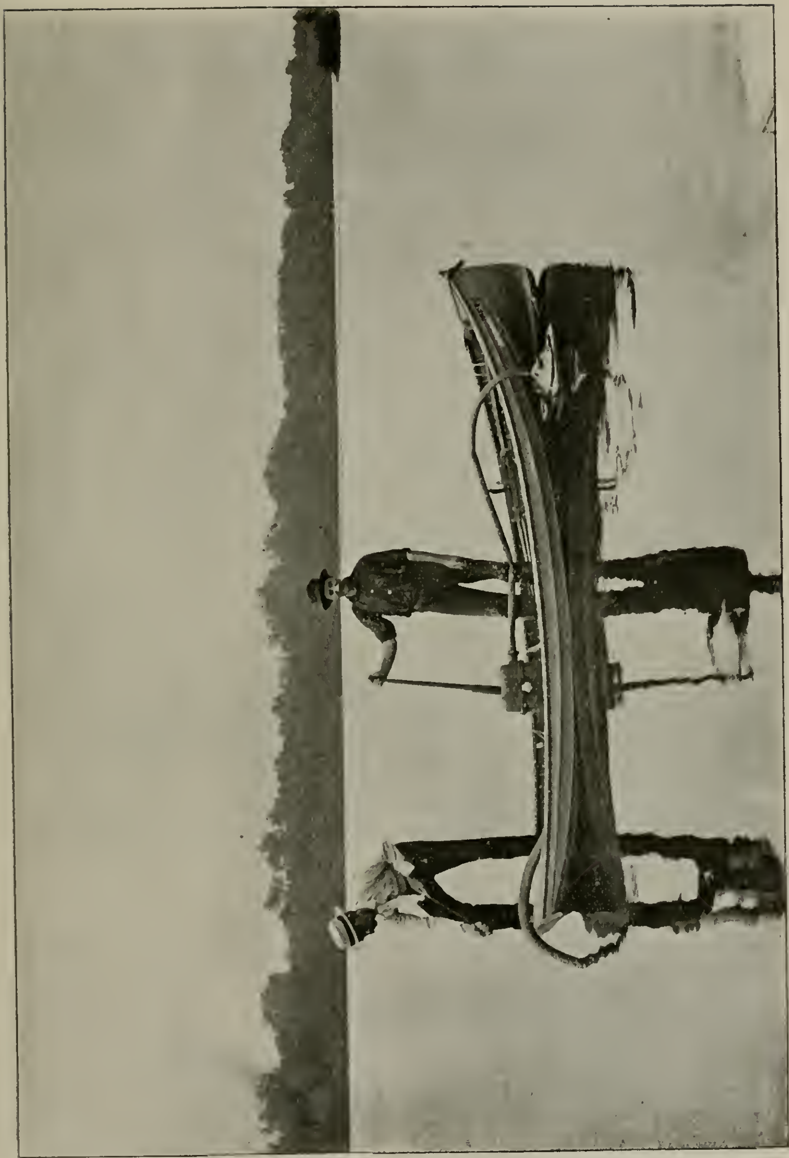

Plate VII.