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Seasonal changes in the haptoral organs and copulatory organ of *Hamatopeduncularia wallagonius* Singh *et. al.* 1995 on the gills of *Mystus seenghala* (sykes) and *Wallago attu*(bleeker and schn.) from Bhola ki jhal reservoir, U. P. India.

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Pragati Rastogi* and Jyoti Singh**

Abstract:

It's a well known fact that monogenean parasite show changes in relation to environmental factors like temperature. Present study revealed the fact that haptoral hard structures and copulatory organ of monogenean parasite Hamatopeduncularia wallagonius in Mystus seenghala (Sykes) and Wallago attu (Bleeker and Schn.) show changes with change in temperature. Worms were collected from the gills of Mystus seenghala (Sykes) andWallago attu(Bloch and Schn) at Meerut at different water temperatures. Samples were taken at monthly intervals of about 30 (± 5) days during the period from December 2008 to February 2012. The main results indicate difference between all season with the help of suitable tables and histograms. Main result shows significant changes in morphometrical variables of haptoral hard parts and copulatory organ with reference to temperature.

Keywords: *Monogenea, Hamatopeduncularia wallagonius, environmental factors, haptoral hard structures,copulatory organ, morphometric variables.*

**Asst. Prof., Dept. of Zoology, Meerut College, Meerut*
***Research Scholar, Department of Zoology, Meerut College, Meerut*

Introduction:

Singh *et. al.,* 1995, described *Hamatopeduncularia wallagonius* from the gills of *Wallago attu*(Bloch and Schn) at Meerut. Highest prevalence of infection was recorded in period from January to March. There are several morphological and anatomical characteristics of monogenea that are used for species determination. Main morphological parameters are morphometric characteristics of attachment apparatus and copulatory organ. Species definition using only the shape and measurement of attachment apparatus and copulatory organ is difficult in similar species, because some measurements often overlap, while shape is variable. Because the mistaken description, of an already existing species as newly discovered occurs, it is important to know which factors affect morphometrical variation to avoid incorrect determination of species (Gusev 1985).

The aim of this study is to investigate the differences of measurements of attachment apparatus and copulatory organs between *H. wallagonius* individuals in different temperatures. It is expected to detect morphometric variation in morphology of *H. wallagonius* considering seasonal changes in water temperature.

Materials and Methods:

Fishes for present investigation were collected from a reservoir Bholakijhal, near Marhi village, Meerut (U. P.). Total 90 specimens were used to study seasonal variations. All specimens were used to study seasonal variations. Mizelle's (1936 and 1938) freezing technique was employed for collecting parasites. Parasites thus collected, were processed for morphometric studies.

Morphometric Analysis:

Worms were washed thoroughly several times with chilled distilled water, to remove any mucous or debris adhering to the parasites. These worms were fixed in hot 4% neutral formaldehyde for at least 8 hours. Worms were washed thoroughly with distilled water. For study of hard parts worms were mounted in glycerol. These worms were observed under microscopes and photographs of hard parts were taken.

Parameters that were measured for morphometric analysis and study of seasonal variation as suggested by Mo (1991, 1991 and 1991) include 15 morphometric parameters of the attachment apparatus and two of the copulatory organ: total length of anchor - la; length of anchor shaft - las; length of anchor point - lap; length of anchor root - lar; length of transverse bar - lb; total basal width of transverse bar - tbwb; basal width of transverse bar - bwb; total median width of transverse bar - tmwb; median width of transverse bar - mwb; maximal distance between processes of transverse bar - mdpb; total length of marginal hooks - lmh; length of marginal hook handle - lh; length of marginal hook sickle - lsi; length of cirrus tube, and width of cirrus tube.

A total 90 specimens of *H. wallagonius* were measured and photographed with help of calibration tool of Motic DMB1 Microscope. It was not always possible to measure all 15 characters, because some prepared specimens were destroyed during the preparation or had deterioratedwith time. Some attachment apparatus had inconvenient position or were unsuccessfully compressed between the coverslip and the slide therefore, have not been recorded. Unequal number of measurements and low number of specimens in autumn period was taken into account during statistical analysis. All data was processed using SPSS version 11 and a computer for calculating the mean and standard error. This data was also processed for probability coefficient and regression analysis. All results obtained were graphically presented. All the measurements are in microns (μ) in table 1 and 2. All temperature readings are in degree Celsius (°C).

Results:

Host	: *Mystus seenghala* (Sykes) and *Wallago attu* (Bleeker and Schn.)
No. of hosts examined	: 22
No. of hosts found infected	: 15
No. of worms collected	: 90
Site of infection	: Gills
Locality	:Bholakijhal (reservoir), Meerut

Singh *et. al.*, 1995, described *Hamatopeduncularia wallagonius* from the gills of *Mystus seenghala* (Sykes) and*Wallago attu*(Bloch and Schn) at Meerut. Analysis of hard parts of species, based on fresh material collected by the author, is given here in as such.

Table 1: Mean minimum and maximum length and widthof cirrus of *H. wallagonius* (December - February)

	Total length (μ)	Width (Proximal end) (μ)
December	63.12, 64.00 (63.60)	9.75, 10.25 (9.96)
January	64.59, 64.82 (64.70)	10.76, 10.95 (10.80)
February	64.11, 64.91 (64.62)	10.31, 10.90 (10.7)

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Table 02: Mean minimum and maximum length of haptoral hard parts of *H. wallagonius* (December – February)

	Dorsal Anchor (µ)				DTB (µ)		Ventral Anchor (µ)				VTB (µ)		Marginal Ho	
	LA	LAS	LA R-Out	LA R-Inr	LB	M WB	LA	LAS	LA R-out	LA R-Inr	LB	M WB	LM H	LH
December	43.69, 56.01; (49.86)	27.57, 31.15; (26.79)	22.83, 28.50; (26.67)	28.14, 30.50; (29.53)	71.22, 79.99; (67.20)	3.06, 6.21; (4.86)	58.56, 62.90; (56.24)	31.11, 32.40; (29.41)	32.36, 35.91; (33.52)	30.01, 37.13; (31.34)	88.37, 97.80; (79.69)	4.16, 5.34; (4.25)	18.20, 21.80; (16.62)	14.64, 16.96; (13.92)
January	50.15, 64.74; (52.16)	24.81, 36.26; (29.79)	23.81, 34.05; (29.70)	25.91, 33.25; (29.53)	69.00, 94.71; (78.44)	4.58, 6.45; (5.67)	58.35, 63.00; (61.30)	33.15, 37.60; (34.39)	31.11, 36.14; (34.80)	30.37, 38.98; (34.73)	85.15, 99.60; (94.78)	3.47, 5.02; (4.79)	16.25, 17.88; (19.55)	13.99, 14.95; (14.26)
February	49.19, 52.23; (50.61)	22.65, 30.90; (28.53)	21.45, 32.94; (28.09)	24.32, 31.13; (29.25)	61.41, 75.80; (77.81)	3.19, 6.34; (5.00)	52.35, 63.20; (61.08)	27.04, 37.96; (31.77)	30.40, 36.57; (33.53)	29.04, 33.00; (34.37)	75.03, 99.29; (89.86)	3.32, 5.47; (4.26)	15.18, 18.08; (17.23)	13.13, 15.79; (15.69)

Table 3: Significant Pearson correlations of *H. wallagonius*

Hard parts having bivariate correlation	*H. wallagonius*
Total Length of cirrus – Width of cirrus	0.999
La - las of Dorsal anchor	0.958
Lb - mwb of Dorsal Transverse Bar	0.671
La - las of Ventral Anchor	0.870
Lb - mwb of Ventral Transverse Bar	0.761
Lmh - lh of Marginal Hooklet	-0.143

Regression analysis

Output obtained from regression analysis of *H. wallagonius* to find out their linear mathematical relationships with all hard parts of cirrus and haptor are as follows:

Cirrus

Length of Cirrus = 50.303 + 1.335 x width of Cirrus

Dorsal anchor

Length of anchor = 29.710 + 0.746 x Length of anchor shaft

Dorsal transverse bar

Length of bar = 23.794 + 9.792 x Median width of transverse bar

Ventral anchor

Length of anchor = 27.715 + 0.999 x Length of anchor shaft

Ventral transverse bar

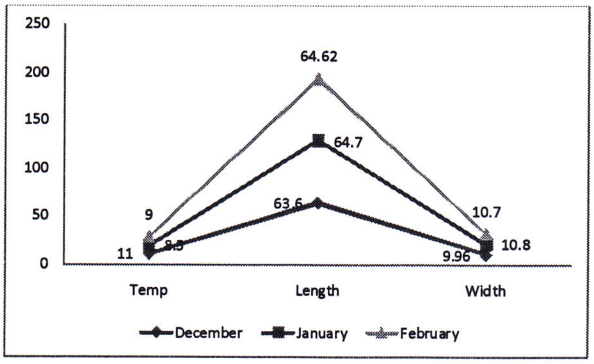
Length of bar = 4.043 + 18.962 x Median width of transverse bar

Marginal hooklet

Length of marginal hooklet = 21.230 + -0.235 x Length of hook handle

Table 4: Mean variations in size of cirrus of *H. wallagonius* with temperature from December 2008- February 2012.

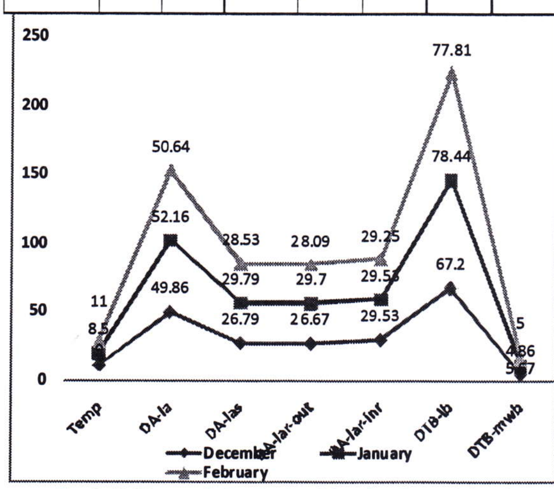
	Temp	Length	Width
December	11.0	63.6	9.96
January	8.5	64.7	10.8
February	9.0	64.62	10.7



Graph 1: Mean variations in size of cirrus of *H. wallagonius* with temperature from December 2008- February 2012.

Table 5: Mean variations in size of dorsal anchor and dorsal transverse bar of *H. wallagonius* with temperature from December 2008- February 2012.

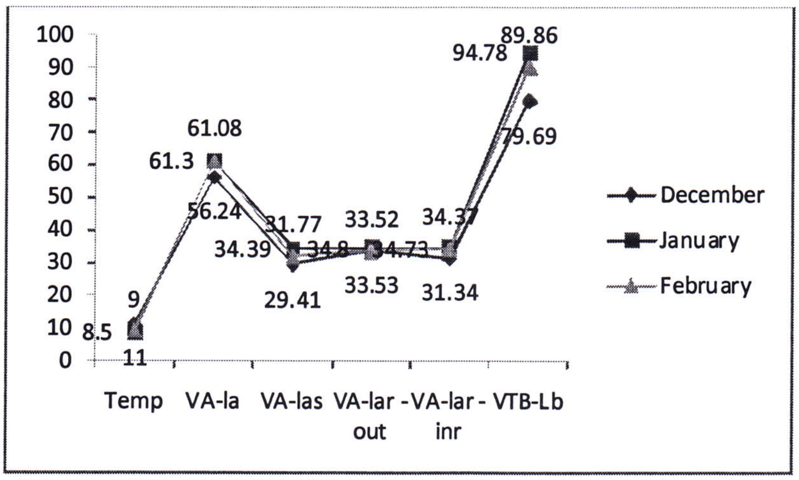
	Temp	DA-la	DA-Las	DA-Lar - out	DA-Lar - inr	DTB-	DTB-
December	11.0	49.86	26.79	26.67	29.53	67.2	4.86
January	8.5	52.16	29.79	29.7	29.53	78.44	5.67
February	9.0	50.64	28.53	28.09	29.25	77.81	5.0



Graph 02: Mean variations in size of dorsal anchor and dorsal transverse bar of *H. wallagonius* with temperature from December 2008- February 2012

Table 6: Mean variations in size of ventral anchor of ventral transverse barsand of *H. wallagonius* with temperature from December 2008- February 2012.

	Temp	VA-la	VA-las	VA-lar - out	VA-lar - inr	VTB-Lb	VTB-Mwb
December	11.0	56.24	29.41	33.53	31.34	79.69	4.25
January	8.5	61.3	34.39	34.8	34.73	94.78	4.79
February	9.0	61.08	31.77	33.52	34.37	89.86	4.26



Graph 3: Mean variations in size of ventral anchor and Ventral transverse bars of *H. wallagonius* with temperature From December 2008- February 2012.

Discussion

Characters measured on each of 90 samples were normally distributed. During present investigation total length and width (proximal end) of **cirrus** have changed. Length and width of cirrus are temperature dependent. It shows significant regression. **Dorsal anchor** are highly flexible. Length of hard parts has great impact in the overall appearance of the hard parts. In December outer root is longer than inner root. Shaft is slightly

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curved. **Dorsal transverse bar** of *H. wallagonius* show changes in shapes and also in size. There is a progressive increase in the median width of dorsal transverse bar with decrease in temperature. As temperature falls again in January and February, median width of dorsal transverse bar increases again and acquires a convex shape. **Ventral anchor** changes its size and shape as temperature changes from December to February. Curvature of shaft increases from December through February. Outer and inner root cannot be demarcated. Anchor shaft is recurved. **Ventral transverse bar** appears long, convex and broad 'V' shaped. **Marginal hooklets** have changed their size and shapes according to changes in temperature of water.

Pearson correlation

Pearson correlations were used to determine if the hard armature of *H. wallagonius* is significantly correlated or not. Out of 14 characters (including cirrus) 11 show a positive correlation with each other. Table 03 shows all spss outputs including their correlation.

Cirrus: Total length and width shows strong correlation with each other, r = 0.999. Correlation is significant at 0.05 levels.

Dorsal anchor's character length of anchor with length of shaft is positively correlated, r = 0.981 and significant at 0.01 level. Length of outer root with inner root is significantly correlated, r = 0.812. Correlation is significant at 0.05 level.

Dorsal transverse bar of *H. wallagonius* shows positive correlation in January and February. Length of transverse bar with maximal distance between transverse process shows positive correlation with each other in January and February, r = 0.916 and r = 0.992 respectively. Correlation is significant at 0.01 levels. Further basal width of transverse bar with median width is not correlated with each other.

Ventral anchor show positive significant correlation in all months. Length of anchor with length of shaft is positively correlated, r = 0.855. It is significant at 0.05 levels. Length of outer root with inner root is significantly correlated in February, r = 0.889. Correlation is significant at 0.01 level.

Ventral transverse bar shows positive correlation. Length of bar with maximal distance between processes is positively correlated to each other in December and January, r = 0.934 and r = 0.992 correlation is significant at 0.01 level. Basal width of ventral transverse bar with median width is not correlated with each other.

Marginal hooklet show positive correlation. Length of hooklet with hook handle is positively correlated, r = 0.922 and correlation is significant at 0.01 level. Length of handle with length of sickle show negative correlation, r = - 0.541 which indicates increase or decrease in value of corresponding length of hook handle will either decrease or increase value of length of sickle.

Regression Analysis

Regression analysis of *H. wallagonius* to find out their linear mathematical relationships with all hard parts of cirrus and Haptor. Regression analysis of *H. wallagonius* helps to find out a linear mathematical relationship between two variables or characters of hard parts of cirrus. Length has been chosen as an independent variable because it can be measured easily. In present study, width is dependent variable. In case of cirrus there is significant regression. In haptor, lengths of dorsal and ventral anchor, length of dorsal and ventral transverse bar is independent variable. Length of marginal hooklet has been chosen as an independent variable because they are easily measured. Lengths of anchor shaft of dorsal and ventral anchor, median width of dorsal and ventral transverse bar are dependent variables. Length of marginal hook handle is dependent variable.

Line graphs

Line graphs of cirrus and Haptor were obtained and represented in the form of Table 4-5; Graph 1-3. Most of histogram diagrams show a wide range of variations in size and shape of cirrus and haptor of *H. wallagonius* with temperature (°C). All characters of dorsal anchor of *H. wallagonius* show correlation with each other. Dorsal anchor is temperature dependant. Length and shaft of anchor are temperature dependent. As temperature increases in December all parameters decrease. Outer and inner root show slight changes according to water temperature. Dorsal transverse bar is inversely related to temperature. As temperature decreases in January length of transverse bar increase. But towards February as temperature rises slightly it decreases. Basal and median width of transverse bar is not temperature dependent. Ventral anchor is also inversely related to water temperature. It means as temperature increases all parameters decrease. Ventral transverse bar is temperature dependent. As temperature increases in December length of transverse bar and its median width increases.

Conclusion:

From this study it is concluded that anchors show a slim and long appearance in colder months of January and February. Whereas, in warm spring month, anchors appear short, stout and curved. Dorsal transverse bar also appears long and slender in colder months and short and stout in warm month. Study also conclude that continuous analysis of haptoral hard parts in different seasons provide great details of those variations which are shown by opisthaptoral hard parts of monogenean parasites. Parasites belonging to different genera also differ in their hard parts. Present study also describes that size of hard parts (cirrus and haptor) of a parasite depend upon seasonal temperature. These seasonal variations increase our knowledge and are very important for improving species descriptions. It helps in resolving some disparity in taxonomy of monogeneans.This study concluded that correlations between hard parts of haptor cannot be used as characteristic feature to differentiate between two genera. In addition regression analysis is carried out to find out a mathematical linear relationship between two variables (hard parts). One variable is independent variable i.e. its value is known. Other variable is dependent i.e. whose value is to be determined. Obtained equations help to find out unknown values of different hard parts of haptor with known value. Results from present statistical study of correlation and regression analysis indicate towards common purpose of degree and direction of relationship between different hard parts of cirrus and haptor. And it also indicates to differentiate among monogeneans genera.

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