RESULTS

Diversity and spatio-temporal distribution of Hilsa in Gujarat

The fish is found in the Persian Gulf, Red Sea, Arabian Sea, Bay of Bengal, Vietnam Sea and China Sea in the riverine natural surroundings covering the Shatil al Arab, and the Tigris and Euphrates of Iran and Iraq, the Indus of Pakistan, the waterways of eastern and western India to be specific the Ganga, Bhagirathi, Hooghly, Rupnarayan, Brahmaputra, Godavari, Narmada, Tapti and other seaside streams, the Irrawaddy of Myanmar, and the Padma, Jamuna, Meghna, Karnafully and other seaside streams of Bangladesh.

Three types of Hilsa specifically *Tenualosa ilisha* (Hamilton), *Tenualosa toli* (Valenciennes), *Hilsa kelee* (Couvier) are caught from the estuaries and seaside waters of India. The ordinary natural surroundings, pattern of transitory propensity, greatest age and development by and large contrast from species to species. Since, these species comprise prized Hilsa fishery, they are liable to substantial angling weight and its unreliable abuse results in decrease in catch. Among these three species, just *Tenualosa ilisha* is widely used as a part of fishery while *Tenualosa toli* and *Hilsa kelee* are barely accessible in Indian waters.

In this objective, the present diversity of Hilsa species has been studied found in the waters of Gujarat. The diversity of the Hilsa (clupeids) has also been done with the literature of secondary data available from the rivers and marine regions of the world. A cartographical representation depicts the availability of Hilsa from the Persian Gulf to the waters of Myanmar has been prepared and also preliminary survey through the marine and freshwater landing centres of Gujarat has been conducted to ascertain the actual diversity of Hilsa. The Hilsa shad is an anadromous species, yet two different ecotypes – a fluvial potamodromous compose and a marine kind has been perceived. The potamodromous stocks seem to stay in the center compasses of the waterways consistently and breed there in. The anadromous stocks, whose ordinary living space is the lower district of the estuaries and the foreshore zones, rise the streams amid the rearing season and come back to the first territory in the wake of generating.

The secondary map generated from different sources and available literature was scrutinized. The availability or by catch of *Tenualosa ilisha* was observed in the places as shown in the map (APPENDIX – II).

While assessing the survey Rivers like Mahi and Tapi have been seen with the presence of Hilsa fishing in the past and presently too though at a very small proportion, but the fishing activity in Narmada River has been much superior to the other rivers of Gujarat and so is mainly focused in the present study. It is the main waterway where Hilsa cruises around 129 kms from the ocean. Perceptions were made on the course of the stream demonstrated that there is not really any substantial scale Hilsa angling past the town of Zanor (65 kms from the ocean) as reefs don't appear to climb past this zone. Yet, it appears to rise to the town of Garudeshwar. Past this town, the waterway bed is rough and subsequently the ebb and flow is substantially more grounded. The high speed of the stream current might be the primary factor for limiting the movement of the Hilsa in this zone. In the past, it has been recorded that in the stream Narmada, Hilsa are found in its lower comes to amid the storm a long time with no point by point writes about producing. Examinations were done in a similar waterway yet at towns of Nikora and Zanor, 65 kms upstream

from the ocean along the course of the stream and around 12 to 25 kms from the town of Bharuch (APPENDIX – III). This zone is somewhat under tidal influence however the water at this piece of the region stays new or salt free. There is a slight increment in water stature amid rainstorm or high spring tide.

Taxonomic and molecular identification of Hilsa.

The Indian shad (*Tenualosha ilisha*) is one of the major fisheries of Gujarat. The present study has been majorly concentrated in Narmada River, as this is one of the primary rivers for inland catch of Hilsa in Gujarat. The specimens caught from the river has been morphologically described and identified based on the characters from standard available identification keys. The data has been presented in the form a factsheet.

Scientific name	Tenualosa ilisha
Common name	Hilsa shad
Local name	Chaksi (Gujarat)
	Kingdom: Animalia
	Phylum: Chordata
	Super Class: Pisces
Classification	Class: Actinopterygii
	Order: Clupeiformes
	Family: Clupeidae
	Genus: Tenualosa
	Species: Tenualosa ilisha
Habit & habitat	Marine; freshwater; brackish; pelagic-neritic;
	anadromous; Tropical; 34°N - 5°N, 42°E - 97°E.
	Indian Ocean: Persian Gulf eastward to Myanmar,
Distribution	including western and eastern coasts of India. Reported from the Gulf of Tonkin, Viet Nam.
Distribution	Reported in Tigris River basin and probably other
	rivers of southern Iran
Deserintion	
Description	Distinct median notch in upper jaw. Gill rakers fine

Table 6. Factsheet of habits and habitats of Tenualosa ilisha

	and numerous, about 100 to 250 on lower part of arch. Fins hyaline. A dark blotch behind gill opening, followed by a series of small spots along flank in juveniles. Color in life, silver shot with gold and purple. Schooling in coastal waters and ascending rivers for as much as 1200 km (usually 50-100 km). Migration though is sometimes restricted by barrages. Hilsa far up the Ganges and other large rivers seem to be permanent river populations. Feeds on plankton, mainly by filtering, but apparently also by grubbing on muddy bottoms. Breeds mainly in rivers during the southwest monsoon (also from January to February to March). Artificial propagation has been partially successful in India. Known to be a fast swimmer, covering 71 km in one day.
Reproduction	Breeds mainly in rivers, upstream to about 50 km or even over 1000 km as in the Ganges (younger fishes may breed in the tidal zone of rivers). In some rivers the migration is restricted by barrages; there is some evidence that Hilsa far up the Ganges and other large rivers, although migrating upstream to spawn, are permanent river populations that do not descend to the sea. The main breeding season is during the southwest monsoon, with a shorter season from January to February or March.
Fin formula	B: vi, D: 18(3/15); P: 15; V: 9, A: 20(2/18); C: 19, L. l: 48, L.tr:18 (Day F., 1885)
Environmental impacts	Threat to over exploitation by human or habitat destruction by natural calamities or human activity.
Threat status	Harmless to human. Catches in India have declined rapidly, and declines have also been reported from Iraq and Bangladesh. Declines are thought to be the result of over-fishing, industrial pollution, which causes large-scale mortality of fish eggs and larvae, and especially dams, such as the Farakka barrage (Ganges River) which impact the species spawning migrations. Artificial propagation of the species by egg stripping has been partially successful in India.
Traditional	Not available

knowledge	
Conservation measures	Least concern (LC). There are no known, species- specific conservation measures in place for Tenualosa ilisha. There are several marine protected areas within its distribution. (IUCN, 2019)
Commercial utilization	Highly use as a food source in market.

The specimens collected from various places of Gujarat were first taxonomically identified with standard available keys and post identification the specimens were subjected to molecular identification by DNA barcoding. The most common gene COI and 16S were chosen for barcoding. Appropriate techniques were adopted for the barcoding. The results obtained from the sequencing of both the genes were used for construction of phylogenetic trees using MEGA 10 software. This trees show an interrelationship in genetic levels of the specimens caught at different areas throughout Gujarat.

Further the sequences were matched with the sequences extracted from NCBI of the same gene done at different places to construct phylogenetic trees of specimens caught at different places.

It can be surely validated that the Hilsa after spawning does not travel into far off areas into sea. The sub populations exist near the estuarine mouth and with the advent of monsoon and flow of fresh water they start their inward migration towards the river.

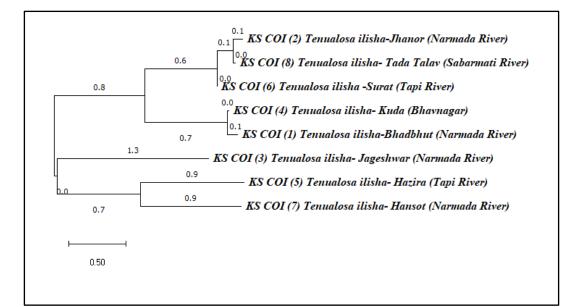


Fig.8. Phylogenetic tree constructed for *Tenualosa ilisha* barcoded from different locations in Gujarat for Cytochrome oxidase I gene.

The evolutionary history was inferred using the Neighbour-Joining method. The optimal tree with the sum of branch length = 6.41336133 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 715 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

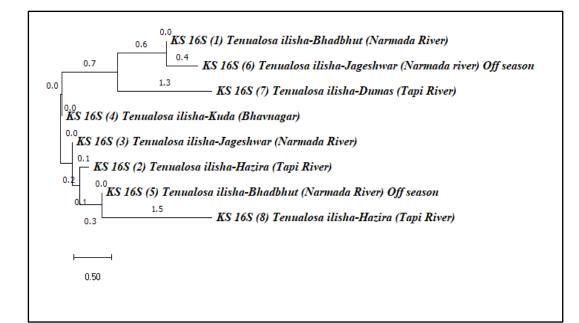


Fig.9. Phylogenetic tree constructed for comparison of *Tenualosa ilisha* barcoded from different locations in Gujarat for 16S rDNA gene.

The evolutionary history was inferred using the Neighbour-Joining method. The optimal tree with the sum of branch length = 43.91328953 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 18 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1301 positions in the final dataset. Evolutionary analyses were conducted in MEGA 10.

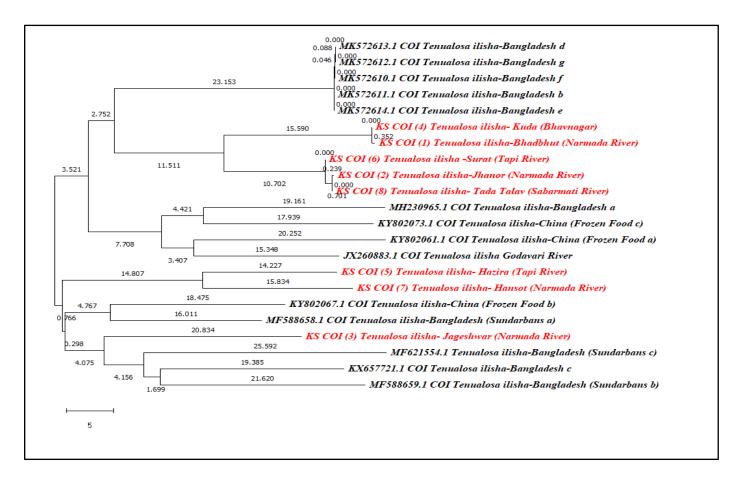


Fig.10. Comparative phylogenetic tree constructed for *Tenualosa ilisha* barcoded from different locations throughout the South East Asian region for Cytochrome oxidase I gene.

The evolutionary history was inferred using the Neighbour-Joining method. The optimal tree with the sum of branch length = 339.46585225 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 22 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 715 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

In this comparative phylogenetic tree, the sequences of COI have been extracted from NCBI. These sequences have been used to do a comparative inter or intra relationship studies of *Tenualosa ilisha* to check whether the species found in these areas have any kind of genetic relationship with each other. Many of the studied sequences have been done to check or comply with the quality of frozen food. The genetic distances of the sequences done during this study were low thus it can be ascertained that all the specimens belonged to the same set of larger populations. They must have further segregated into smaller sub populations surviving in the estuaries of each river travelling into the freshwater zones for spawning and breeding. A clear difference of 2.752 genetic distances can be seen among the species caught in Bangladesh and from Gujarat, India clearly implying the difference of sub-set populations.

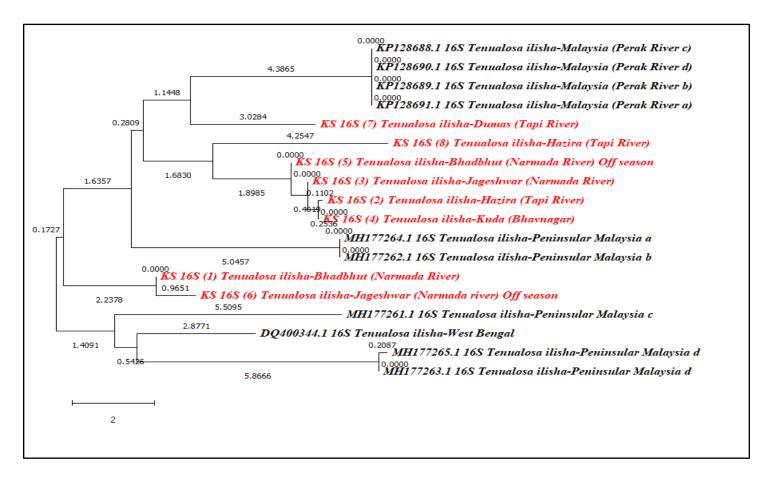


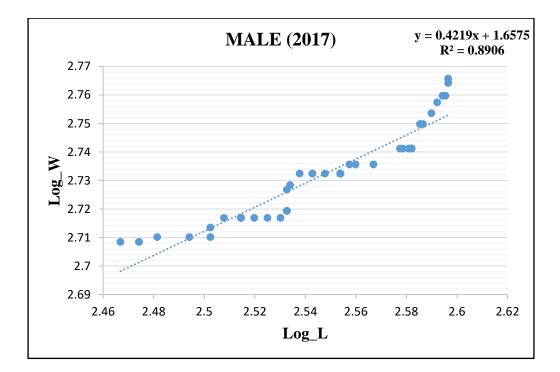
Fig.10. Comparative phylogenetic tree constructed for *Tenualosa ilisha* barcoded from different locations throughout the South East Asian region for 16S rDNA gene.

The evolutionary history was inferred using the Neighbour-Joining method. The optimal tree with the sum of branch length = 43.91328953 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 18 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1301 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

This comparative tree shows the sequenced data of 16S rDNA gene from the current study and the data from other places for the same gene. This additional gene was done to further validate the genetic variation or inter relationship of the species *Tenualosa ilisha* caught at different place. Here, the data is mostly from further South East Asian countries like Malaysia. The separate clade formation in both the data set clearly shows the variation in genetic makeup of the single species formed out of separated topographical locations over the years because of subsistence of the populations in the estuarine zone itself. Also, in this study samples were taken from Jageshwar, the mouth of Narmada River during the off season (non-monsoon period) to authenticate if the subpopulations were near each riverine zone, to which it was found to be true as it was formed in a single clade with the Hilsa sample found in Narmada River.

Fishery ecology and stock assessment of Hilsa in Narmada River

Understanding the fishery ecology and the population of any species is very important to ascertain the biodiversity status of any ecosystem and for the case of aquatic ecosystem in a particular species.



Length-weight relationship

Fig.11. Length-Weight relationship of male *Tenualosa ilisha* in Bhadbhut, Narmada River (2017)

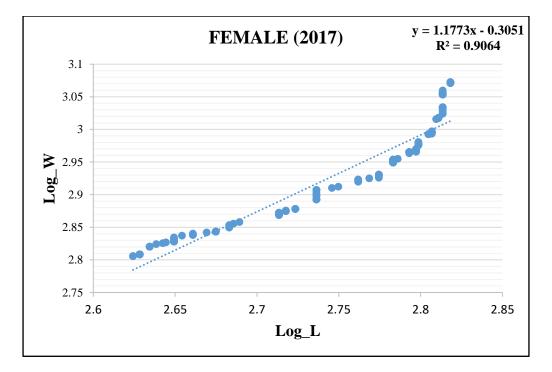


Fig.13. Length-Weight relationship of female *Tenualosa ilisha* in Bhadbhut, Narmada River (2017)

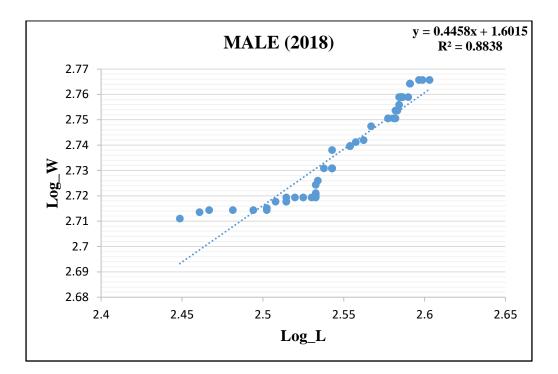


Fig.12. Length-Weight relationship of male *Tenualosa ilisha* in Bhadbhut, Narmada River (2018)

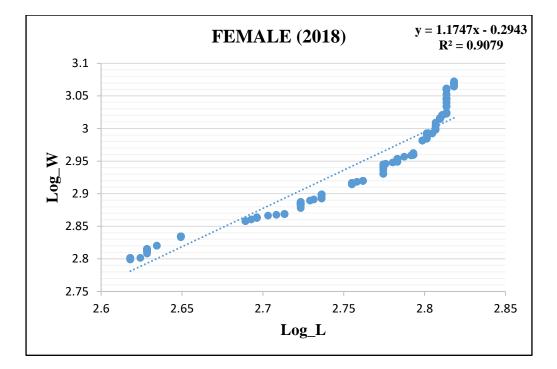


Fig.14. Length-Weight relationship of female *Tenualosa ilisha* in Bhadbhut, Narmada River (2018)

Tenualosha ilisha (Hilsa), only fisheries exhibiting anadromous behavior in the entire Indian subcontinent travels to the freshwater regimes during the monsoons and when the rivers are mostly in high floods. It has been observed that the fishes entering during that season has almost lost their feeding habits and until breeding and spawning do not search for food. Comparatively, the male fishes showed a positive allometric growth to the females and also it was observed that the male population was almost half of the total female fishes.

The R^2 values in both the seasons were 0.89 and 0.90 shows the significance in the values recorded. The length-weight relationship in the present study showed a negative allometric growth for the females as their bodies showed more body weight because of the presence of large mass of eggs post breeding season than the length of the body.

Condition factor

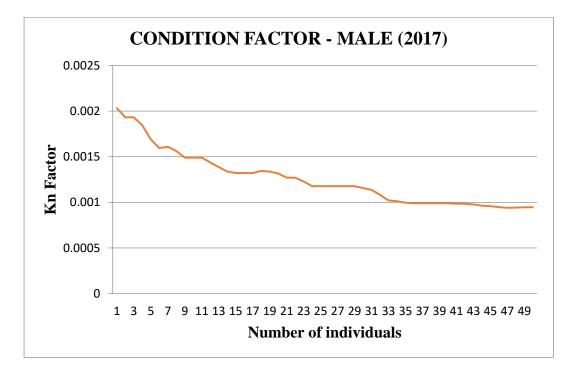


Fig.15. Condition factor (Kn) of male *Tenualosa ilisha* in Bhadbhut, Narmada River (2017)

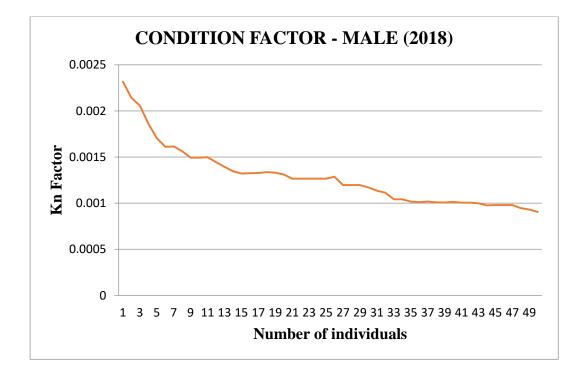
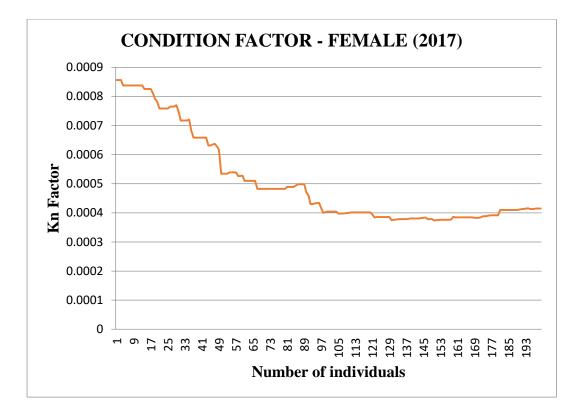
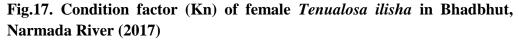


Fig.16. Condition factor (Kn) of male *Tenualosa ilisha* in Bhadbhut, Narmada River (2018)





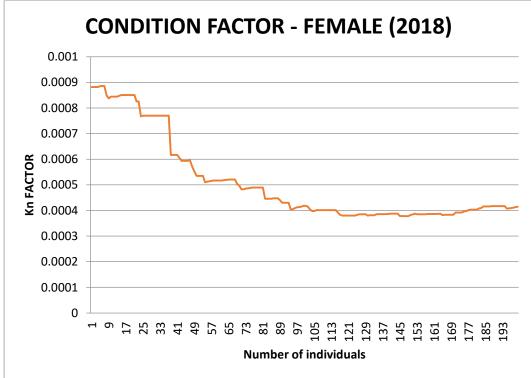


Fig.18. Condition factor (Kn) of female *Tenualosa ilisha* in Bhadbhut, Narmada River (2018)

RESULTS

In this study, the condition factor of males and females have been described separately. The condition factor (Kn) values shows a graph which predicts that the fishes arriving for spawning with the onset of monsoon are healthier and have rich fat reserves as they have remain well fed before beginning their anadromous journey. During the monsoons, after the eggs are released probably because of high flow of water, high turbidity of Narmada river system, the adult fishes are unable to feed and hence their condition worsens until they reach back to the mouth of the estuary.

The lower Kn values suggested that the reproductive season was over. This indicates that the species is prepared for reproduction sometime before that period (August). It should be emphasized that during the other months the Kn value remained constant with only slight variation.

The condition factor increases starting from July (the reproductive period is resumed), reaching its highest values in August – September and ends by October.

The values of the condition factor (Kn) vary according to seasons and are influenced by environmental conditions. The same may be occurring in the environment under study since the floodplain is influenced by many biotic and abiotic factors, which favour the equilibrium of all the species in the ecosystem.

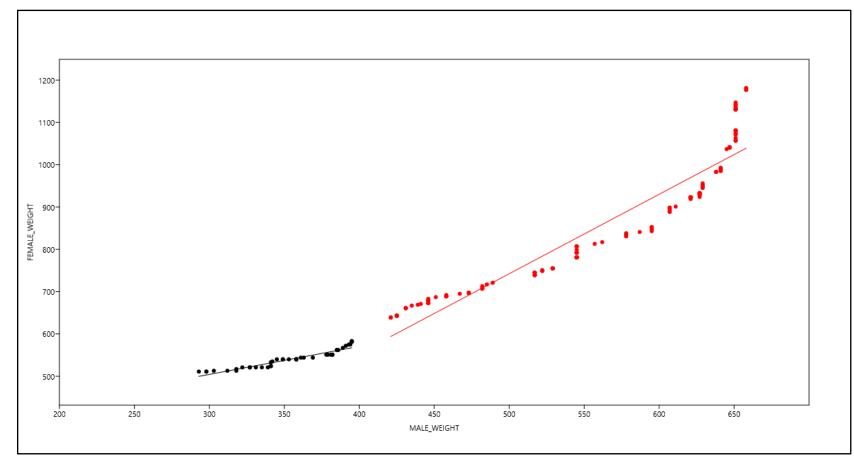


Fig.19. One – way ANCOVA for male and female length and weight relationship of *Tenualosa ilisha* at Bhadbhut, Narmada River, Gujarat in 2017

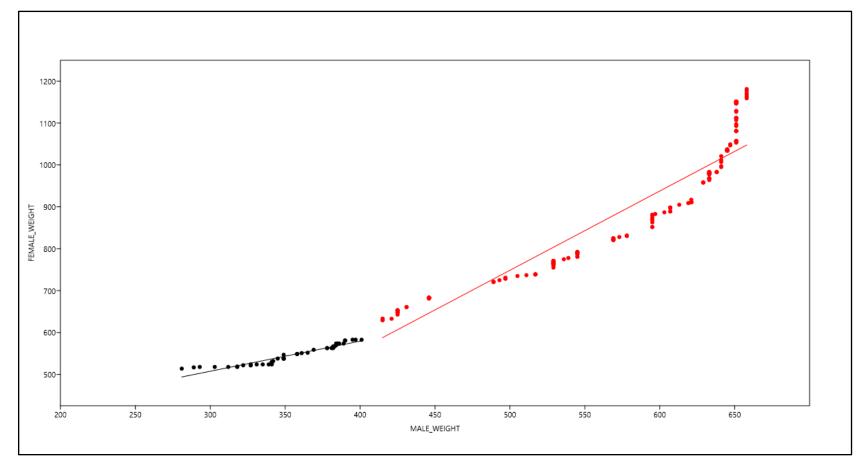


Fig.20. One – way ANCOVA for male and female length and weight relationship of *Tenualosa ilisha* at Bhadbhut, Narmada River, Gujarat in 2018

	Sum of sqrs	df	Mean square	F	p (same)
Adj. mean:	65206.9	1	65206.9	25.16	1.008E-06
Adj. error:	640130	247	2591.62		
Adj. total:	705337	248			

Homogeneity (equality) of slopes:

F:	25.5	7			
p (same)	8.33E	-07			
			Mean	Adjusted mean	Slope
Male W	eight		540.76	850.8	0.66288
Female V	Veight		865.87	788.36	1.8784

Test for equal means, adjusted for covariate (2018)

	Sum of sqrs	df	Mean square	F	p (same)
Adj. mean:	72549	1	72549	27.1	4.05E-07
Adj. error:	661144	247	2676.7		
Adj. total:	733693	248			

Homogeneity (equality) of slopes:

F:	25.0)5			
p (same)	1.065E	E-06			
	L		Mean	Adjusted mean	Slope
Male W	'eight		546.46	863.84	0.71763
Female V	Veight		877.72	798.37	1.8911

It represents the inter-relationship of the linear growth of length of male and female Hilsa with the differential values of weight in each graph. It can be clearly seen that the weight and length are dependent of each other and the linear pattern of the graph proves the point that both the parameters grow simultaneously along with each other.

LENGTH (in mm) 2017 LENGTH (in mm) WEIGHT (in g) WEIGHT (in g) Ν 50 200 200 50 293 421 639 Min 511 658 Max 395 583 1181 Sum 17747 27038 113152 173173 Mean 354.94 540.76 565.76 865.865 4.14969 2.901732 5.570743 11.13633 Std. error Variance 421.0024 24803.58 860.9963 6206.636 20.51834 78.7822 Stand. dev 29.34274 157.4915 Median 358 540 595 852 334 521 517 739 25 prcntil 75 prcntil 382 551 641 986.5 Skewness -0.3730244 0.3856622 -0.517101 0.321834 -0.8450178 -0.6386398 -1.162461 -1.016193 Kurtosis Geom. mean 353.7209 540.3816 559.9609 851.8439 Coeff. var 8.266957 3.794353 18.18893 13.92502

Table.7. Summative statistical analysis showing the relationship of male and female Hilsa, *Tenualosa ilisha* in correlation with the length and weight of year 2017 at Bhadbhut, Narmada River, Gujarat

and weight of year 2018 at Bhadbhut, Narmada F 2018 M LENGTH (in mm) Ν 50 281 Min 401 Max 17696 Sum 353.92 Mean

able.8. Summative statistical analysis showing the relationship of male and female Hilsa, <i>Tenualosa ilisha</i> in correlation with the length Ind weight of year 2018 at Bhadbhut, Narmada River, Gujarat						
2018	M LENGTH (in mm)	M WEIGHT (in g)	M LENGTH (in mm)	M WEIGHT (in g)		
Ν	50	50	200	200		
Min	281	514	415	630		
Max	401	583	658	1181		
Sum	17696	27323	113648	175543		
Mean	353.92	546.46	568.24	877.715		
Std orror	4 33023	3 265054	5 710746	11 /805		

Std. error	4.33023	3.265054	5.719746	11.4895
Variance	937.5445	533.029	6543.098	26401.72
Stand. dev	30.61935	23.08742	80.88942	162.4861
Median	349	542.5	595	881
25 prentil	334	524	517	739
75 prentil	383.25	567.75	641	1010.75
Skewness	-0.446951	0.211407	-0.6376237	0.184504
Kurtosis	-0.5221718	-1.453695	-0.9994998	-1.148579
Geom. mean	352.5797	545.9843	562.0651	862.7522
Coeff. var	8.651489	4.224906	14.23508	18.5124

RESULTS

The statistical analysis has been done with the help of PAST software. The analysis done is Analysis of Co-Variance (ANCOVA) for the length-weight data set of male and female Hilsa caught during the monsoonal season of 2017 and 2018 (Fig. 19 & Fig. 20

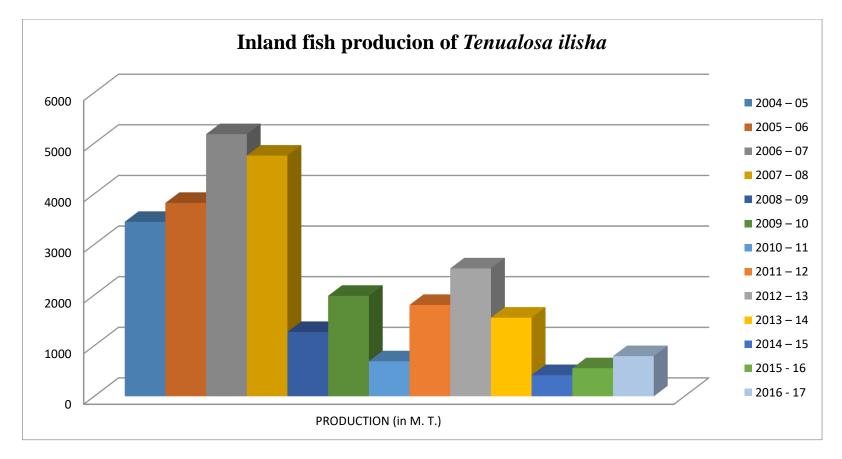
A summative statistical analysis has been done for the parameters of length and weight as shown in Table 7. The factor of skewness and kurtosis shows a negative relation between the length and weight proving the point many present outliers falling away from the linear value. This represents the data that the migration of Hilsa has not been uniform or increasing in the monsoon season during each year. The fishes only at the nearby estuarine zone during the off season travel inward toward the Narmada River with the advent of fresh water outflow but with the increased level of turbidity or sedimentation, the anadromous migration slows down with only the already those females with spent eggs present in the river. Freshly gravid females might be avoiding such polluted waters and lay eggs directly in the brackish water zone thus thereby decreasing the catch of Hilsa in inland water zones.

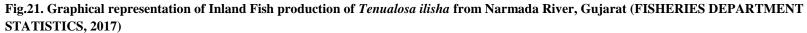
Also the presence of heavy metals in the water might be the additional reason for such a low catch of Hilsa with ever increasing Hilsa in the inland waters.

SR. NO.	SPECIES	YEAR	PRODUCTION (in M. T.)
1		2004 - 05	3448
2		2005 - 06	3821
3		2006 - 07	5180
4		2007 - 08	4756
5		2008 - 09	1272
6		2009 - 10	1987
7	Tenualosa ilisha	2010 - 11	693
8		2011 - 12	1806
9		2012 - 13	2528
10		2013 - 14	1554
11		2014 - 15	419
12		2015 - 16	556
13		2016 - 17	794

Table.9. Inland fish production of Tenualosa ilisha in Bharuch district

The inland fish production data for *Tenualosa ilisha* has been obtained from the annual handbook of Fisheries Statistics (2017) by Commissioner of Fisheries, Government of Gujarat. This is a secondary data which has been incorporated to emphasise on the stock and population estimation in Narmada River, Gujarat. It clearly shows a steady decline in the catch of Hilsa.





Physico – chemical analysis

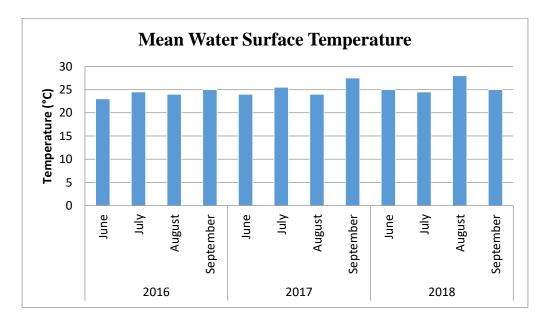


Fig.22. Mean water surface temperature observed during the migratory period of *Tenualosa ilisha* in the monsoon from the year 2016 – 2018 in Bhadbhut, the major landing centre along Narmada River, Gujarat

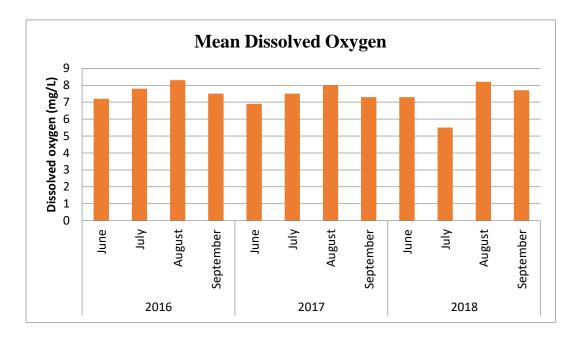


Fig.23. Mean dissolved oxygen observed during the migratory period of *Tenualosa ilisha* in the monsoon from the year 2016 – 2018 in Bhadbhut, the major landing centre along Narmada River, Gujarat

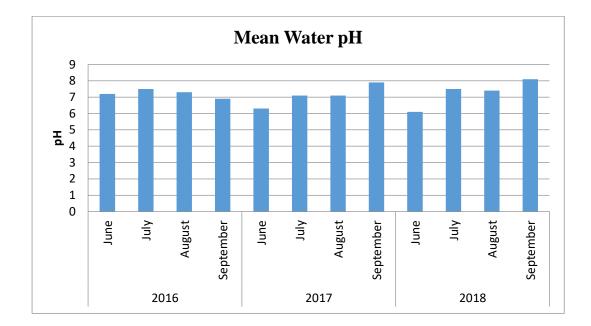


Fig.24. Mean pH observed during the migratory period of *Tenualosa ilisha* in the monsoon from the year 2016 – 2018 in Bhadbhut, the major landing centre along Narmada River, Gujarat

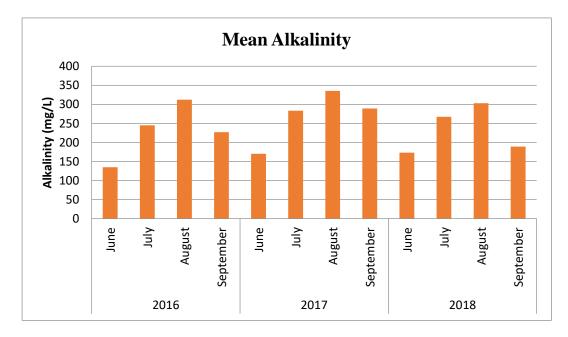


Fig.25. Mean alkalinity observed during the migratory period of *Tenualosa ilisha* in the monsoon from the year 2016 – 2018 in Bhadbhut, the major landing centre along Narmada River, Gujarat.

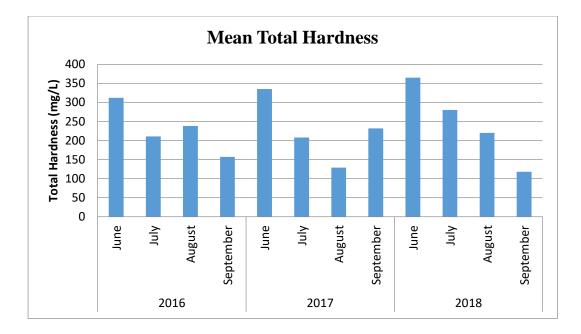


Fig.26. Mean Total Hardness observed during the migratory period of *Tenualosa ilisha* in the monsoon from the year 2016 – 2018 in Bhadbhut, the major landing centre along Narmada River, Gujarat.

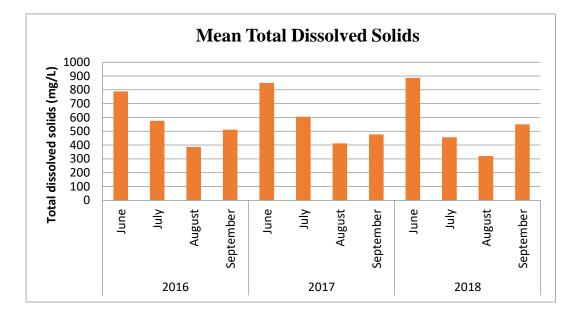


Fig.27. Mean total dissolved solids observed during the migratory period of *Tenualosa ilisha* in the monsoon from the year 2016 – 2018 in Bhadbhut, the major landing centre along Narmada River, Gujarat.

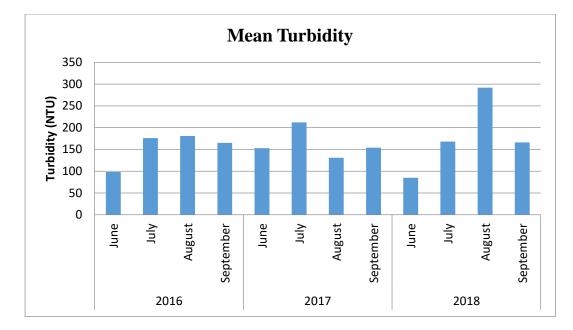
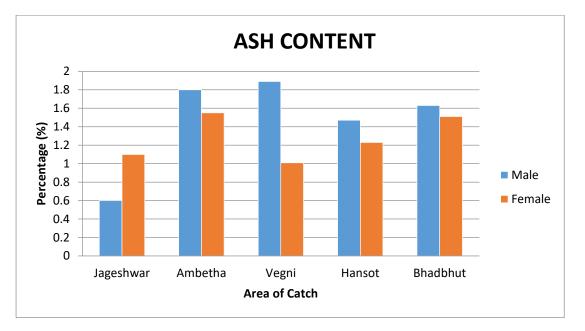


Fig.28. Mean turbidity observed during the migratory period of *Tenualosa ilisha* in the monsoon from the year 2016 – 2018 in Bhadbhut, the major landing centre along Narmada River, Gujarat.

The physico-chemical parameters of water in Bhadbhut, the major landing centre of *Tenualosa ilisha* in Narmada River were assessed for three years – 2016, 2017 and 2018 during the period of monsoon season starting from June to September. The water temperature, dissolved oxygen and pH were observed having no significant changes during the entire study period but the total hardness and total dissolved solids were observed with high variation. The reasons of this variation can be attributed to the effluent discharge in the waters of Narmada River from the industries in and around Bharuch and Ankleshwar. The presence of heavy metals and other contaminants, also the higher sedimentation rate from the upper reaches of Narmada River adds on to the increasing values of turbidity every year as seen in the graphical representation.

Bio-assay studies of Hilsa in relation to migration in Narmada River



Proximate analysis

Fig.29. Ash content (%) variation for male and female Hilsa collected from different landing centres along the Narmada River, Gujarat in the monsoon of the year 2016.

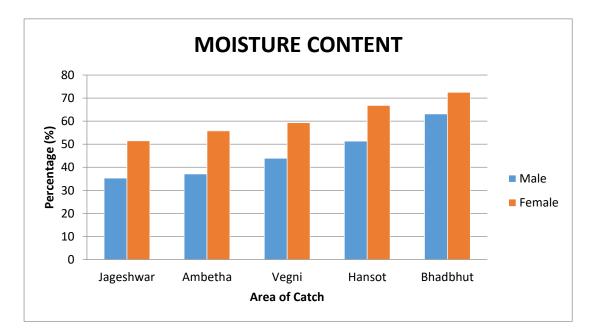


Fig.30. Moisture content (%) variation for male and female Hilsa collected from different landing centres along the Narmada River, Gujarat in the monsoon of the year 2016.

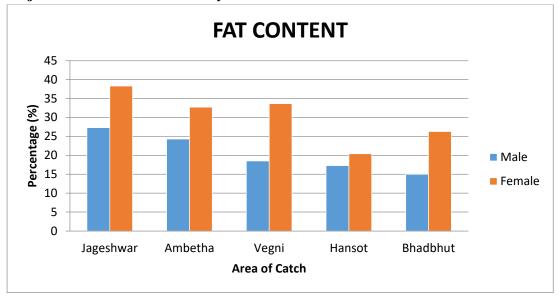


Fig.31. Fat content (%) variation for male and female Hilsa collected from different landing centres along the Narmada River, Gujarat in the monsoon of the year 2016.

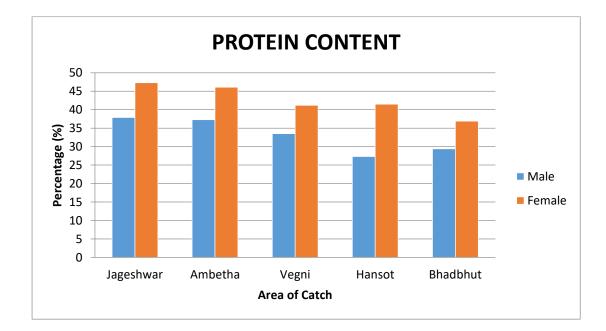


Fig.32. Protein content (%) variation for male and female Hilsa collected from different landing centres along the Narmada River, Gujarat in the monsoon of the year 2016.

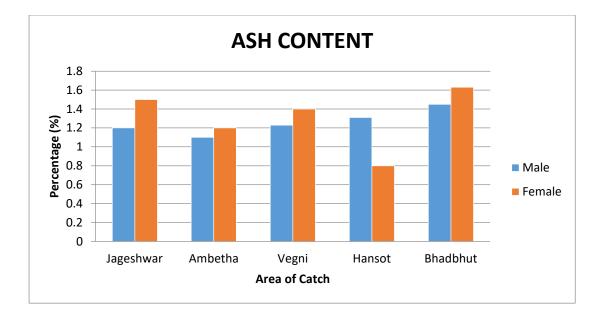


Fig.33. Ash content (%) variation for male and female Hilsa collected from different landing centres along the Narmada River, Gujarat in the monsoon of the year 2017.

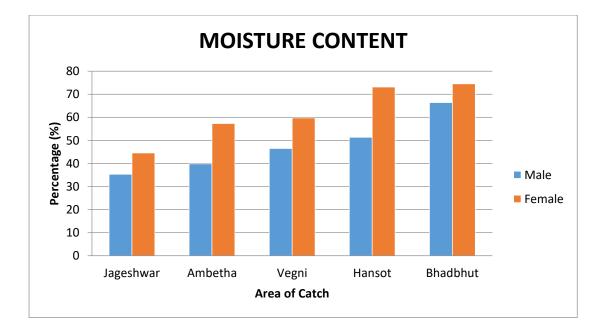


Fig.34. Moisture content (%) variation for male and female Hilsa collected from different landing centres along the Narmada River, Gujarat in the monsoon of the year 2017.

RESULTS

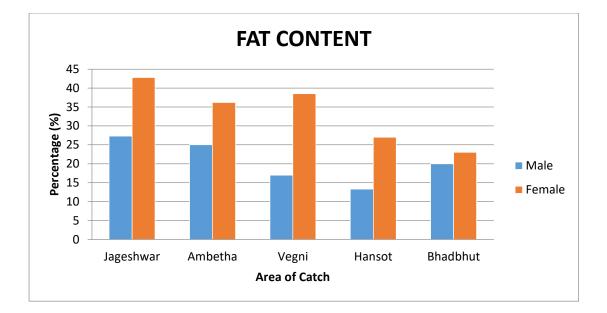


Fig.35. Fat content (%) variation for male and female Hilsa collected from different landing centres along the Narmada River, Gujarat in the monsoon of the year 2017.

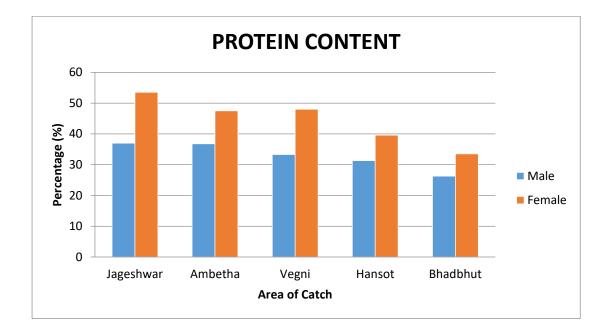


Fig.36. Protein content (%) variation for male and female Hilsa collected from different landing centres along the Narmada River, Gujarat in the monsoon of the year 2017.

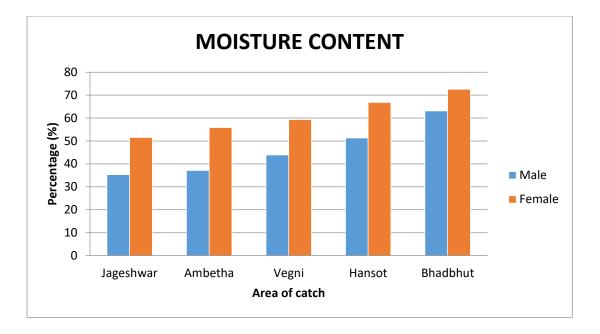


Fig.37. Moisture content (%) variation for male and female Hilsa collected from different landing centres along the Narmada River, Gujarat in the monsoon of the year 2018.

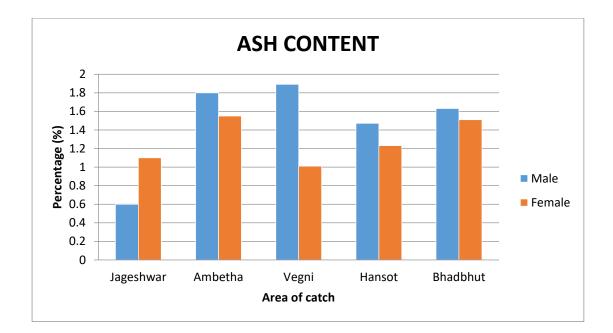


Fig.38. Ash content (%) variation for male and female Hilsa collected from different landing centres along the Narmada River, Gujarat in the monsoon of the year 2018.

RESULTS

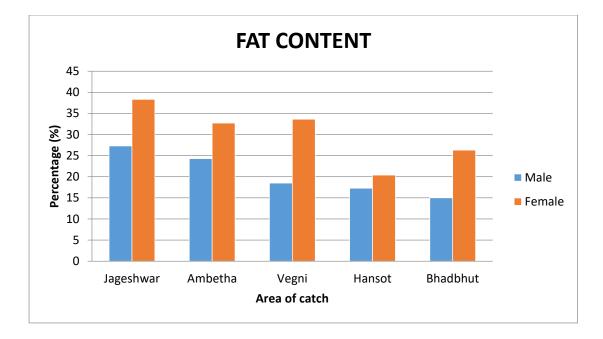


Fig.39. Fat content (%) variation for male and female Hilsa collected from different landing centres along the Narmada River, Gujarat in the monsoon of the year 2018.

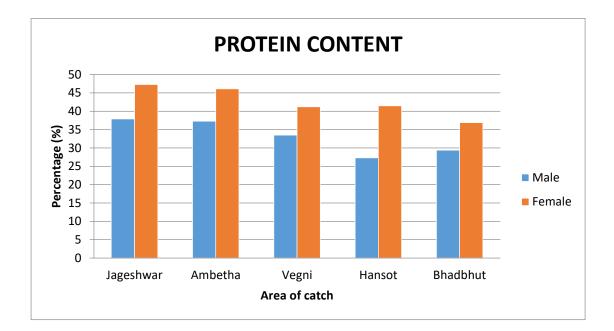


Fig.40. Protein content (%) variation for male and female Hilsa collected from different landing centres along the Narmada River, Gujarat in the monsoon of the year 2018.

The analysis of proximate composition of *Tenualosa ilisha* was studied in this objective. It was clearly observed that with change of place and according to size during inward migratory route, the proximate composition changes considerably. The assessment for the protein, fat, moisture and ash content was obtained from the muscular part of Hilsa.

Protein was an important source of human daily diet. Fisheries sector provides an overall 23% of the world animal's protein intake to which Asian countries contribute nearly 26% (Sen, 2010). Hilsa was also a protein rich fish. In different size groups of fishes were found and that were subdivided into male and female Hilsa. The moisture content varied from 35 - 67 % in male Hilsa while in females the variation was observed from 42 - 74%. According to Dempson *et al.* (2004), the lower the percentage of water in fish muscle, the greater are the availability of lipid and protein contents and so would be the higher energy content of the fish.

With reference to the ash content of fish muscle, around 0.5 - 1.6% was observed in male and in female it was observed for 1.1- 1.65%. It can be ascertained from the present result that Hilsa good source of minerals like calcium, potassium, zinc, iron and magnesium.

The protein content was found to be highest when the fish enters the mouth of Narmada River at Jagheswar and lowest at Bhadbhut. With the advance of the fish through the migratory route into the river, it loses the energy deposit stored in the form of fats and so the results obtained shows a decline content of fat content from 27 - 15% in males on an average while in females it goes down from 37 - 35% in every year while it moves from Jageshwar to Bhadbhut, the principal landing centre in Narmada River.

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Gonadosomatic Index and Hepatosomatic Index

The gonadosomatic index (GSI) is the most reliable and scientifically approved indicator because it gives a correct time span regarding season of spawning. The utility of gonadosomatic index as the indicator of the reproductive activity has been discussed (Skasena, 1987).

Relative fecundity, measured either as egg output per unit weight or as gonosomatic index, decreases interspecifically with female size in fishes (Sadovy, 1996). The frequency of spawning also decreases with species size, with smaller species much more likely to be daily spawners. One way to consider more periodic spawning, whether it is restricted to a lunar period or a yearly period, is that the benefits for spawning at a particular time of year outweigh two potential costs: not surviving to the next reproductive period, and morphological limitations on how many eggs can be developed at a given time. For larger species, lower instantaneous mortality rates could lessen any costs of delaying reproduction, tipping the balance in favour of seasonal reproduction. This topic has not been much pursued and represents a profitable area of collaboration between behavioural biology and physiology.

Gonadosomatic index indicates gonad development and maturity. External morphological features were used to distinguish the matured females.

The bulged abdomen of the female fish was easily distinguished as in matured condition. For each fish, total length (TL) was measured with a measuring scale to the nearest centimetre and body weight (BW) were measured by an electronic balance. The gonads were dissected out and weighed to the nearest 0.01 g.

The gonadosomatic index (GSI) was determined using the formula (Render *et al.*, 1995):

$$GSI = (GW/BW) \times 100$$

where, GW = gonad weight, BW = body weight of fish.

Hepatosomatic index (HSI) value is a very important parameter which indicated the fish maturity condition, general health of the Hilsa. The HSI as an indicator of energy status (Wootten *et. al.*, 1978) and its negative correlation with GSI (Singh and Singh, 1983 & 1984 and Singh *et. al.*, 2005) are indicative of importance of liver capacity to store glycogen, physiological condition, reproduction activity, feeding habit and food availability (Tavares-Dias *et. al.*, 2000).

The calculation of hepatosomatic index can be calculated as follows:

$$HSI = LW \ge 100/BW$$

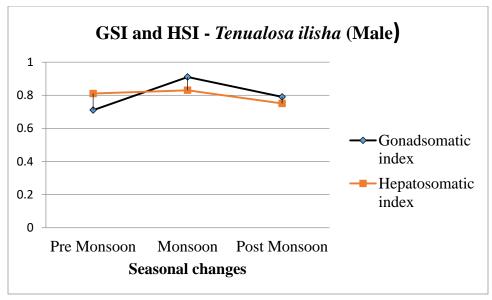
Where, LW = Liver weight and BW = Body weight.

Male Hilsa	Length (cm)	Width (cm)	Body Weight (g)	Liver weight (g)	HSI	Testis weight (g)	GSI	Testis Length (cm)
	Pre-monsoon							
Range	22 - 37.5	4.3 - 8.9	149 - 557	1.23 - 3.13	0.32 - 1.16	0.21 - 4.51	0.14 - 1.07	1.03 - 5.9
Mean	27.56	6.13	365.11	2.39	0.81	2.25	0.71	2.89
SD	3.85	1.38	123.65	1.14	0.23	1.98	0.31	1.55
				Monsoon		·		
Range	26 - 39	5.7 - 9.3	187 - 637	1.31 - 3.82	0.41 - 1.33	2.13 - 7.39	0.41 - 1.35	2.30 - 6.95
Mean	32.56	6.3	355.13	2.56	0.83	4.77	0.91	4.77
SD	3.85	1.12	123.56	0.89	0.15	2.39	0.28	1.8
				Post Monsoo	n			
Range	21 - 33	4.8 - 6.9	135 - 453	0.95 - 2.96	0.66 - 0.83	1.15 - 4.12	0.56 - 1.19	1.23 - 1.89
Mean	26.33	5.13	256.12	1.81	0.75	1.89	0.79	1.57
SD	4.9	1.13	15.65	0.96	0.10	1.03	0.19	0.21

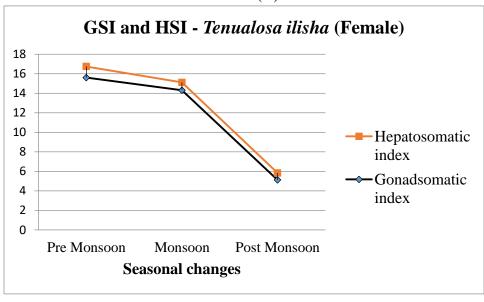
Table 10: Morphometric and reproductive biological information on male Hilsa considered for study.

Female Hilsa	Length (cm)	Width (cm)	Body Weight (g)	Liver weight (g)	HSI	Ovary weight (g)	GSI	Ovary Length (cm)
Pre-monsoon								
Range	29 - 43.3	5.1 - 10.23	292 - 856.3	2.9 - 7.6	0.5 - 1.56	21.5 - 89.7	1.9 - 23.11	2.5 - 14.3
Mean	31.7	7.9	471.3	4.33	1.14	43.1	15.6	7.88
SD	6.13	2.89	234.9	1.56	0.8	28.13	3.79	2.99
Monsoon								
Range	35.5 - 53.6	6.3 - 13.9	445 - 1015.5	2.44 - 8.8	0.49 - 1.33	15.6 - 103.8	2.89 - 28.6	4.7 - 15.1
Mean	47.3	8.9	691.5	5.77	0.81	65.1	14.3	9.2
SD	4.9	2.13	196.3	1.19	0.33	27.5	3.87	2.09
Post Monsoon								
Range	33.5 - 41	6 - 7.9	389 - 780	3.11 - 7.8	0.35 - 0.86	11.15 - 61.3	2.8 - 7.89	3.5 - 8.8
Mean	36.3	6.51	496	5.32	0.71	45.12	5.12	6.9
SD	5.01	0.98	215.51	2.12	0.15	23.85	2.33	1.56

Table 11: Morphometric and reproductive biological information on female Hilsa considered for study.
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(B)

Fig.41. Comparative values of Gonadosomatic and Hepatosomatic index values of Male and Female *Tenualosa ilisha* in Narmada River, Gujarat

The females attain larger sizes than the males. It is important to note that Hilsa has two breeding season (Hora, 1938). Islam (1989) reported that spawning of Hilsa takes place almost around the year, but the major spawning appears to take place in October – November, with subsidiary spawning in June – July and

February – March. The former two are considered 'summer spawning' and the latter is 'winter spawning'. Present study was done in monsoon season only (June to September) and Post monsoon (October to mid-February. The ovaries of this fishes are very large at the monsoonal time even up to 100 g weighed ovary is found. The testis tissues are less weighed compared to ovarian tissues. The ovary is a paired structure. Each of this pair is a sac like elongated structure. The colour of ripe ovary is pinkish and soft, whereas spent one is flaccid and reddish in colour and in a regressive or, recovering stage. Both the ovaries are not of same size.

Hilsa with its unique anadromous migratory behaviour shows very much differentiating changes in both male and female specimens. The GSI and HSI levels always more in females while in comparison to males. Though while observed closely, in females both the indexes reduce down drastically for its spawning and breeding behaviour and in post monsoon after the eggs are completely spent, the levels reach drastically down. It can be attributed to the lower levels of energy and availability of less food and higher turbidity in the river, the food resources are only available when the fishes reach the mouth of the river.