The relationship between haematocrit and some biological parameters of the Indian shad, *Tenualosa ilisha* (Family Clupeidae)

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Jawad, L. A., Al–Mukhtar, M. A. & Ahmed, H. K., 2004. The relationship between haematocrit and some biological parameters of the Indian shad, *Tenualosa ilisha* (Family Clupeidae). *Animal Biodiversity and Conservation*, 27.2: 47–52.

Abstract
The relationship between haematocrit and some biological parameters of the Indian shad, Tenualosa ilisha *(Family Clupeidae).—* Haematological parameters have been recognised as valuable tools for the monitoring of fish health. Here we analyse the relationship between haematocrit and body length, sex and reproductive state in the Indian Shad *Tenualosa ilisha.* Haematocrit value showed a quadratic relationship to fish size (body length), incrementing as the fish body length increased up to 400 mm, after which it decreased. Male fish showed a higher haematocrit value than females. Haematocrit appeared to be higher in the pre–spawning period than in the spawning phase, but then increased slightly in the post–spawning period.

Key words: Haematology, *Tenualosa ilisha*, Basrah, Iraq.

Resumen
Relación entre el hematocrito y algunos parámetros biológicos del sábalo de la India, Tenualosa ilisha *(Familia Clupeidae).—* Se ha demostrado que los parámetros hematológicos constituyen una valiosa herramienta para controlar la salud de los peces. En este artículo se analiza la relación entre el hematocrito y la longitud del cuerpo, el sexo y el estado reproductivo del sábalo de la India *Tenualosa ilisha.* Se ha encontrado una relación cuadrática entre el valor del hematocrito y el tamaño del pez (longitud del cuerpo), en aumento con la longitud del cuerpo, hasta los 400 mm, para después empezar a disminuir. Los valores del hematocrito de los peces machos son más elevados que los de las hembras. Parece que el hematocrito es más elevado en el periodo anterior al desove que durante el mismo, aunque en el período posterior se registra un ligero aumento.

Palabras clave: Hematología, *Tenualosa ilisha*, Basora, Irak.

(Received: 1 II 00; Conditional acceptance: 8 II 01; Final acceptance: 10 XI 03)

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ISSN: 1578–665X

Introduction

Haematological parameters have been recognised as valuable tools for the monitoring of fish health (Bhaskar & Rao, 1984; Schuett et al*.,* 1997) and in helping fishery biologists interpret physiological responses to environmental stress, information which is specially relevant when comparing studies of different fish species living in contrasted habitats (Fasihuddin & Kumari, 1990; Ivanc et al., 1996; Zhiteneva et al., 1997; Leonard & McCormick, 1999; Zhiteneva, 1999). It has been observed that blood parameters such as haematocrit, haemoglobin concentration and RBC count are related to environmental factors such as water temperature and salinity (Graham, 1997). Additionally, the relationship between haemoglobin and oxygen differs between loading and unloading sites and shows adaptations not only to environmental conditions but also to metabolic requirements, both of which govern oxygen availability and transport to tissues (Weber & Wells, 1989). Such adaptations may involve quantitative changes in total Hb content, or qualitative changes in Hb–oxygen–binding properties, and may appear both at the inter– and intra–specific level (Weber & Wells, 1989). Thus, the remarkable diversity of oxygen transport properties results from evolutionary processes through subtle sequence differences in haemoglobin that appear to match the varied metabolic demands of animals with the environmental oxygen supply (Wells, 1999).

In spite of the vast number of reports on the haematology of the different species of fish, only a few studies have investigated the relationship between haematological parameters and aspects of fish biology such as body length, sex and the reproductive period (Pandy et al., 1976; Zhiteneva & Goroslovskaya, 1986; Canfield et al., 1994).

The aim of this work was to determine the relationship between haematocrit and biological parameters in the Indian shad *T. ilisha*.

Materials and methods

The Shatt al–Arab River is formed by the confluence of the two major rivers in Mesopotamia, the Tigris and Euphrates at Qarmat Ali, 160 km north of the Arabian Gulf. This river flows in a south–eastern direction toward the Arabian Gulf. Water temperature shows a marked seasonal variation reaching a maximum in July (32ºC) and a minimum in December (15ºC) (Al–Hassan & Hussain, 1985).

The Indian shad, *Tenualosa ilisha,* is an anadromous clupeid fish, and represents one of the most important fisheries in the estuarine waters and some distance up stream (Sarma, 1984). In the Arabian Gulf area, this species is found along the Iranian side of the gulf and moving northward toward the estuary of Shatt al–Arab River in Iraq and other rivers in Iran (Al–Hassan, 1993). *T. ilisha* ascends Shatt al–Arab River and reaches the great marsh area just north of Basrah city, Iraq. The upper limit of its northern distribution is Al-Hammar Marsh (180 km north of Basrah city).

T. ilisha has a multispawn ability and has a long spawning season, which may last from May to August (Husain et al., 1991). Its absolute fecundity has been estimated to be in the range of 450,000–1,600,000 eggs per female (Jabir & Faris, 1989). It may reach 2,000,000 depending on the size of the fish (Hussain et al., 1991). It is well known that fecundity of the fish correlates positively with the square length of the fish (Wootton, 1990). Maturing individuals entering the Shatt al–Arab River range between 200 mm and 550 mm in total length, while a few immature fish enter the same waterway as small as 220 mm in length (Jabir & Faris, 1989).

Fish specimens (N = 400) were collected from Shatt al–Arab River at Basrah city, Iraq, in 1990. Immediately after capture, blood was sampled at the collection site by cutting the caudal peduncle without anaesthesia in small fish, and by direct sampling from the heart with a hypodermic needle for the larger specimens. They were later measured to the nearest mm. To determine haematological values, the blood was kept in tubes containing EDTA as an anticoagulant and in an icebox with ice. They were processed within a few hours of collection. The haematocrit value or packet cell volume was determined with a microhematocrit pipette. Blood samples were centrifuged for 5 min (12,000 rpm). Haematocrit value was determined according to the method described in Al–Abood & Al–Hassan (1988). Haematocrit was measured for each individual fish. Specimens (389) were dissected to determine sex and stage of maturity. Three stages were recognised, pre–spawning (fish with developing gonads that might contain eggs of sperms), spawning (fish with fully developed gonads containing well–developed eggs or sperms) and post–spawning individuals (fish with spent gonads).

Results

The relationship between body length and haematocrit

The haematocrit value differed according to the total length of the fish (ANOVA, $F_{5,381}$= 5136, P < 0.001) (table 1). Multiple pair–wise comparisons suggested that all the length groups differed from each other (Tukey tests all P < 0.001) with the exception of the 200$^+$mm length group in comparison with the 500$^+$mm group (TUKEY test, P = 0.99). Haematocrit value showed a polynomial relationship (quadratic regression) to the length of the fish (Ht = –5.7 + 0.2801 x L – 0.000387 x L^2; R^2 = 0.78, P < 0.001, $F_{2,384}$ = 6.78, P < 0.001). Fish in the smallest size group (0–100 mm) had the lowest values for haematocrit (table 1). This increased as the fish length increased up to a certain level, so that the fishes in the size group 301–400 mm showed the highest haematocrit values, after which no further increase was noted.

The relationship between fish haematocrit, sex and spawning period

Males showed a higher haematocrit value than females, independently of body length (tables 2, 3). Haematocrit value was higher at the beginning of maturation, it then decreased in April when the fish started to spawn, and increased slightly in the post-spawning period (tables 3, 4). Pre– and post–spawning periods had a higher Ht level than the spawning period (table 5). Pre– and post–spawning periods did not differ in Ht level (table 5). This seasonal variation was similar in both sexes (two–way ANOVA, interaction Sex x Period not significant; *F*–value 0.03; df 2,69; p. 97; table 3, fig. 1). When sex and spawning period were taken into account, the relationship of haematocrit and fish length disappeared (table 3).

Table 1. Total length and blood haematocrit value of *Tenualosa ilisha:* BL. Body length groups (in mm); *N.* Number of fishes; ML. Mean length (in mm ± SE); Ht. Haematocrit value (% ± SE).

Tabla 1. Longitud total y valor del hematocrito sanguíneo de Tenualosa ilisha: *BL. Grupos de longitug corporal (en mm);* N. *Número de peces; ML. Longitud media (en mm ± EE); Ht valor del hematocrito (% ± EE).*

BL	*N*	ML	Ht %
0–100	75	78.65 ± 0.84	13.22 ± 0.83
101–200	85	137.15 ± 0.85	24.85 ± 0.70
201–300	90	262.40 ± 0.07	37.74 ± 0.08
301–400	74	330.19 ± 0.81	52.05 ± 0.80
401–500	35	445.53 ± 0.18	43.23 ± 0.19
501–600	30	534.50 ± 0.12	24.17 ± 0.13

Discussion

The general trend in the relationship between blood haematocrit and body length is the longer the fish, the higher the haematocrit in *Cyprinus carpio,* for example, Murachi (1959) found that haematocrit increased as the fish length increased. Similar results were obtained for *Clarius batrachus* (Joshi & Tandon, 1977).

Male *T. ilisha* showed higher blood haematocrit values than females in all the length groups studied. This is in agreement with results from other fish species (*Telapia zilli*, Ezzat et al. (1973) [Ezzat et al., 1973]; *Cyprinus carpio* Fourie & Hattingh (1976) [Fourie & Hattingh, 1976]; *Cyprinion macrostomus* Al–Mehdi & Khan (1984) [Al–Mehdi & Khan, 1984]; *Amphiprous cuchia* Banerjee (1986) [Banerjee, 1986]. Fouri & Hattingh (1976) suggested that the differences in haematocrit between the two sexes are genetically determined, although Raizada et al. (1983) considered that the differences might be due to the higher metabolic rate of males compared to females. Our results support this suggestion, which has been related to an increase in fish activity with an increase in size (Chaudhuri et al., 1986).

It is generally stated that the blood haematocrit value in fish increases during the spawning season (Joshi & Tandon, 1977; Khan, 1977; Leonard &

Table 2. Sex and blood haematocrit value, Ht (% ± SE) of *Tenualosa ilisha: BL. Body length; N.* Number of fishes.

Tabla 2. Sexo y valor del hematocrito sanguíneo, Ht (% ± EE) de Tenualosa ilisha*: BL. Longitud corporal;* N. *Número de peces.*

	N		Ht			
BL	M	F	M	F	*t*–test	*P*
0–100	46	29	12.42 ± 0.04	11.08 ± 0.05	2.34	< 0.05
101–200	45	40	24.87 ± 0.85	23.28 ± 0.05	2.41	< 0.05
201–300	48	32	34.67 ± 0.07	33.62 ± 0.08	2.22	< 0.05
301–400	34	40	52.78 ± 0.83	50.73 ± 0.08	2.35	< 0.05
401–500	23	12	45.22 ± 0.12	41.91 ± 0.03	2.19	< 0.05
501–600	16	14	23.79 ± 0.18	23.49 ± 0.09	2.24	< 0.05

Table 3. ANCOVA on the relationship between fish sex, spawning period and length (as covariate) of *Tenualosa ilisha: S. Sex; Sp. Spawning period; L. Length*.

Tabla 3. ANCOVA de la relación entre sexo, el período de desove y la longitud (como covariable) de Tenualosa ilisha*: S. Sexo; Sp. Periodo de desove; L. Longitud.*

Factors	*F*–value	df	*P*
S	266.74	1,46	0
Sp	139.17	2,46	0
L	0.04	2,45	0.95
S x Sp	0.03	2,69	0.97
S x L	0.01	2,62	0.97
Sp x L	0.03	2,60	0.98
S x Sp x L	0.02	2,68	0.97

McCormick, 1999). This increase has been interpreted in relation to the high–energy requirements of fish during the breeding season. On the other hand, Sano (1963) and Einszporn–Orecka (1970) reported a marked reduction in haematocrit during gonadal development in both sexes of cultured trout, interpreted as a result of the depletion of nutritive substances during spawning. This agrees with the finding in the present study in *T. ilisha*. During the spawning season, the water temperature in Shatt al–Arab River is at its highest level (July, 32ºC), resulting in less oxygen content in the water which might cause the rise in haematocrit value. Hence, two factors are probably responsible for the rise in haematocrit value: a physiological factor evoked by a high energy demand during the breeding season and an environmental factor induced by the rise in water temperature.

Several authors have shown how environmental factors such as water temperature have a direct effect on different blood parameters such as haematocrit through their effect on the haemoglobin oxygen–binding properties and thus on oxygen trans-

Table 4. Blood haematocrit, Ht (% ± SE), fish sex and different spawning periods of *Tenualosa ilisha:* Pre–Sp. Pre–spawning period; Sp. Spawning period; Pst–Sp. Post–Spawning period.

Tabla 4. Hematocrito sanguíneo, Ht (% ± EE), sexo y distintos períodos de desove de Tenualosa ilisha: *Pre–Sp. Periodo previo al desove; Sp. Periodo de desove; Pst–Sp. Periodo posterior al desove.*

		Ht		
Sex	*N*	Pre–Sp	Sp	Pst–Sp
Males	33	34.36 ± 0.18	33.38 ± 0.15	34.29 ± 0.18
Females	42	33.52 ± 0.16	31.12 ± 0.16	33.45 ± 0.16

Table 5. Post hoc comparisons (Tukey test) within and between spawning periods and within and between sexes: Pre–Sp. Pre–spawning period; Sp. Spawning period; Pst–Sp. Post–Spawning period.

Tabla 5. Comparaciones post hoc *(test de Tukey) en los períodos de desove y entre los mismos, y en los sexos y entre los mismos: Pre–Sp. Periodo previo al desove; Sp. Periodo de desove; Pst–Sp. Periodo posterior al desove.*

	Male			Female		
	Pre–Sp	Sp	Pst–Sp	Pre–Sp	Sp	Pst–Sp
Male						
Pre–spawning		0.0003	1.0000	0.0012		
Spawning			0.0003		0.0009	
Post–spawning						0.0004
Female						
Pre–spawning					0.0000	0.9992
Spawning						0.0001

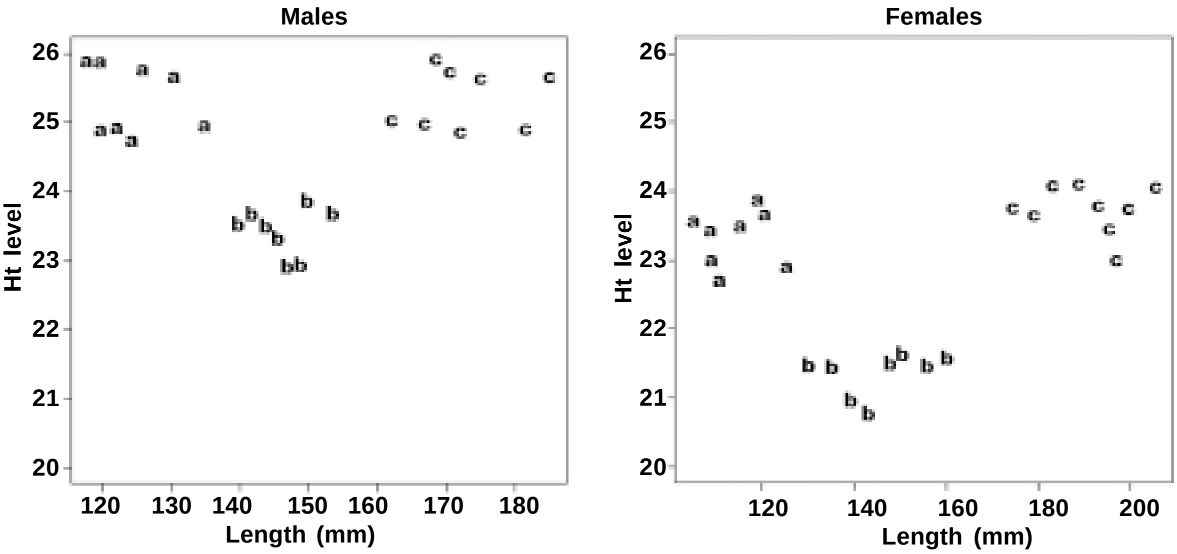

Fig. 1. Length versus haematocrit value by gender and spawning period of *T.ilisha:* a. Pre–spawning period; b. Spawning period; c. Post–spawning period.

Fig. 1. Longitud según el valor del hematocrito, por género y período de desove de T. ilisha: *a. Período anterior al desove; b. Período de desove; c. Período posterior al desove.*

port (Di Prisco & Tamburrini, 1992; Wells, 1999). On the other hand, *T. ilisha* is a migratory fish and enters rivers at the stage when the fish are ready to lay their eggs. Such activity leads this fish species to face changes in the external salinity which in turn produces changes in the distribution of water masses and in the total amount of haemoglobin (Parry, 1961). Increases in osmoregulatory work might be expected to produce an increase in the blood oxygen carrying capacity, which in turn would bring about a significant change in the haematocrit value, by the same reasoning as for temperature. However, changes in water balance will cause an osmotic effect in the red blood cell, finally increasing haematocrit (Cameron, 1970).

Different rates of fish activity demand different levels of metabolic activity. Such activity requires several physiological adjustments. These include haematological parameters (Putman & Freel, 1978), which play a significant role in the increase of blood supply to the muscle through their variation. Haematocrit is one of those parameters that showed a general correlation with fish activity. *T. ilisha* might need to be more active and thus increase its metabolic rate if environmental changes such as water quality or destruction of breeding niches occur in its natural habitat. The recent diversions in Shatt al–Arab River directions have led not only to changes in the water quality of the lower reaches, ultimately changing water salinity, but also to the disappearance of major marsh areas where *T. ilisha* usually lay their eggs (Munro & Towron, 1997). Haematological parameters may therefore be of value in monitoring the effects of habitat changes on fish biology.

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