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Ecophysiological Indicators of Stress in Female Persian Sturgeon, *Acipenser persicus*

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Abstract: The study on the ecophysiological impacts of stress in female Persian sturgeon broodfishes revealed that the average cortisol level varried in all broodfish caught at the Sefidrud River (163.38±21.2ng mL^{-1}), Sefidrud River estuary (260.27±19.7ng mL^{-1}) and Gorganrud River estuary (179.38±20.8ng mL^{-1}). The variations in serum glucose levels were 140.65±12.05mg dL^{-1}, 144.44±5.9mg dL^{-1} and 126.24±11mg dL^{-1}, respectively. The maximum level of cortisol (781 ng mL^{-1}) and serum glucose (281 mg dL^{-1}) were observed during the transport of broodfish from the capture stations to the hatcheries and the minimum level of cortisol (25.2 ng mL^{-1}) and serum glucose (33 mg dL^{-1}) were observed during the confinement of broodfishes in the breeding ponds. The rhythmic and significant variations of cortisol level during capture, handling, transport and confinement of female Persian sturgeon broodfish in all three regions can be regarded as a valuable indicator of stress.

KEY WORDS: Stress, Cortisol, Glucose, *Acipenser persicus*, Broodfish

Introduction

Capture and transport of Persian sturgeon broodfishes during their spawning migration inflicts severe stress on them. According to Donaldson (1981) environmental factors such as handling, crowding, anesthetics, confinement and pollutants can induce stress in fishes. The impact of stress induced by these factors is stronger in female broodfishes. Schreck (1981) and Wedemeyer & McLeary (1981) have stated that owing to the hyperglycemic characteristic of cortisol in response to increase in glyconeogenesis and glycogenolysis (Pickering *et al.*,

1982; Barton *et al.*, 1985) a combined study of cortisol and blood serum glucose is the most appropriate stress index in various fishes. Also Specker & Schreck (1980) have proved that handling and transport cause a rise in cortisol levels in fish blood serum. Most studies on fish stress have been made on fishes in captivity (Barton & Iwama, 1991 ; Pickering, 1992), and limited information is available on the impacts of stress in the wild populations of fish (Pankhurst & Dedual, 1994).

Owing to the economical importance of the Persian sturgeon as an endemic species of the South Caspian Sea, the significance of rehabilitation of their stocks and also since among the five endemic species of the South Caspian basin the largest number of fingerlings released into the sea (22,586,417 fingerlings in 1999) belongs to this species, the ecophysiological impacts of stress was studied for the first time on Persian sturgeon broodfish during different stages of their capture, handling, transport and confinement. This study was conducted to achieve ecophysiological patterns such as identifying the variations in serum cortisol and glucose, and also to determine the changing trends in stress indicators at different stages (capture, handling, transport and confinement) (Bahmani, 1999).

Materials and Methods

Sampling stations and frequency:

Persian sturgeon broodfish were collected from 4 regions: Sefidrud River (broodfish caught from the Sefidrud River, n=20), Sefidrud River estuary (broodfish caught from the sea, n=52), Shahid Marjani sturgeon hatchery (broodfish caught at the Gorganrud River estuary, n=11) and Kurenski breeding ponds of Shahid Beheshti hatchery (broodfish kept in confinement). Samples were collected during different periods including: capture, handling, transport and confinement from 1996 to 1998. Broodfish caught from the Sefidrud River and its estuary had to be carried a distance of 100 Km and those caught from the Gorganrud River estuary had to be carried a distance of 700 Km to reach to the Shahid Beheshti hatchery (carried on trucks supplied with oxygen).

Catch Method:

Broodfish in the river were caught using beach seine nets and those from the sea were caught using gillnets. All broodfish were tagged on capture.

Physiological examinations:

Blood samples collected from the caudal vein (during each stage) were transferred to sterile tubes for serological examinations. The sexual maturity of broodfish was determined using the formula given below (Lutes *et al.*, 1987 ; Bahmani *et al.*, 2000):

Polarization Index (PI) = a/b x 100

Where: a = distance of nucleus from egg membrane,
b = distance between animal and vegetal pole

Biometric measurements were conducted on the captured broodfish and their age was determined from the first spine of the pectoral fin.

Weight of each egg was determined using a digital balance (0.001 g precision) and total number of eggs was calculated by counting the number of eggs per gram. After confinement in Kurenski breeding ponds, broodfish were administered Gonadotropin Releasing Hormone (GnRH) agonist and their response to breeding was recorded in order to estimate the effects of catch, transport, handling and confinement on their breeding potential.

Cortisol was determined by the RIA method using the Incstar kit and the detector with the I^{125} tracer (Abraham, 1974).

Serum glucose was determined by the POD (Peroxidase) GOD (Glucose oxidae) enzymatic method using spectrophotometer at 546 nm wavelength (Pickering *et al.*, 1982).

Statistical analysis:

Results were analysed statistically using ANOVA, MANOVA, SPSS, Statgraph and Excel softwares.

Results

The total weight, fork length, age and weight of eggs in broodfish examined in this study are presented in Table 1.

Table 1: Morphometric measurements of Persian sturgeon broodfish (1996-98)

	Catch region	Mean	Min.	Max.	SD	Variance	SEM
Fork length (cm)	Sefidrud River (n=20)	159.98	133.5	177.5	12.28	150.79	1.59
	Sefidrud River estuary (n=52)	154.17	115.5	179.5	12.78	165.63	1.03
	Gorganrud River estuary (n=11)	162.18	153.5	173.0	5.94	165.63	1.03
Total weight (Kg)	Sefidrud River	27.95	16	40.5	6.49	42.12	0.84
	Sefidrud River estuary	24.31	16	34.0	5.06	25.60	0.41
	Gorganrud River estuary	28.64	26	35.5	2.83	8.01	0.49
Age (year)	Sefidrud River	21.05	14	27	3.64	13.25	0.47
	Sefidrud River estuary	21.05	14	27	3.64	9.73	0.47
	Gorganrud River estuary	20.09	17	23	20.5	4.20	0.36
Weight of egg (g)	Sefidrud River	0.014	0.011	0.018	0.00	0.00	0.00
	Sefidrud River estuary	0.015	0.010	0.021	0.00	0.00	0.00
	Gorganrud River estuary	0.016	0.014	0.019	0.00	0.00	0.00
No. of Egg (g^{-1})	Sefidrud River	59.2	45	89	10.97	120.34	1.42
	Sefidrud River estuary	58.46	40	92	8.10	65.61	0.65
	Gorganrud River estuary	57.09	45	66	6.62	43.82	1.15

The variation in cortisol and glucose levels during capture, handling, transport and confinement of broodfish caught in the Sefidrud River, its estuary and Gorganrud River estuary are presented in Tables 2.

Table 2: Biological variables(serologic)in Persian sturgeon broodfish (1996-98)

a. Sefidrud River (n=20)

Indicator	Stage	Mean	Min.	Max.	Range	SD	Variance	SEM
Glucose	Catch	136.5	66.0	219	153	40.7	1637.8	9.05
(mg dL^{-1})	Transport	176.75	82.0	281	199	56.51	3193.4	12.64
	Confinement	108.7	33.0	209	176	64.73	4189.9	14.47
Cortisol	Catch	159.92	48.6	522.2	473.6	128.1	1640.6	28.64
(ng mL^{-1})	Transport	257.38	127.8	579	451.2	107.96	11577.8	24.14
	Confinement	73.75	25.2	194.4	169.2	49.3	2430.5	11.02

b. Sefidrud River estuary (n=52)

Indicator	Stage	Mean	Min.	Max.	Range	SD	Variance	SEM
Glucose	Catch	153.67	85	260	175	39.06	1525.7	5.47
(mg dL^{-1})	Transport	168.62	85	269	184	44.8	2008.8	6.22
	Confinement	111.21	48	246	198	46.0	2116.9	6.38
Cortisol	Catch	257.51	74.6	564.9	490.3	127.9	16381.4	17.92
(ng mL^{-1})	Transport	343.67	60.4	781.6	721.2	167.0	27899.0	23.16
	Confinement	179.58	30.6	720	689.4	134.9	18187.2	18.70

c. Gorganrud River estuary (n=11)

Indicator	Stage	Mean	Min.	Max.	Range	SD	Variance	SEM
Glucose	I confinement	141.64	95	214	119	36.12	1304.65	10.89
(mg dL^{-1})	Transport	158.0	71	208	137	40.99	1680.18	12.36
	IIConfinement	79.09	41	146	105	32.07	1028.48	9.67
Cortisol	I confinement	136.55	84.6	209.1	124.5	32.98	1087.68	9.94
(ng mL^{-1})	Transport	297.84	161	564.6	403.6	121.85	14847.4	36.74
	IIConfinement	103.76	30.6	193.3	162.7	52.7	2777.3	15.89

There was a significant difference in the variations of cortisol in specimens from the Sefidrud River ($P= 0.010$, $F=7.7851$) (Fig. 1).

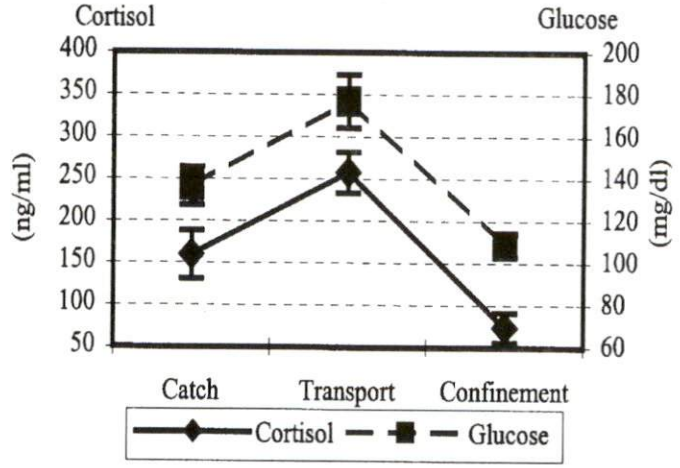

Fig. 1: Variations in cortisol and glucose levels in of broodfish caught at Sefidrud River

The variations of cortisol levels during different stages in specimens caught at the Sefidrud River estuary also showed significant difference (P=0.0000, F=16.8016) (Fig. 2).

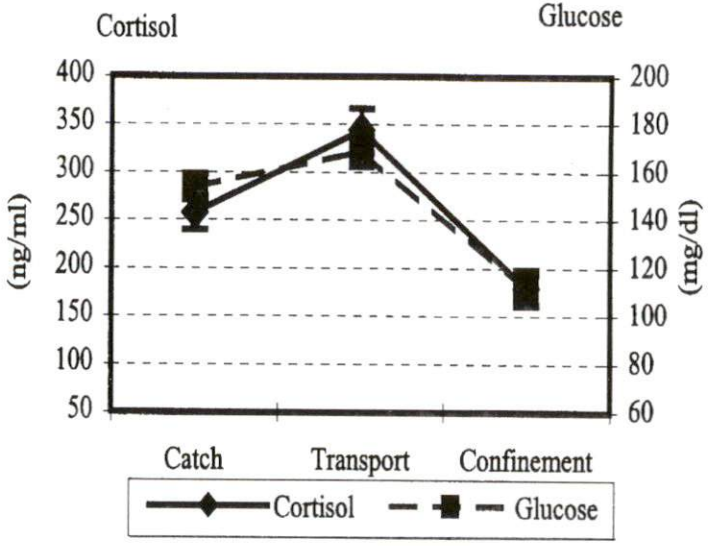

Fig. 2 : Variations in cortisol and glucose levels in broodfishes caught at Sefidrud River estuary

Significant difference was also observed in variation of glucose levels in brood fish caught in the Sefidrud River estuary (P= 0.0000, F=24.4245).

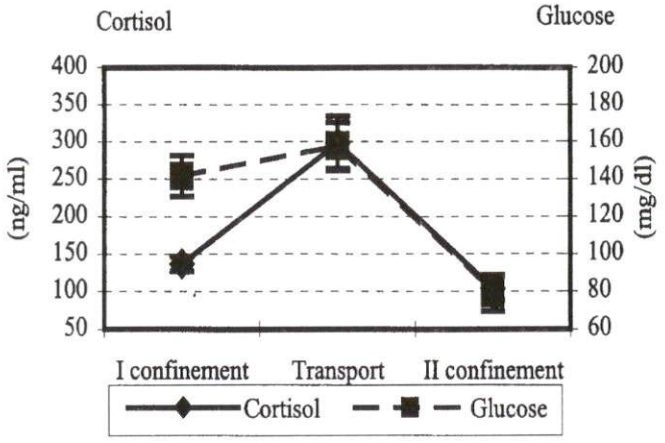

Fig. 3 : Variation of cortisol and glucose levels in broodfishes caught at the Gorganrud estuary

The broodfish caught in the Gorganrud estuary also showed significant difference in cortisol (P=0.0000, F=19.0314) (Fig. 3) as well as serum glucose levels (P= 0.0000, F=14.2626).

Maximum stress was happened during the broodfishes transport and the minimum was caused during their confinement in breeding ponds. Broodfish caught in the Sefidrud River were also in a better physiological state comparing to those caught from the river estuary.

Study of blood indices in relation to reproduction in broodfish:

The mean serum glucose level variations of broodfish caught in the Sefidrud River used for artificial breeding and those which were not suitable for artificial breeding were 136.167, 173.5 ; 90.41 mg dL^{-1} ; 137, 181.6 and 136.12 mg dL^{-1} respectively during capture, transport and confinement and the mean cortisol level variations in the two groups were 169.29, 283.31 ; 66.8 ng mL^{-1}; 145.87, 218.47 and 84.16 ng mL^{-1}, respectively.

The mean serum glucose level variations in broodfish caught in the Sefidrud River estuary used for artificial breeding and those not suitable for artificial breeding were 174.5, 190.2 ; 90.2 mg dL^{-1} ; 148.4, 163.47 and 116.21 mg dL^{-1}, respectively during capture, transport and confinement and cortisol levels in those

two groups were 285.78, 345.03 and 130.79 ng mL^{-1}, and 249.61, 343.34 and 191.19 ng mL^{-1}, respectively.

In broodfish caught in the Gorganrud River estuary, used for artificial breeding and those which were not suitable for artificial breeding the mean variations in serum glucose levels were 142.75, 163.75 ; 78.75 mg dL^{-1} ; 141, 154.71 and 79.28 mg dL^{-1} during capture, transport and confinement, respectively. The mean cortisol level in two groups during the same stages were 130.85, 248.67 and 60.67 ng mL^{-1} and 139.81, 325.92 and 128.38 ng mL^{-1}, respectively.

It is quite evident that broodfish used for artificial breeding from all three regions showed lower levels of cortisol during their confinement in Kurenski ponds. Tables 3, 4 and 5 show the average levels of serologic factors, state of (GnRH) agonist administration and breeding rates in the broodfish studied.

Table 3: Mean levels of serologic parameters in Persian sturgeon broodfish

	Sefidrud River	Sefidrud River estuary	Gorganrud River estuary
Cortisol (ng mL^{-1})	163.38	260.27	179.38
Glucose (mg dL^{-1})	140.65	144.44	126.24

Table 4 : The percentage of gonadotropin agoinst injected broodfish (after confinement in ponds)

	Sefidrud River	Sefidrud River estuary	Gorganrud River estuary
Injected	100 %	95.7 %	100 %
Not injected	-	4.3 %	-

Table 5 : Percentage of breeding (succded in artificial spawning)

	Sefidrud River	Sefidrud River estuary	Gorganrud River estuary
Injected	60 %	19.6 %	36.4 %
Not injected	40 %	80.4 %	63.6 %

Discussion

Comparisons conducted on sturgeons caught in the Sefidrud River estuary showed significant difference in cortisol concentrations during capture. High mean values of 257.51 ng mL^{-1} (SEM=17.92) of cortisol during capture in broodfish caught in the Sefidrud River estuary comparing to the mean values of 159.92 ng mL^{-1} found in breeders caught in the Sefidrud River are the result of the type of fishing gear used to capture these fish. Broodfish in the river were caught using beach seine nets (for a period of half of hour) whereas the catch in the sea was done using gillnets (maximum period of 24 hours). Fishes caught in the sea may remain trapped in gillnets for a whole day. Also since broodfish were caught during the early stages of their migration into the river, owing to the better spawning conditions found upstream and their adaptation to the river conditions, stress (in other words, blood serum cortisol level) will drop. On the other hand this chance is lost by broodfish which are caught downstream. Thus not being able to reach the river upstream for natural spawning results in the failure in reproduction and spawning in species particularly vulnerable species like sturgeon (Kynard, 1997).

Bukovskaya & Bayunova (1989) studied the effects of acute stress on Russian sturgeon, *A. gueldenstaedtii* broodfish. Barton & Iwama (1991) studied the physiological changes produced of stress on the artificial breeding and rearing of *Polyodon spathula*. Bullis (1993) studied the impacts of stress after the capture and handling in *Polyodon spathula*, *A. transmontanus*, *A. medirostris* and *A. fulvescens*. Semenkova *et al.* (1997) studied the effects of stress in *A. gueldenstaedtii* and *A. stellatus* broodfish held in captivity and proved that stimuli

caused by capture, handling, transport, stocking density and confinement caused severe stress in fishes.

Cortisol is the main corticosteroid hormone which increases in most fishes under stress (Barton & Iwama, 1991). Therefore cortisol or 11ß, 17, 21-trihydroxy-4-Pregene-3,20-dione is the most important stress hormone identified in fish (Cuisset *et al.*, 1995). Serum cortisol concentrations were elevated during acute stress and returned to their basal levels within 24 hours or more. Catecholamines, on the other hand, showed faster variations (Pickering & Pottinger, 1995). However no reliable data is available for sturgeons.

The study on handling and transport of broodfish showed severe stress inflicted on them leading to a significant increase in stress indicator (cortisol) in broodfish caught in the Sefidrud River which reaches a value of 257.38ng mL^{-1} (SEM=24.14) and in broodfish caught in the Sefidrud River estuary. These levels reach 343.67 ng mL^{-1} (SEM=23.16) during their transport to the Shahid Beheshti hatchery. Specker & Schreck (1980) have also proved that handling and transport caused blood serum cortisol increase in fishes. Most of the studies have been conducted on fishes under captive breeding (Barton & Iwama, 1991; Pickering, 1992), and very limited information are available on the impacts of stress in wild populations (Pankhurst & Dedual, 1994).

Studies conducted by Barannikova *et al.*, (1997) on the concentrations of serum cortisol during migrations in *A. gueldenstaedtii, A. stellatus* and *Huso huso* have indicated that during feeding migrations, serum cortisol concentration dropped to 22.2±3.7 ng mL^{-1} whereas during their spawning migration to the Volga River, elevated to 126.5±39.1 ng mL^{-1}. So, it seems that cortisol is the primary hormone related to migrations, sexual maturity and osmoregulation in sturgeons. Similarly during early spawning migrations the cortisol concentrations reached 109.8±11.7 ng mL^{-1} in female Russian sturgeon breeders, 170.2±28.5 ng mL^{-1} in female Stellate sturgeon breeders and 165.4 ng mL^{-1} in female great sturgeon breeders.

It is worthy to note that very little information are available on the chemical characteristics of blood in cartilaginous fishes and their variations and vulnerability during stress (McDonald & Millgan, 1992 ; Cataldi *et al.*, 1998). Also no studies have been conducted on the impacts of stress during transport and confinement of sturgeon broodfish. Therefore data obtained from this study call for further. As we have seen due to the us of fishing gear and also the physiological state of each broodfish, the levels of cortisol found in broodfish

caught in the Sefidrud River are lower than those found in the Sefidrud River estuary. This shows that the significant difference in cortisol concentrations in the two groups of broodfish is mainly because of the harmful effects of the fishing gear and also to some extent on the physiological state of the broodfish in their migration route (in the sea).

Elevation in cortisol concentrations during transport totally depends on the mode of transport of broodfish to the hatcheries. The distance, coverage, stocking density of broodfish and time of transport are among the effective factors in increasing stress at this stage.

As mentioned earlier, the mean cortisol concentration in broodfish caught at the Gorganrud estuary was 136.55 ng mL^{-1} (SEM=9.94). However when transferred to the Shahid Beheshti hatchery the mean cortisol concentration elevated to 297.84 ng mL^{-1} (SEM=36.74). Thus it is quite evident that due to the acute stress inflicted on these broodfish during their confinement and transport under specific conditions (loaded on trucks) it is unlikely that their cortisol levels will return to their basal levels (at the time of capture) or slightly higher, and thus leads to chronic stress in the broodfish. Therefore cortisol concentrations will not decrease as long as unsuitable physiological conditions exist in Persian sturgeon broodfish (during transport and handling). After passing through a state of acute stress and reaching a peak point, the broodfish gradually adapt themselves to the prolonged state of confinement and the rising course in cortisol concentrations in response to stress during transport stabilizes to a certain extent.

Density is another important factor in increasing stress during transport (Donaldson, 1981; Pickering & Pottinger, 1987). This is clearly seen in broodfish that are carried from Gorgan to the Shahid Beheshti hatchery in Rasht. Persian sturgeon broodfish were kept in high densities (11 broodfish in each tank) without water renewal for an extended period of 12-14 hours. Studies show that high stocking densities can result in interruption in the activity of the HPI (Hypothalamus Pituitary Interrenal) axis in these fishes (Pickering & Pottinger, 1987). It appears that physical and chemical characteristics of water particularly increase of water temperature and fluctuations in oxygen concentrations in prolonged stress conditions during the transport of broodfish from the Gorganrud estuary. This indicates interactions among various physical and environmental stress and effects of synergism in stabilizing stress in fishes.

Also there are variations in cortisol concentrations in salmon blood specimens collected in darkness and those collected during the day (Pickering *et al.*, 1982). Cortisol concentrations examined in fishes during the dark hours were significantly higher than those examined in fishes during the day. The difference in cortisol concentrations during the day and night has also been reported for other species. Elevated concentrations of cortisol (564.6 ng ml^{-1}) in fish caught at the Gorganrud estuary after their transport to the Shahid Beheshti hatchery (blood samples collected at midnight) also agree with this. The cortisol concentrations of sturgeons during dark hours have not been studied so far.

Mean values of cortisol in the three groups of broodfish studied after 1 to 5 days in confinement in Kurenski ponds at the Shahid Beheshti hatchery showed declines in cortisol concentrations. Comparison of the three groups showed that the most stable and lowest concentrations of cortisol belonged to the broodfish caught in the Sefidrud River with a mean value of 73.75 ng mL^{-1} (SEM=11.02). Broodfish caught in the Gorganrud estuary that were first transferred to the ponds at the Shahid Marjani hatchery and after a short confinement transferred to the Shahid Beheshti hatchery showed a mean cortisol concentration of 103.76 ng mL^{-1} (SEM=15.89) and were in a better physiological state as compared to broodfish caught at the Sefidrud River estuary that showed an average cortisol concentration of 179.58 ng mL^{-1} (SEM=18.7).

Glucose:

Our results showed similar variations in cortisol and glucose concentrations in the three studied groups of female Persian sturgeon broodfish. Glucose concentrations showed significant difference (P=0.0000). Usually variation in corticosteroids and serum glucose under stress conditions are a result of severe environmental stimuli. There is evidence of hyperglycemic response of cortisol to glycogenolysis and glyconeogenesis (Pickering *et al.*, 1982; Wendelaar Bonga, 1993). The highest mean serum glucose concentration in broodfish during this study was observed during handling and transport 281 $mgdL^{-1}$. Results obtained by Staurnes *et al.*, (1994) on *Gadus morhua* and Pottinger (1998) on *Cyprinus carpio* also support this view that stimuli during transport give rise to general response to stress in fishes, elevated concentrations of cortisol and glucose being the most obvious of them. Schreck (1981), Wedemeyer & McLeary (1981) also believed that a combined study of cortisol and blood serum glucose is the most

suitable indicator to different types of stress in fishes (Barton et al., 1985). Results from this study also demonstrate this view.

Effects of stress on breeding condition in broodfish:

Researchers believe that the induction of environmental stress and response of the HPI axis to handling, indicates acute stress in wild species held in confinement leads to a state of chronic stress in these fishes (Pottinger *et al.*, 1999). Therefore it appears that employing rational and correct measures in reproduction management of sturgeon broodfishes can play a significant role in enhancing the quality and quantity of artificial breeding.

Comparison of breeding conditions in broodfish indicate that most of the broodfish studied in the three regions showed cortisol concentrations in the range of 100-300 ng mL^{-1}. Broodfish caught in the Sefidrud River showed the highest rate of propagation (60 %) whereas the lowest rate of propagation (19.6 %) belonged to broodfish caught at the Sefidrud River estuary. It is evident from the results that lesser stress and better physiological state was found in broodfish caught in the Sefidrud River. Broodfish caught in the Sefidrud River estuary (caught at sea) lacked the suitable physiological for artificial breeding. Broodfish caught at the Gorganrud estuary showed 36.4 % of successful propagation.

Comparison of the weight of each egg in the broodfish showed that in the majority of broodfish studied in the three regions the weight of eggs ranged from 0.015-0.018 g.

The mean cortisol concentration in these broodfish as well as in those used for artificial breeding, ranged from 100-300 ng mL^{-1}.

Campbell *et al.* (1992) showed that recurrence of any type of disturbance and change in aquatic ecosystems gives rise to stress and ultimately to a significant decrease in ovary size and consequently a decrease in egg size, lowering in fecundity and disturbance in ovulation. Breeding male broodfish under stress with female fish also under stress leads to the decline in viability and survival rate of the resulting generation, and increases mortality of larvae, and hatching time in eggs. The results of the present study also demonstrate this view.

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