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Optimum growth, enzymatic and biochemical reactions of stellate sturgeon, Acipenser stellatus juveniles in response to the feeding frequency and exposure to environmental salinity

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ABSTRACT

The salinity and feeding frequency are two fundamental challenges in breeding stellate sturgeon, *Acipenser stellatus* juveniles both for aquaculture and restocking purposes. We evaluated growth indices, biochemical and digestive enzymes, osmoregulation indices, and gill tissues of 810 stellate sturgeons (10.96 ± 0.89 g in weight and 12.64±0.96 cm in length) in 9 treatments including three salinity levels with three types of feeding during eight weeks of rearing. The treatments included two, four and six times (2T, 4T and 6T) of feeding in combination with zero, 6 and 12 ppt salinities (0‰, 6‰ and 12‰). The main growth indices and food efficiency exhibited an upward trend by increasing in salinity and feeding frequency. The average final weight and length in 6T + 12‰ were higher than in other treatments. In addition, the highest average condition factor was measured in 4T + 6‰ ($p < 0.05$). The average food conversion ratio of all treatments decreased compared to 2T + 0‰. Alkaline phosphatase (ALP) in 2T + 0‰, alanine aminotransferase (ALT) in 6T + 6‰, 2T + 12‰, 4T + 12‰, 6T + 12‰, and aspartate aminotransferase (AST) in 4T+12‰ and 6T+12‰ were higher than in the other treatments ($p < 0.05$). The highest amount of amylase was measured in 2T + 6‰ and lipase in 6T + 0‰, 2T + 6‰, and 4T + 12‰ ($p < 0.05$). The results of the investigated indices showed that it is not necessary to increase meals at higher salinities. In addition, raising the salinity of the breeding environment to 6 ppt is favorable for safety factors and fish growth.

Keywords: Stellate sturgeon, Feeding frequency, Salinity, Physiology.
Article type: Research Article.

INTRODUCTION

Sturgeon aquaculture has received much attention worldwide due to a continuous decrease in catch yield (Jafari *et al.* 2021; Fathollahi *et al.* 2021; Bakhshalizadeh *et al.* 2022; Mir Rasekhian *et al.* 2022). Sturgeons are bred in different environments both in the Iranian inland, and coastal areas of the Caspian Sea, floating cages in the Caspian Sea, or dam reservoirs. Fresh or brackish water are used in all these reservoirs. The feeding indices such as the type of food, the amount of food, and the frequency of feeding are among the crucial factors in enhancing the production of stellate sturgeon. Recently, efforts have been made to breed sturgeon in fresh and saltwater environments. Salinity is an

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important ecological parameter that affects the growth and survival of aquatic organisms, however, it is subjected to drastic changes in coastal areas, in response to climate fluctuations. Therefore, knowing the effects of different salinities on each species to determine the best ones is an essential condition in commercial aquaculture species to tolerate physiological changes following alterations in salinity (Farshadian *et al.* 2019). Like other aquatic animals, sturgeon feeding can critically affect production as it might alter the growth, reproduction, and longevity of fish. Therefore, it is possible to make sturgeon breeding economically efficient with proper feeding, and by improving the growth indices (Hung 2017). Feed costs are the most significant operating cost in hatcheries (Lee *et al.* 2016). Thus, optimizing the feeding strategy is essential to maximize growth and feed conversion, minimize size heterogeneity, and limit waste and abatement costs. The optimal feeding frequency for fish may vary based on species, age, height, environmental factors, and food quality. Optimum feeding frequencies for fish range from constant feeding for African catfish larvae, *Clarias gariepinus* (Aderolu *et al.* 2010) to just one meal every other day for sea bass *Dicentrarchus labrax*. The information suggests that the feeding frequency can be determined similarly for different fish sizes (Varsamos *et al.* 2005). Some studies examined the effect of salinity and feeding frequency on growth of different species of sturgeons (Giberson & Litvak 2011; Andrei *et al.* 2017). Lee *et al* (2016) reported that changing the nutritional status of white sturgeon (*Acipenser transmontanus*) lowered the salinity tolerance of young sturgeons.

Given the development of sturgeon culture in different fresh and saltwater resources, it is essential to understand the effect of feeding frequency in different salinities of the farmed sturgeon. Sturgeons usually live in brackish waters, so obtaining an optimal feeding strategy and providing the highest growth per unit of feed cost can be considered an influential and vital factor for the development of rearing these species. Therefore, this study aimed to investigate the effect of feeding frequency and salinity on growth indices, liver enzymes, digestive enzymes, antioxidant activities, gill tissue, and osmotic compatibility of farmed stellate sturgeon, *A. stellatus*.

MATERIALS AND METHODS

Rearing condition

A total of 810 stellate sturgeon with an average weight of 12.24 g were kept in fresh water for one week at the Guilan sturgeon research station in the southern part of the Caspian Sea to adapt to the conditions. The fiberglass tanks had covers with a valve providing food and sunlight access. The Caspian Sea and well water supplied brackish and fresh water. Three levels of salinity (0, 6, 12 ppt) and three levels of feeding frequency in total 9 treatments (each with 3 replicates) were considered as follows (Vaz *et al.* 2015) (Table 1):

Table 1. Feeding times of stellate sturgeon juveniles in different treatments

Treatment	Feeding times
2T + 0‰	6, 18
4T + 0‰	6, 12, 18, 24
6T + 0‰	6, 10, 14, 18, 22, 2
2T + 6‰	6, 18
4T + 6‰	6, 12, 18, 24
6T + 6‰	6, 10, 14, 18, 22, 2
2T + 12‰	6, 18
4T + 12‰	6, 12, 18, 24
6T + 12‰	6, 10, 14, 18, 22, 2

Zero salinity was prepared by well water (0.0-0.5 ppt): 6 salinities by combining the sea and well water (5-7 ppt), while 12 salinities (11-13 ppt) completely by seawater. During the breeding period, the water temperature (19.5 ± 1.2 °C), dissolved oxygen (5.60 ± 0.44 mg L^{-1}), and percentage of oxygen saturation (60.47 ± 3.6 mg L^{-1}) were measured by digital oxygen meter (Oxi 3205 SET3, WTW, Germany) on the daily basis. Water pH (7.83 ± 0.08) by a pH meter (370, Jenway, England) was measured weekly. In addition, aeration was performed by an air blower (ACO-010, RESUN, China with an oxygenation capacity of 0.135 m^3/min).

The growth and survival calculations

The total length was measured with an accuracy of 0.1 cm and weight with an accuracy of 1.0 g. The growth status of fish and the performances of the treatments of growth indicators were calculated as follows:

1. Food conversion ratio (FCR) (Hevroy *et al*, 2005)

$FCR=F/(Wt-W0)$

2. Specific growth ratio (percentage per day) SGR: (Kamil and Taha, 2022)

$$S.G.R=(Lnwt-Lnwo)/t\times 100$$

3. Percentage of body weight increase (%BWI): (Hung *et al.*1989)

$$\%BWI=(Bwf-Bwi)/Bwi\times 100$$

4. Daily growth (gram/day) GR: (Hung *et al.* 1989)

$$G.R=(Bwf-Bwi)/n$$

5. Condition factor (CF): (Ojolick *et al.* 1995)

$$CF=(Bw/TL^3)\times 100$$

6. Feed efficiency (FE)

FE= 100 × (BWf—BWi) /Tf; where BWf and BWi are the final and initial average weight and Tf is total consumed food (Hung *et al.*1989).

Blood and serum sampling

At the end of the rearing period, blood samples were taken from stellate juveniles to determine the liver and digestive enzymes. About 24 h before blood sampling, fish feeding was stopped, and three samples were randomly selected from each replicate. 2 mL blood was collected from the caudal vein at the base of the anal fin and divided into 2 Eppendorf tubes, one of them was heparinized. The samples were centrifuged (10 min, 4°C, at 3000 rpm). The supernatant of serum was separated and kept at -80℃ to be used later to measure the biochemical parameters (Kazemi *et al.* 2005).

Liver enzyme activity

Aspartate aminotransferase (AST)

Aspartate aminotransferase (AST) was measured using a kit from Pars Azmoun (Karaj, Iran) and an autoanalyzer (Prestige 24i) with an enzymatic method (IFCC: International Federation of Clinical Chemistry). The results were presented at a wavelength of 340 nm. The basis of the test is the reaction of L-aspartate with 2-oxaloglutarate, which AST catalyses this reaction. Then the resulting oxaloglutarate produces malate. In this reaction, NADH has light absorption, which is directly related to the increase of oxaloglutarate, therefore, quantitative analysis of AST enzyme is possible (Liu *et al.* 2016).

Alanine aminotransferase (ALT)

Alanine aminotransferase was measured by the IFCC enzyme method using an autoanalyzer (Prestige 24i; Pars Azmoun diagnostic kit, Karaj, Iran). The absorbance was read at a wavelength of 340 nm. The basis of the experiment was the reaction of l-alanine with 2-oxaloglutarate, which ALT catalyses this reaction. The resulting pyruvate produced L-lactate. In this reaction, NADH has light absorption that is directly related to the increase of pyruvate (Liu *et al.* 2016).

Alkaline phosphatase (ALP)

Alkaline phosphatase was measured by photometric method (DGKC: Deutsche Gesellschaft fur Klinische Chemie) using Pars Azmoun (Karaj, Iran) kit. The absorbance value of the samples was read after 1 min at 405 nm. The average optical absorption differences after 1, 2 and 3 min were multiplied by 2757. In the case of control group, TruLab P and TruLab N of Pars Azmoun Company were used separately (Shahsavani *et al.* 2008).

The activity of digestive enzymes

Amylase

Amylase was measured through kinetic colorimetric and using an alpha-amylase kit (Dialab, Austria) with a spectrophotometer (UV/VIS 6505 model, Jenway, England) using standard solutions substrate, and buffer with a wavelength of 405 nm (Hekmatpour *et al.* 2023).

Lipase

Lipase activity was prepared by the reaction mixture including buffer, DTNB probe, and lipase substrate then was read by spectrometry and by the Lipase (Liquid stable) kit (Bionik company, Tehran) with a spectrophotometer (model -UV/VIS 6505, Jenway company, England) at a wavelength of 580 nm (Hekmatpour *et al.* 2023).

Protease

It was read in the supernatants using spectrometric measurement method by measuring the absorbance of acidic solution components at 275 nm and using a biochemistry kit (made in Iran) with a spectrophotometer (model UV/VIS-6505, Jenway Company, England) at a wavelength of 450 nm (Hastuti & Subabdiyono 2020).

Antioxidant activities

Catalase (CAT)

It was read and measured using the 96/84 Tests Catalase Activity (CAT) Assay kit (ZellBio GmbH, Germany) and the colorimetric method. The amount of absorbance was calculated using an ELISA reader (model RS232, BIOTEK, USA; Abhijith *et al*. 2016).

Glutathione Peroxidase (GPX)

It was measured using the Assay kit (96/48 Tests) Glutathione Peroxidase (GPX; ZellBio GmbH, Germany) by colorimetric method. The results were read using an ELIZA reader device (model RS232, BIOTEK, USA; Peglia & Valentine 1967).

Superoxide dismutase (SOD)

It was measured using the Super Oxide Dismutase (SOD) Assay Kit (96/48 Tests, ZellBio GmbH, Germany), by colorimetric method. The results were read using an ELIZA reader device (model RS232, BIOTEK, USA) (Abhijith *et al*. 2016).

Statistical analysis

The normality of the data in groups and replicates was checked by the Shapiro-Wilk test. One-Way ANOVA and Duncan's tests were run to compare the groups in the treatments due to the normality of the data. A multifactorial test was used to compare the reciprocal effects of feeding frequency and salinity on various indices of blood, immunity, and stress. All statistical analyses were carried out by SPSS version 20.

RESULTS

Growth indices

The results of the last biometric data showed that the final length, weight, percentage of weight gain, average daily growth, specific growth rate, average weight gain, and food efficiency increased by higher salinity and feeding frequency (Table 2). The average final weight and length in 6T +12‰ was significantly higher than in the other treatments, while the lowest final weight and size was observed in 2T + 0‰ (Table 2). The highest average CF rate was observed in 4T + 6‰ while the lowest in 6T + 0‰ and 6T = 6‰. Based on the one-way analysis of variance and Duncan's test, statistically significant differences were observed between treatments in all the above indices ($p < 0.05$). The average food conversion ratio in all treatments significantly decreased compared to 2T + 0‰. Based on the one-way analysis of variance and Duncan's test, all treatments exhibited statistically significant differences with 2T + 0‰ ($p < 0.05$), however, they did not differ with each other significantly ($p > 0.05$). Based on the multifactorial test of feeding frequency, salinity, and their interaction, the weight and length of young fish were improved while the food conversion ratio reduced ($p < 0.05$). Feeding frequency and the level of salinity separately influenced the percentage of weight gain, average daily growth, specific growth rate, average weight gain, and food efficiency ($p < 0.05$), however, their interaction did not affect these indices ($p > 0.05$). The frequency of feeding, the level of salinity, and their interaction did not influence the CF ($p > 0.05$; Table 2).

Liver enzymes (ALP, ALT and AST)

The results show that the amount of alkaline phosphatase (ALP) among the liver enzymes of blood serum in 2T + 0‰ was significantly higher than in the other treatments. The lowest level was observed in 6T + 6‰ and 6T + 12‰. Alanine aminotransferase (ALT) values in the blood serum of fish in 6T + 6‰, 2T + 12‰, 4T + 12‰, and 6T + 12‰ were higher than in the other treatments, while the lowest in 6T + 0‰. The amount of aspartate aminotransferase (AST) in fish blood serum was larger in 4T + 12‰ and 6T + 12‰ than in the other treatments, while the lowest in 6T + 0‰ and 4T + 6‰. Moreover, the statistical analysis with one-way analysis of variance and Duncan's test showed a statistically significant difference between the treatments ($p < 0.05$). The multivariate analysis test showed that the feeding frequency ($p < 0.05$), the level of salinity ($p < 0.05$), and their interaction ($p < 0.05$) influenced the concentrations of liver enzymes in blood serum of the fish (Table 3).

Digestive enzymes

The results showed that the digestive enzymes levels varied in different treatments, and the highest amylase level was observed in 2T + 6‰, while the lowest in 6T +12‰. On the other hand, the highest lipase level was recorded in 6T + 0‰, 2T + 6‰, and 4T + 12‰, while the lowest in 2T + 0‰ and 6T + 12‰. Based on ANOVA and Duncan's test, a statistically significant difference was observed between the enzymes levels among the treatments ($p < 0.05$). The maximum amount of protease was recorded in 2T + 6‰, 2T = 12‰, and 4T + 12‰, while the lowest in 2T + 0‰, 4T + 0‰, 6T + 6‰, and 6T + 12‰. A multi-factor analysis test showed that feeding frequency ($p > 0.05$) and salinity level ($p > 0.05$) did not affect lipase level, while their interaction ($p < 0.05$) influenced it. The feeding frequency ($p < 0.05$) and salinity ($p < 0.05$) and their interaction ($p < 0.05$) also influenced the amylase and protease levels (Table 4).

Antioxidant activities

The results showed that the amount of CAT in 2T + 12‰ was significantly higher than in the other treatments while the lowest in 6T + 12‰. The highest amount of glutamine peroxidase (GPX) was recorded in 4T + 12‰, while the lowest in 6T + 12‰. Furthermore, the maximum SOD was related to 6T + 0‰, 2T + 6‰, and 4T + 12‰, while the lowest to 6T + 12‰. Based on a one-way analysis of variance and the Duncan test, a statistically significant difference was observed between the values of antioxidant factors in treatments. Regarding the interaction effect of salinity and feeding frequency on the antioxidant activity based on the multifactorial analysis, the frequency ($p > 0.05$) and the salinity levels were not effective separately ($p > 0.05$), however, their interaction influenced antioxidant activity ($p < 0.05$; Table 5).

Table 2. The effect of salinity and feeding frequency on growth indices of stellate sturgeon juveniles.

Treatment	Final weight (g)	Final length (cm)	Condition factor (CF)	Food conversion factor	Body weight increase (% day^{-1})	Daily growth	Specific growth ratio (% day^{-1})	Feed efficiency
2T + 0‰	21.27 ± 1.45 dABC	1.39 ± 0.36 cABC	0.21 ± 0.009 ab	2.34 ± 0.05 aABC	110.99 ± 17.21 aAB	1.85 ± 0.28 cAB	1.19 ± 0.13 cAB	110.99 ± 17.21 bAB
4T + 0‰	29.23 ± 2.13 bcABC	23.42 ± 0.44 bABC	0.23 ± 0.01 ab	1.27 ± 0.09 bABC	171.17 ± 22.29 abAB	2.85 ± 0.37 abAB	1.61 ± 0.14 abAB	171.17 ± 22.29 abAB
6T + 0‰	26.51 ± 1.25 cABC	23.40 ± 0.35 bABC	0.21 ± 0.006 b	1.39 ± 0.02 bABC	143.44 ± 19.06 bcAB	2.39 ± 0.32 bcAB	1.44 ± 0.11 bcAB	143.44 ± 19.07 bcAB
2T + 6‰	28.43 ± 0.94 bcABC	23.41 ± 0.39 bABC	0.22 ± 0.006 ab	1.18 ± 0.007 bABC	162.93 ± 13.34 abAB	2.72 ± 0.22 abAB	1.59 ± 0.08 abAB	162.93 ± 13.34 abAB
4T + 6‰	29.53 ± 1.28 bcABC	22.99 ± 0.50 bABC	0.25 ± 0.002 b	1.11 ± 0.07 aABC	174.49 ± 11.78 abAB	2.91 ± 0.19 abAB	1.67 ± 0.07 abAB	174.49 ± 11.78 abAB
6T + 6‰	32.66 ± 0.81 abABC	25.70 ± 0.88 aABC	0.20 ± 0.06 b	0.93 ± 0.03 bABC	196.46 ± 8.03 aAB	3.27 ± 0.13 aAB	1.81 ± 0.04 aAB	196.46 ± 8.03 aAB
2T + 12‰	30.89 ± 1.68 bcABC	23.86 ± 0.50 bABC	0.23 ± 0.01 ab	1.08 ± 0.09 bABC	175.70 ± 18.69 abAB	2.93 ± 0.31 abAB	1.65 ± 0.1 abAB	175.71 ± 18.69 abAB
4T + 12‰	31.51 ± 1.51 abABC	24.49 ± 0.47 bABC	0.21 ± 0.005 ab	1.02 ± 0.08 bABC	189.15 ± 15.15 abAB	3.15 ± 0.25 abAB	1.74 ± 0.09 abAB	189.15 ± 15.15 abAB
6T + 12‰	35.46 ± 1.74 aABC	25.20 ± 0.44 aABC	0.22 ± 0.005 ab	0.87 ± 0.06 bABC	208.39 ± 13.008 aAB	3.47 ± 0.22 aAB	1.86 ± 0.007 aAB	208.39 ± 13.008 aAB

Non-shared lowercase letters indicate differences between treatments ($p < 0.05$). Letter A indicates the effect of the number of feeding times ($p < 0.05$), letter B shows the result of salinity ($P<0.05$), and letter C demonstrates the interaction of the number of feeding times and salinity.

Table 3. Changes in liver enzymes of stellate sturgeon juvenile fish in response to changes in salinity and feeding frequency

Treatments	Alkaline phosphatase (U L^{-1})	Alanine aminotransferase (U L^{-1})	Aspartate aminotransferase (U L^{-1})
2T + 0‰	505.33 ± 31.24 ^{aABC}	27 ± 2.30 ^{bABC}	285.67 ± 8.35 ^{cdABC}
4T + 0‰	433 ± 28.01 ^{abABC}	24.67 ± 0.33 ^{bcdABC}	264.33 ± 11.61 ^{deABC}
6T + 0‰	396 ± 2.89 ^{bcABC}	21 ± 0.58 ^{dABC}	243 ± 15.50 ^{eABC}
2T + 6‰	364.67 ± 3.76 ^{bcABC}	25.33 ± 1.45 ^{bcABC}	263.67 ± 7.86 ^{deABC}
4T + 6‰	422.67 ± 22.26 ^{abcABC}	21.67 ± 1.45 ^{cdABC}	247.33 ± 13.69 ^{eABC}
6T + 6‰	322 ± 26.27 ^{cABC}	31.33 ± 0.33 ^{aABC}	309.67 ± 3.53 ^{bcABC}
2T + 12‰	336.33 ± 26.87 ^{bcABC}	31 ± 1.15 ^{aABC}	306.33 ± 2.73 ^{bcABC}
4T + 12‰	370.67 ± 31.16 ^{bcABC}	31.33 ± 1.20 ^{aABC}	334.67 ± 12.55 ^{abABC}
6T + 12‰	327.67 ± 6.23 ^{cABC}	33.33 ± 1.20 ^{aABC}	353.67 ± 4.81 ^{aABC}

Non-shared lowercase letters indicate differences between treatments (P<0.05). Letter A indicates the effect of the number of feeding times ($p < 0.05$), letter B shows the result of salinity ($p < 0.05$), and letter C demonstrates the interaction of the number of feeding times and salinity.

Table 4. Alterations in the digestive enzyme levels of stellate sturgeon juveniles in different salinity treatments and feeding frequencies.

Treatments	Amylase (U L^{-1})	Lipase (U L^{-1})	Protease (U L^{-1})
2T + 0‰	35.67 ± 1.41 ^{cdABC}	11.60 ± 0.49 ^{eC}	15.33 ± 0.88 ^{cABC}
4T + 0‰	34.40 ± 0.42 ^{cdABC}	12.57 ± 0.46 ^{deC}	14.67 ± 0.88 ^{cABC}
6T + 0‰	38.10 ± 0.85 ^{bcABC}	17.77 ± 0.63 ^{aC}	18.67 ± 0.88 ^{bABC}
2T + 6‰	49.43 ± 3.98 ^{aABC}	17.07 ± 1.15 ^{abC}	22.67 ± 1.20 ^{aABC}
4T + 6‰	36.80 ± 2.17 ^{bcABC}	13.90 ± 0.35 ^{deC}	16.67 ± 0.88 ^{abABC}
6T + 6‰	33.77 ± 0.87 ^{cdABC}	13.23 ± 0.64 ^{abC}	14.33 ± 0.33 ^{cABC}
2T + 12‰	38.80 ± 0.65 ^{bcABC}	15.37 ± 0.29 ^{bcC}	22.33 ± 0.88 ^{aABC}
4T + 12‰	41.63 ± 0.80 ^{bABC}	17.60 ± 0.49 ^{aC}	23 ± 1.15 ^{aABC}
6T + 12‰	30.47 ± 0.58 ^{dABC}	11.97 ± 0.29 ^{eC}	14 ± 0.58 ^{cABC}

Non-shared lowercase letters indicate differences between treatments ($p < 0.05$). Letter A indicates the effect of feeding frequency ($p < 0.05$), letter B the result of salinity ($p < 0.05$), and letter C the interaction of feeding frequency and salinity.

Table 5. The response of the antioxidant activity of stellate sturgeon juveniles to changes in salinity and feeding frequency

Treatment	CAT (U L^{-1})	GPX (U L^{-1})	SOD (U L^{-1})
2T + 0‰	136.33 ± 1.76 ^{cdC}	263.33 ± 21.65 ^{eC}	43.33 ± 0.88 ^{abC}
4T + 0‰	142 ± 2.08 ^{abC}	284.33 ± 22.98 ^{deC}	44.67 ± 2.72 ^{abC}
6T + 0‰	143 ± 2.89 ^{abC}	348 ± 6.56 ^{abcC}	52 ± 0.58 ^{aC}
2T + 6‰	143.67 ± 1.45 ^{abC}	365.33 ± 15.05 ^{abC}	53.33 ± 0.88 ^{aC}
4T + 6‰	140 ± 2.30 ^{dbcC}	304.33 ± 5.20 ^{cdeC}	43 ± 0.58 ^{abC}
6T + 6‰	144.67 ± 1.45 ^{abC}	317.67 ± 19.94 ^{bcdC}	46.33 ± 2.19 ^{bC}
2T + 12‰	146 ± 0.58 ^{aC}	305 ± 10.41 ^{cdeC}	46.66 ± 0.88 ^{bC}
4T + 12‰	139 ± 1 ^{bcC}	369.33 ± 18.28 ^{aC}	52.67 ± 1.45 ^{aC}
6T + 12‰	132.67 ± 0.88 ^{dC}	274.67 ± 13.32 ^{deC}	41 ± 0.58 ^{cC}

Non-shared lowercase letters indicate differences between treatments ($p < 0.05$). Letter A indicates the effect of the feeding frequency ($p < 0.05$), letter B the result of salinity ($p < 0.05$), and letter C d the interaction of feeding frequency and salinity.

DISCUSSION

According to the results, raising the feeding frequency from 2 to 6 times a day and, at the same time elevating the salinity from zero to 12 ppt improved the growth performance and nutrition indices. The highest final weight, final length, percentage of body weight gain, average daily growth, daily growth rate, specific weight gain, and food efficiency were recorded in 6T + 12‰. Increasing in the feeding frequency and salinity affected the weight gain, so that by the simultaneous increase of meals and salinity, the weight and length of juveniles upraised ($p < 0.05$). Numerous investigations proved the effects of feeding frequency and salinity independently and in

combination, which also depend on species and breeding conditions. Giberson & Litvak (2011) evaluated Atlantic sturgeon, *A. oxyrinchus* and short-nose sturgeon, *A. brevirostrum* in Canada and found that increasing in the feeding frequency from one to four and eight times a day enhanced growth indices in short-nose sturgeon fish, however, no difference was recorded in Atlantic Sturgeon. Similarly, elevated feeding frequency of rainbow trout from two to eight times in cold seawater conditions in Turkey exhibited positive results (Türker & Yildirim 2011). Andrei *et al.* (2017) concluded that feeding frequency (one, two, three, and four times per day) displayed other effects on the growth and feeding performance of Russian sturgeon (*Acipenser gueldenstaedii*). Feeding once and twice a day had the best growth performance compared to three and four times daily. Zolfaghari *et al.* (2011) studied Persian sturgeon fingerlings, *Acipenser persicus* and reported that feeding four times a day exhibits better results than three times. Booth *et al.* (2008) stated that increasing the meal from 1 to 4 times a day enhanced the feeding efficiency and growth of the Australian snapper, *Pagrus auratus*. By comparing the treatments of 1, 2, 3, and 4 feeding times per day, Aderolu *et al.* (2010) reported that in cultured African catfish, 3 times per day exhibited the best growth performance.

The number of feeding times affects the growth performance, and the exact number of these times was variable depending on the fish species and probably the environmental conditions such as water temperature and salinity, which should be determined specifically for each species. Higher salinities on stellate sturgeon significantly upraised growth indices, which can exhibit different results in different species. Investigating on long-term salinity performance of 0, 10, and 20 ‰ revealed that the growth and survival rates during fish keeping were significantly reduced (Dawood *et al.* 2021). The effect of water salinity on performance and some physiological responses of yellowfin seabream *Acanthopagrus latus* 12.5 g and Barramundi, *Lates calcarifer* (33.5 g) and increasing salinity from 6 to 12 ‰ improved the growth performance of yellowfin seabream, and the increase of more than 12‰ gradually mitigated the growth of fishes (Torfi Mozanzadeh *et al.* 2021). In addition, elevated survival and specific growth rate (SGR) in salinities 7, 14, and 21 ppt were reported in American shad, *Alosa sapidissima* compared to the control group ($p < 0.05$; Liu *et al.* 2016). Notothenioid fish, *Eleginops maclovinus* of the Sub-Antarctic region had the highest growth rate at 15 ppt salinity during experiments at salinities of 5, 15, and 35 ppt, where the environmental salinity was close to the isosmotic point (Vargas-Chacoff *et al.* 2015). The weight gain and food conversion ratio were significantly affected by salinity following the comparison of the effects of 0, 5, 10, and 20‰ salinities on the growth of short-nose sturgeon (*A. brevirostrum*). Breeding fish in 0‰ salinity showed a significant elevation in weight and a better food conversion ratio than in the other salinities. Fish grown in 20‰ salinity showed the lowest growth performance. Therefore, salinity can impede the growth of short-nose sturgeon and indicates the importance of zero salinity in the commercial breeding operation of this species (Jarvis *et al.* 2001). Comparing the research results with earlier research showed that the change in feeding frequency and rearing environment (fresh and saltwater) can have different effects depending on the type of species and rearing conditions. Since the stellate sturgeon is anadromous, the brackish water of the Caspian Sea provided better conditions for the growth and development of this fish. Setting the feeding six times a day caused a significant difference in the growth indices with other treatments. Therefore, the Caspian Sea is a suitable habitat for breeding this fish and also the feeding management functions well.

The levels of liver enzymes (ALT and AST) increased by higher salinity and feeding frequency apart from ALP, was higher in 2T + 0‰ than in the other treatments. The highest levels of these enzymes were observed in 4T + 12‰. Any liver damage can increase these three main liver enzymes (ALT, AST, ALP) levels. When the metabolic process is abnormally slow, the risk of liver damage will be elevated (Rubenstein & Laine 2004). Although AST and ALT upraise in liver lesions, ALT was a more specific and defining enzyme. The results showed that the effect of salinity on liver enzymes was more significant than the feeding frequency. In a study by Dawood *et al.* (2021), long-term salinity performance of 0, 10, and 20‰ showed that fish grown at 20‰ and subjected to hypoxic stress exhibited the highest levels of ALT, AST, and ALP than in the other groups. Physiological responses of yellowfin seabream (*A. latus*) to acute salinity challenge included the cortisol and ALP elevation in all groups after 2 h ($p < 0.05$) and returned to the baseline value within 24 h (Farshadian *et al.* 2019). The effect of different salinities on American shad caused a significant elevation in the specific activity of ALP (Liu *et al.* 2016). Different levels of salinity can affect the activity of enzymes, and an increase in salinity can lead to more serum ALT (Shalaby 2005). The activity of serum enzymes in giant sturgeon, *Huso huso* raised in freshwater and brackish water ponds showed that salinity can elevate serum enzyme activity (Rajabipour *et al.* 2009). The logical procedure for the elevation of transferase enzymes is the release of aminotransferases from

damaged cells. The damaged cells release their content, including aminotransferase, into the bloodstream causing these enzymes to upraise in the serum (Martinez-Porchas *et al.* 2011). The results in the present study showed more amylase in 2T + 6‰ and 4T + 12‰, lipase in 6T + 0‰, 2T + 6‰, and 4T + 12‰, and protease in 2T + 6‰, 2T + 12‰, and 4T + 12‰ than in the other treatments. The amount of all three digestive enzymes in 6T + 12‰, was lower than in the other treatments. The number of feeding frequency and salinity levels did not affect the lipase level, however, their interaction influenced its concentration. On the contrary, the number of feeding times and the level of salinity and their interaction affected the amount of amylase and protease, since digestive enzymes (protease, amylase and lipase) indicated food consumption and growth differences (Gomez-Requeni *et al.* 2013). The activities of digestive enzymes among different fish species are affected by age, the amount and type of diet (Debnath *et al.* 2007), pH, and optimal temperature (Xiong *et al.* 2011).

The complete effectiveness of the whole digestive process mainly depends on the structure and function of digestive enzymes (Gisbert *et al.* 2009). Digestive processes and enzyme activities are usually synonymous indicating similar activities (Rungruangsak-Torrissenen *et al.* 2006). It has been proven that the relatively higher activity of digestive enzymes improves growth performance (Mohammadian *et al.* 2017). Thus, better growth performance in treatments with more frequency and higher salinity in this research shows the effect of digestive enzymes on growth indices. In terms of feeding, the frequency of 3 times and 4 times a day did not significantly affect the secretion of digestive enzymes in *Oreochromis niloticus* (Thongprajukaew *et al.* 2017). Among the digestive enzymes in *Acanthopagrus latus* cultured at 24 and 35‰ salinities, total protease activity was the highest, while its amount was the lowest in fish cultured at a salinity of 6‰ ($p < 0.05$). Lipase activity in yellowfin seabream gradually increased and then dropped with greater water salinity up to 35%. Further, total protease and lipase activities in *L. calcarifer* were elevated by more salinity from 6 to 24%, and then gradually decreased ($p < 0.05$). Liu *et al.* (2016), reported that different salinities on American shad, *A. sapidissima* caused the highest specific lipase activity in the control group ($p < 0.05$). Digestive proteases are synthesized as inactive zymogens in pancreatic cells and are activated by selective proteolysis when released in the intestinal lumen. Changes in water salinity may affect zymogen activation, as it occurs outside the cell boundaries in the intestinal lumen (Jobling, 1995). The effect of salinity on trypsin production and activity might act as a common regulatory mechanism for activating all zymogens (Moutou *et al.* 2004). The difference in the obtained results can primarily depend on the type of fish species. The GPX in 4T,12‰, 2T,6‰, and SOD in 6T,0‰, 2T,6‰, 4T,12‰, and CAT in 4T,12‰ had the highest amount. All three above enzymes were lowest in 2T,0‰ and 6T,12‰. In addition, the number of times of feeding and the amount of salinity alone did not affect the number of antioxidant enzymes, but their mutual effect did. There are antioxidant mechanisms in fish to counter the adverse effects of free radicals, such as enzymes related to antioxidant defense, including CAT, GPX, and SOD. These enzymes and other cell compounds create a defensive barrier against oxidative conditions that occur in breeding conditions and due to stress caused by the high density of aquatic animals (Speers-Roesch & Ballantyne 2005; Trenzado *et al.* 2006). CAT enzyme is found in peroxisomes and cell membrane organelles, which contain a variety of enzymes effective in metabolic reactions related to the stimulation of the antioxidant defense system.

CAT enzyme is a primary defense barrier against oxidants and facilitates the elimination of free radicals produced by H_2O_2 and metabolizes them into molecular oxygen and water (Hao & Chen 2012). Nutritional and environmental factors alter antioxidant activity in fish. Torfi Moazenzadeh *et al.* (2021) showed that the action of CAT in the liver of *A. latus* reduced with higher salinity, and the activities of GPX and SOD were gradually elevated by greater salinity in the liver ($p < 0.05$). In the case of *L. calcarifer*, CAT and GPX decreased significantly from 6 to 24%, and their activity gradually upraised from 35 to 48% ($p < 0.05$). However, SOD activity significantly rose from 6 to 24% and then fell significantly in its liver. Different salinities caused a significant elevation in SOD and CAT in American shad, *A. sapidissima* (Liu *et al.* 2016). With more salt, the antioxidant enzyme levels of Adriatic sturgeon, *Acipenser naccarii* also increased (Martinez-Alvarez *et al.* 2002). Starvation also augmented catalase and superoxide dismutase activity in Adriatic sturgeon and rainbow trout (Furne *et al.* 2009).

CONCLUSION

The results showed that growth indices improve by more salinity, and the highest weight gain was observed at 6T + 12‰. The highest amounts of digestive enzymes in the treatments with feeding frequency were simultaneously

higher with more frequent feeding. The highest antioxidant activity was also seen in the treatments with greater salinity. In contrast, liver enzymes varied across treatments.

The largest Osmoregulation indices were observed in treatments with higher salinity. Therefore, less stress is expected for stellate sturgeon in breeding with salinities close to the Caspian Sea water. This species can be bred in salinities as low as 12 ppt, and more frequent feeding helps this fish tolerate different environmental conditions.

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