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Effects of the Amplitude and Frequency of Salinity Fluctuations on the Growth Performance of Juvenile Tongue Sole (*Cynoglossus semilaevis*)

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Key words: salinity fluctuations, growth, food consumption, apparent digestibility, *Cynoglossus semilaevis*

Abstract

The effects of amplitude (2, 4, 6, 8 ppt) and frequency (2, 4, 8 days) of salinity fluctuations on the growth, body composition, and energy budget in juvenile tongue sole (*Cynoglossus semilaevis*) were investigated for 64 days. Results showed that the frequency and amplitude, as well as the interaction between them, significantly affected the specific growth rate. The tongue sole had higher specific growth rates at the amplitudes of 4 and 6 ppt and frequencies of 4 and 8 days than tongue sole in other treatments or the unfluctuating control. In these treatments, food consumption, food conversion efficiency, and apparent digestion in terms of energy were also significantly higher than in the control. The growth rate of the juvenile tongue sole was a quadratic function of the salinity amplitude at various frequencies and was described by the equation $G = \beta_0 + \beta_1(SA) + \beta_2(SA)^2$, where G represents the specific growth rate on a 64-day basis, SA is salinity amplitude in ppt, β_0 is the intercept on the G axis, and β_1 and β_2 are the regression coefficients, respectively. The optimal salinity amplitudes for the best growth at salinity fluctuation frequencies of 2, 4, and 8 days were estimated to be 3.54, 4.89, and 4.74 ppt, respectively, suggesting that commercial farmers can rear juvenile tongue sole in moderate salinity fluctuations to achieve better growth performance.

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Introduction

Salinity is one of the most important abiotic factors affecting the growth of aquatic organisms and has complex and wide-ranging biological effects (Brett and Groves, 1979; Gaumet et al., 1995; Kumlu et al., 2000; Boeuf and Payan, 2001). In intensive systems where salinity can be altered, commercial interests determine the optimum salinity level for each fish species. The idea that aquatic organisms grow better under constant salinity rather than fluctuating salinity is generally accepted and therefore most aquatic organisms are cultured in water of a constant salinity. However, fluctuating salinities can positively influence the growth of aquatic organisms (Stroganov 1962; Konstantinov and Martynova, 1993).

Tongue sole (*Cynoglossus semilaevis* Güther, 1873), Actinopterygii, Cynoglossidae, is a commercially important and rare native fish species in the Bohai, Yellow, and East China Seas of China, and inhabits coastal waters and estuaries (Masuda et al., 1984). However, the wild population has sharply decreased due to over-fishing and annual output is now less than one ton (Jiang and Wan, 2005). Tongue sole has become an important indoor intensively-cultured species in China due to its high nutritional and economic value. Research on tongue sole has focused on food habits and life history cycles, breeding, and embryonic development (Dou, 1993, 1995a,b; Du et al., 2004; Liu et al., 2005; Ma et al., 2005, 2007), karyotype, genetic diversity (Zhuang et al., 2006; Liao et al., 2007; Liu et al., 2007, 2008, 2010), influence of water temperature and ration (Fang et al., 2010), and compensatory growth (Tian et al., 2010). The objective of this study was to examine the effects of frequency and amplitude of fluctuating salinities on growth and food consumption in tongue sole.

Materials and Methods

Fish acclimation. Juvenile tongue sole were provided by Mingbao Aquatic Product Co., Ltd., Yantai, P.R. China, and transferred to the laboratories at the Aoshanwei Research Centre of Ocean University of China. They were acclimated to seawater (30 ppt) in a 1000-l fiberglass tank for at least one week before the experiment during which they were fed commercial pellets (Guangzhou Yuequn Technology Co., Ltd, P.R. China) to satiation twice a day at 07:00 and 18:00 (Table 1).

Experimental design. The effects of three fluctuating frequencies (every 2, 4, or 8 days) and four fluctuation amplitudes (2, 4, 6, or 8 ppt) were compared to an unfluctuating control. The salinity of the control was a constant 30 ppt. An example of a 4-day salinity fluctuation of 6 ppt is shown in Fig. 1. There were five replicates for each treatment, and each replicate had six juveniles. After acclimation, fish were starved for 24 h to evacuate the gut. Normal healthy fish were anesthetized using MS-222 (60 mg/l) to reduce stress, weighed, and assigned to one of the 65 rectangular glass aquaria (55 × 30 × 35 cm) used in the experiment.

On day 1 of the experiment, all fish were held at the control salinity (30 ppt). Salinity was subsequently changed as per the experimental design. The fish were fed as described for acclimation above. During the experiment, 30% of the water in each aquarium was replaced every other day to ensure suitable water quality. Temperature, pH, and ammonia nitrogen were monitored daily and were 18-22°C, 8.0, and <0.2 mg/l, respectively. A simulated natural photoperiod (14 h light:10 h dark) was maintained.

To obtain water of the desired salinity, seawater was filtered through a sand filter, and low-salinity water was made by diluting the sand-filtered seawater with fully aerated tap water. High-salinity water was made by adding sea salt to the sand-filtered seawater. Moderate

Table 1. Chemical (%) and energy (KJ/g) contents of commercial pellets fed to tongue sole juveniles.

Protein[1]	54.25
Ash[2]	<16.00
Fat[2]	≥12.00
Moisture[3]	≤4.12±0.03
Fiber[2]	<3.00
Calcium[2]	>2.30
Phosphorus[2]	>1.50
Energy[4]	15.36±0.11

[1] according to N × 6.25; N content determined by Vario ELIII Elemental Analyser (Elementar, Germany)
[2] provided by manufacturer
[3] after oven-drying to a constant weight at 70°C
[4] measured by PARR1281 Calorimeter (PARR Instrument Co., USA)

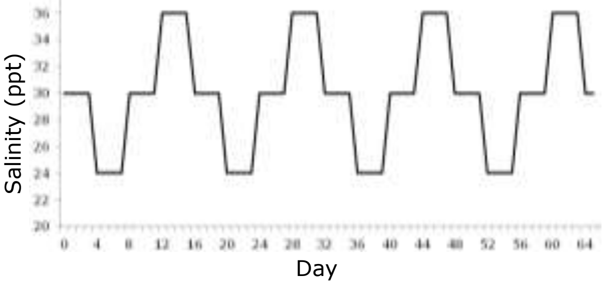

Fig. 1. Example of salinity fluctuation of 6 ppt every 4 days. The pattern was similar for frequencies of 2 and 8 days, and amplitudes of 2, 4, and 8 ppt.

aeration was continuously provided to maintain dissolved oxygen above 6 mg/l, stopped 30 min before collecting feces and uneaten food, and resumed immediately after collection. Food consumption was calculated as the difference in dry weight between the amount of food supplied and amount of uneaten food.

Data calculation. The experiment was conducted from September 27 to December 1, 2011. At the end of the experiment, all fish were starved for 24 h, weighed, and dried at 70°C. Specific growth rates in terms of weight gain (SGR_w; %/d) and energy (SGR_e; %/d) were determined as $100(\ln W_t - \ln W_0)/t$ and $100(\ln E_t - \ln E_0)/t$, where W_t and W_0 are the final and initial wet body weights in g, E_t and E_0 are the final and initial energy contents in the fish body, and t is the feeding duration in days. Relative specific growth rate (RSGR; %) was determined as $100[SGR(s + \Delta s) - SGRs]/SGRs$, where SGRs is the SGR at 30 ppt, Δs is the amplitude of salinity fluctuation, and $s + \Delta s$ indicates the fluctuating salinity.

Food conversion efficiency in terms of weight (FCE_w; %) and energy (FCE_e; %) were determined as $100(DW_t - DW_0)/C_w$ and $100(E_t - E_0)/C_e$, where DW_t and DW_0 are the final and initial dry body weights in g, and C_w and C_e are food intakes in g. Apparent digestibility rates in terms of weight (ADR_w; %) and energy (ADR_e; %) were calculated as $100(C_w - F_w)/C_w$ and $100(E_t - E_0)/E_t$, where F_w is total fecal production in g (Sun et al., 2006).

The effects of fluctuating salinity amplitudes and frequencies on SGR were tested using the quadratic polynomial function: $G = \beta_0 + \beta_1 (SA) + \beta_2 (SA)^2$ where G represents the specific growth rate on a 64-day basis, SA is salinity amplitude in ppt, β_0 is the intercept on the G axis, and β_1 and β_2 are the regression coefficients.

Statistical analysis. Statistical analysis of the data was performed with a statistical package (SPSS 16.0 for Windows, SPSS Inc., Richmond, CA, USA). The assumption of homogeneity of variances was tested for all data, which were log-transformed if necessary. The interaction between salinity amplitude and fluctuations on growth and food consumption was tested using two-way analysis of variance (ANOVA). Significant ANOVA was followed by Duncan's multiple comparison to determine differences between treatments. Differences were considered statistically significant if $p<0.05$.

Results

There were no mortality, health disturbances, or alterations in behavior in any treatment. There were no significant differences in intial body weight between treatments (Table 2). Two-way ANOVA showed that both the frequency and the amplitude of salinity fluctuation significantly affected the final weights of the tongue sole juveniles ($p<0.01$) and that there was a significant interaction between frequency and amplitude of salinity fluctuations ($p<0.01$). The fish in the 8 day/±6 ppt treatment attained the highest final body weight. Food consumption increased with increased frequency and amplitude of salinity till it reached an optimum level. However, there was no interaction between frequency and amplitude of salinity.

Two-way ANOVA analysis showed that frequency and amplitude of the salinity fluctuations significantly affected the specific growth rates ($p<0.01$) and that there was a significant interaction between the frequency and amplitude of salinity. When both SGR_w and SGR_e were considered, frequencies of 4 and 8 days and amplitudes of 4 and 6 ppt produced significantly higher growth rates than the control and other treatments (Fig. 2). The relative specific growth rate differed with frequency and amplitude (Fig. 3).

Table 2. Growth and feed efficiency in tongue sole (*Cynoglossus semilaevis*) reared in salinity fluctuations occurring at different intervals (days) and amplitudes (±ppt), means±SE.

Salinity changes in...		*Weight (g)*		*Food conversion efficiency (%)*		*Apparent digestibility rate (%)*		*Food consumption (g/d/ind)*
Frequency (days)	*Amplitude (ppt)*	*Initial*	*Final*	*Weight*	*Energy*	*Weight*	*Energy*	
Control		7.55±0.27	17.39±0.70[bc]	93.73±1.29[ab]	18.97±0.52[b]	76.19±1.73[ab]	58.11±0.11[b]	0.14±0.01[a]
2	2	7.41±0.19	17.52±1.24[bc]	89.31±5.61[ab]	15.60±0.99[a]	82.62±1.42[c]	56.23±2.29[b]	0.14±0.00[a]
2	4	7.59±0.14	17.45±0.94[bc]	93.43±9.13[ab]	19.54±1.82[b]	81.94±3.64[bc]	59.55±1.18[b]	0.15±0.01[ab]
2	6	7.60±0.18	16.13±0.88[b]	94.08±5.21[ab]	22.81±1.28[b]	80.38±2.83[bc]	59.56±0.95[b]	0.14±0.00[a]
2	8	7.77±0.17	14.91±0.67[a]	81.73±5.02[a]	14.15±0.91[a]	79.47±1.45[b]	44.24±1.48[a]	0.14±0.01[a]
4	2	7.43±0.09	17.21±1.09[bc]	101.34±5.79[bc]	21.96±1.20[b]	79.32±2.67[b]	63.07±0.50[b]	0.15±0.01[ab]
4	4	7.53±0.02	18.72±0.76[c]	108.98±2.98[c]	27.53±0.68[c]	81.90±3.47[c]	68.29±0.52[bc]	0.16±0.00[b]
4	6	7.57±0.07	18.55±1.13[c]	109.87±4.84[c]	30.43±1.36[c]	76.44±1.49[ab]	68.90±0.50[bc]	0.16±0.01[b]
4	8	7.77±0.09	15.02±0.27[a]	87.60±3.63[ab]	19.05±0.79[b]	79.71±1.45[b]	52.40±0.40[ab]	0.14±0.01[a]
8	2	7.49±0.17	17.57±0.69[c]	102.60±5.14[bc]	25.22±1.20[bc]	79.62±1.51[b]	64.81±0.72[b]	0.16±0.00[b]
8	4	7.62±0.18	19.37±0.90[c]	110.21±3.31[c]	31.88±0.83[c]	74.52±1.14[a]	71.21±0.41[c]	0.17±0.01[bc]
8	6	7.50±0.10	19.42±1.15[c]	112.23±2.01[c]	33.59±0.47[c]	74.34±1.86[a]	72.88±0.80[c]	0.18±0.01[c]
8	8	7.56±0.20	15.05±0.44[a]	98.42±3.91[b]	25.31±0.94[bc]	78.61±1.03[b]	58.06±0.38[b]	0.15±0.01[a]

Data in a column with different superscripts significantly differ (Duncan's multiple comparisons, $p<0.05$) from other treatments at the same frequency.

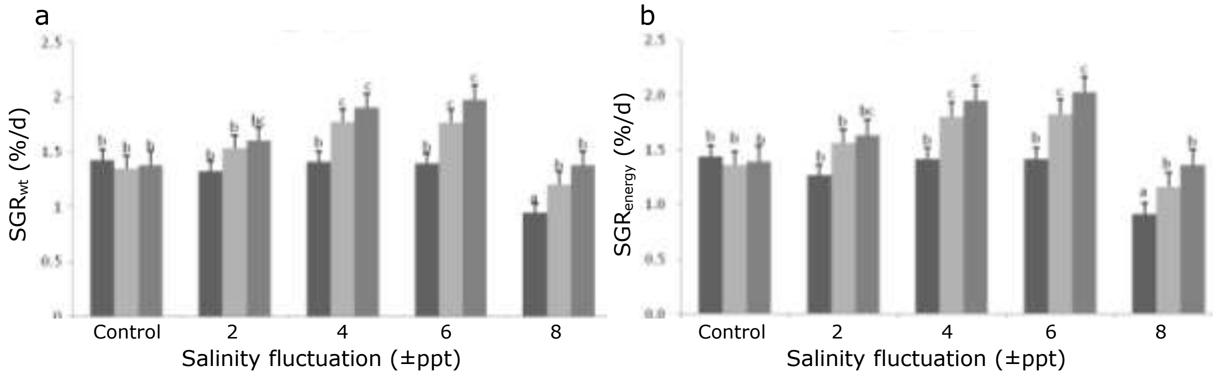

Fig. 2. Specific growth rate in terms of (a) wet weight (SGR_w; %/day) and (b) energy (SGR_e; %/day) of tongue sole (*Cynoglossus semilaevis*) reared in salinity fluctuations occuring at different intervals (day) and amplitudes (ppt) during a 64-day experiment. Means with different letters indicate significance differences ($p<0.05$). Error bars represent standard errors. 2d 4d 8d

Equations for regression relationships of specific growth rates to salinity amplitudes at different fluctuation frequencies show that the optimal salinity amplitude for growth at fluctuation frequencies of 2, 4, and 8 days is 3.54, 4.89, and 4.74 ppt, respectively (Table 3).

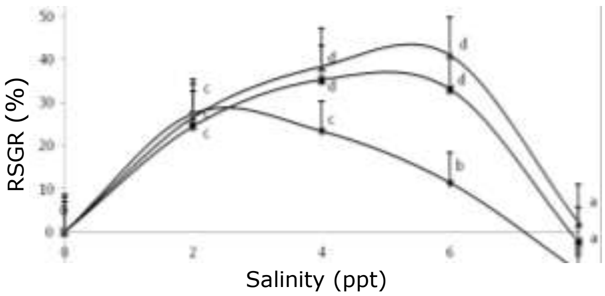

Fig. 3. Relative specific growth rate (RSGR) in terms of body weight of juvenile *Cynoglossus semilaevis* raised in salinity fluctuations at different intervals (days) and amplitudes (ppt). Error bars represent standard errors. 2 d 4 d 8 d

Food conversion efficiency. Food conversion efficiencies in terms of wet weight (FCE_w) and energy content (FCE_e) were significantly affected by frequency and amplitude of salinity but there was no significant interaction between frequency and amplitude (for FCE_w, $p = 0.854$; for FCE_e, $p = 0.923$). FCE at 8 ppt was significantly lower than at other amplitudes, while there were no significant differences in FCE at 2, 4, or 6 ppt.

Table 3. Regression relationships* between specific growth rate (G) of tongue sole (*Cynoglossus semilaevis*) and salinity amplitudes (SA; in ppt) at different fluctuation frequencies.

Frequency (days)	β_0	Std. error	β_1	Std. error	β_2	Std. error	R^2	*F*	*P*
2	1.080	0.042	0.138	0.025	-0.020	0.003	0.745	24.810	<0.05
4	1.035	0.037	0.201	0.022	-0.025	0.003	0.843	45.612	<0.05
8	1.031	0.036	0.218	0.021	-0.026	0.003	0.862	53.280	<0.05

* $G = \beta_0 + \beta_1(SA) + \beta_2(SA)^2$, where β_0 = intercept on specific growth rate axis and β_2 = regression coefficient

Apparent digestibility. The apparent digestibility rates ranged 74.34-82.62% in terms of weight and 44.24-72.88% in terms of energy and were significantly affected by the frequency and amplitude of salinity fluctuations (for ADR_w, $p<0.05$; for ADR_e, $p<0.01$). There was no significant interaction between frequency and amplitude on ADR_w or ADR_e. ADR at two-day intervals was higher than at 4 or 6-day intervals or in the control, while there were no significant differences between days 4 and 8.

Discussion

While the idea that aquatic organisms grow better under constant salinity is generally accepted, the positive influence of salinity fluctuations on the growth of aquatic organisms have been reported in Russian sturgeon, *Acipenser gueldenstaedti* (Stroganov, 1962) and juvenile Russian sturgeon, *A. gueldenstaedti*, common carp, *Cyprinus carpio*, and white amur, *Ctenopharyngodon idella* (Konstantinov and Martynova, 1993). Salinity fluctuations of 0.2 ppt considerably accelerated the growth of juvenile fish in comparison to their growth in either freshwater or water with a salinity of 2 ppt (Stroganov, 1962; Konstantinov and Martynova, 1993). Tongue sole is euryhaline and able to adapt to a wide range of salinities (0-40 ppt) in a short time period (Tian et al., 2011). In the present study, fluctuations of 4-6 ppt at frequencies of 4 and 8 days resulted in significantly higher growth rates than the constant salinity of 30 ppt. However, the fluctuation amplitude of 8 ppt and frequency of 2 days did not improve the growth beyond that of the control; on the contrary, it seemed that this combination of frequency and amplitude reduced growth. Thus, the general conclusion that salinity fluctuations produce higher or lower growth rates than a constant salinity depends not only on amplitude but also on the frequency of the fluctuations.

Investigations probing into the effects of salinity fluctuations on growth in aquatic animals have produced variant results. In *Litopenaeus vannamei* juveniles maintained in flutuations of 5 ppt every 4 days, food consumption and growth were higher than in shrimp maintained at a constant 20 ppt or other levels (Feng et al., 2008). The digestive gland weight of *Marsupenaeus japonicus* increased after a salinity change, suggesting that salinity changes may affect digestibility and thereby affect growth (Marangos et al., 1989). The present study indicates that fluctuating frequencies and amplitudes of salinity significantly affect food consumption, food conversion efficiency, and even apparent digestion rates of juvenile tongue sole. The best growth performance (final weight, SGR), food consumption, food conversion efficiency (FCE_w, FCE_e), and apparent digestion rate in terms of energy content of the fish body (ADR_e) were obtained with the amplitudes of 4 and 6 ppt and the frequencies of 4 and 8 days.

Better growth performance is always the aim of aquaculture farmers. According to results from the present study, moderate salinity fluctuations of 4-6 ppt at a frequency of 4-8 days improve growth in juvenile tongue sole without a significant decrease in food conversion or digestion rate. Thus, commercial tongue sole farmers could obtain the fastest growth by culturing the fish indoors or in ponds near estuaries or creeks where they could take advantage of tidal salinity fluctuations. This information can be utilized in farm site selection and salinity maintenance to maximize commercial productivity.

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