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Effect of Two Substrate Types on Feeding Efficiency by Juvenile Chinese Sturgeon *Acipenser sinensis* on Barcheek Goby *Rhinogobius giurinus*

Xiaolian Gu[1], Jianyi Liu[2,3], Sikai Wang[2,3], Tao Zhang[2,3], Feng Zhao[2,3*], Ping Zhuang[2,3*], Ming Duang[4*], Boyd Kynard[5]

[1]Shanghai Natural History Museum, Shanghai Science & Technology Museum, Shanghai, China
[2]East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai, China
[3]Scientific Observing and Experimental Station of Fisheries Resources and Environment of East China Sea and Yangtze Estuary, Ministry of Agriculture of China, Shanghai, China
[4]Key Laboratory of Breeding Biotechnology and Sustainable Aquaculture, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China
[5]BK-Riverfish, LLC and Environmental Conservation Department, University of Massachusetts, Amherst, MA, USA
Email: Xlgu_1972@hotmail.com, *zhaof@ecsf.ac.cn, *pzhuang@ecsf.ac.cn, *duanming@ihb.ac.cn

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Abstract

We conducted experiments in an artificial stream tank with wild juvenile Chinese sturgeon *Acipenser sinensis* captured in the Yangtze Estuary to test the null hypothesis that their feeding efficiency on Barcheek goby *Rhinogobius giurinus* was not affected by sand vs. smooth glass substrate. Gobies are among the most common prey eaten by wild juvenile *A. sinensis* in the estuary. Test results found neither substrate type significantly affected feeding efficiency by juveniles. Previous research found a strong innate habitat preference of *A. sinensis* for sand substrate. The present results indicate that the preference for sand is not related to efficiently capturing *R. giurinus* on sand, but is an adaptation predisposing juvenile *A. sinensis* to seek sandy substrate where *R. giurinus* and other benthic forage are the most abundant in the Yangtze Estuary.

Keywords

Acipenser sinensis, Feeding Efficiency, Sand Substrate, Behavior

1. Introduction

Chinese sturgeon *Acipenser sinensis* is a Category I (nationally protected) species in China, and IUCN (International Union for the Conservation of Nature) lists the species as critically endangered [1]. *A. sinensis* has a complex life history with adults historically spawning in the upper reaches of the Yangtze River in fall and larvae and juveniles taking about 6 months to migrate downstream to the Yangtze Estuary [2]. Migrant juveniles arrive at the estuary beginning in mid-May and remain to forage for 4 - 5 months (early-May to late-September) before migrating to the sea [3].

The Yangtze Estuary provides the migration route to the sea and an important feeding ground for juvenile *A. sinensis* [4]. Juveniles in the estuary are mainly distributed on the sandy bottom in the brackish-water zone [2] [3]. Gobies, amphipoda, and polychaetes are the top three main foods of juvenile in the Yangtze Estuary; however, gobies are the forage with the greatest index of relative importance IRI = 1856.99; IRI = {[mass percentage (%) + number percentage (%)] × frequency (%) × 10^4} and percent of weight (50.54%), number (28.13%), and frequency (51.85%) [5].

In a previous study, we found juvenile preferred sand substrate over gravel or rubble [6]. Several species of sturgeons also prefer or mostly occur over sand substrate [7]-[11]. Sand substrate of the Yangtze Estuary has a great biomass of benthic animal (5.47 $g \cdot m^{-2}$ and 28.33 $ind \cdot m^{-2}$ in summer) [12]. Thus, do juvenile *A. sinensis* prefer sand because they have a high feeding efficiency on sand substrate compared to other substrate types or because sand contains the greatest abundance of forage?

The literature suggests that substrate type affects feeding of sturgeons. Experiments conducted in glass bottom aquaria found feeding of juvenile lake sturgeon, *A. fulvescens*, was poor on silt substrate [9]. Further, decreased specific growth rates and increased mortality were found for young cultured green sturgeon, *A. medirostris*, reared on sand substrate [13]. Information on foraging and substrate type is important for conservation of wild, in the dredged Yangtze Estuary, where deposition of dredge spill alters natural substrate. In the present study, we tested the null hypothesis that sand vs. smooth glass substrates had no effect on feeding efficiency by juvenile *A. sinensis* on *R. giurinus.*

2. Materials and Methods

2.1. Test Fish

Juvenile *A. sinensis* used in the study were by-catch, collected with gill nets in the Yangtze Estuary in the summer, 2006, by fisherman. Juveniles were transported to the Laboratory of the Administration Agency of Shanghai Yangtze Estuarine Natural Reserve for Chinese Sturgeon, Shanghai, China. Prior to tests, juveniles were held in six rectangular fiberglass tanks (length × width × height = 2 m × 1.5 m × 1 m), supplied with aerated and filtered water (26˚C - 29˚C) pumped from the Yangtze Estuary.

During rearing, juvenile fish were fed 1.5% of fish weight twice each day with an aquatic worm, *Limnodrilus hoffmeister*, plus a dry commercial pellet diet (crude protein ≥ 50%, crude fat ≥ 10%, crude ash ≤ 16% and crude fibre ≤ 2%). Uneaten food and feces were removed twice each day. Water quality was DO ≥ 7 mg·l^{-1} and ammonia ≤ 1 mg·l^{-1}. The local natural day length (photoperiod) was provided to test fish for Chongming Island, China (N: 31.73, E: 121.40). Water temperature was the natural temperature (range, 26˚C - 29˚C; ±1˚C in a day) and was monitored daily in all tanks.

One replicate of 10 fish was chosen to measure fish size (TL and body weight) after testing in order to avoid disturbing fish. Juveniles varied little in size (Mean ± SD TL = 27.3 ± 3.8 cm) and (Mean ± SD body weight = 80.3 ± 4.7 g). *R. giurinus* was collected using a seine net in the Yangtze Estuary. Size of *R. giurinus* tested follows: TL = 2.0 - 2.5 cm. This same size range of *R. giurinus* was found in the stomach of wild juvenile *A. sinensis* captured at Yangtze Estuary [5]. *R. giurinus* were held before testing in a holding tank (200 L) supplied with additional air. After tests, the surviving *R. giurinus* were released in the estuary.

2.2. Test Tank and Procedure

Feeding tests were carried out in a long oval fiberglass stream tank (length: width: height = 300 cm: 60 cm: 60 cm; **Figure 1**). Water flow was created by a water pump connected to the tank with a 5-cm diameter pipe 10 cm above the bottom. Water velocity was 18 - 35 cm·s^{-1} (mean = 31 cm·s^{-1}) measured at five positions 50 cm apart from each other on each side of the tank.

During experiments, light intensity on the bottom was 260 lux (low daylight intensity) provided by two 40 watt fluorescent lamps 2 m above the tank. The test tank was isolated from disturbance by humans with a black adiaphanous curtain. The glass substrate was tested first; then, a 1-cm-thick layer of sand was placed over the entire tank bottom, and tests were conducted on the effect of sand substrate on predation of *R. giurinus* by juvenile *A. sinensis*.

Because of the high level of protection for *A. sinensis*, our experiments were limited to only 60 juveniles. We tested the 60 in groups of 10 fish per replicate: 30 fish (three replicates) tested for foraging over sand and 30 fish (three replicates) tested for foraging over glass. To minimize holding stress to fish, all *A. sinensis* and *R. giurinus* in each replicate were quickly released to the estuary after testing.

A smooth glass bottom artificial substrate is not present in the Estuary, which has only silt, sand, and a mixture of these substrates. All these substrates provide

Figure 1. Plan view of test tank used for feeding efficiency tests. Key: A = water pump; B = pump regulator; C = Arrows showing inflow location and current direction around the tank. (During sand substrate test, 1-cm thick sand was placed over the entire tank bottom.)

a smooth top surface. Because we wanted to evaluate the role of sand alone as it affected foraging by juvenile *A. sinensis* on *R. giurinus*, any smooth substrate would have been acceptable to test vs. sand.

Prior to testing, fish were not fed for 48h. At the beginning of each test replicate, a group of 10 juveniles was randomly selected from a tank with 60 *A. sinensis*. The 10 fish were removed from the holding tank by netting, and then, fish were introduced together into the test tank. After a 2 h acclimation period for test juveniles, 60 *R. giurinus* were introduced into the test tank with juveniles.

Duration of each feeding test was 4 h (7:00-11:00 hours) to prevent the possible effect of a diel foraging rhythm on feeding intensity. When each test finished, juveniles were returned to the holding tank by net. The water in the test tank was drained and the number of surviving *R. giurinus* was counted. During tests, no Chinese sturgeon was injured or died from handling. All were released later per direction of the Shanghai Yangtze Estuarine Natural Reserve for Chinese Sturgeon.

2.3. Data Analysis

The feeding consumption (Fc) was used to index feeding efficiency of juvenile Chinese sturgeon on Barcheek goby [2] [3]:

$$Fc = N_{gt0} - N_{gt1}$$

In the formula, N_{gt0} is the number of *R. giurinus* introduced before the experiment; N_{gt1} is the number of surviving gobies after the test.

Normality test was carried out by Lilliefors Test. If normality test is failed ($\alpha < 0.05$), then the Mann-Whitney Test was used to test significance of the difference of Fc between the two treatment groups (sand vs. glass) [14]. All statistics tests were run with SPSS17.0 software. All result showed with mean ± standard deviation (M ± SD) and significant level was $\alpha < 0.05$.

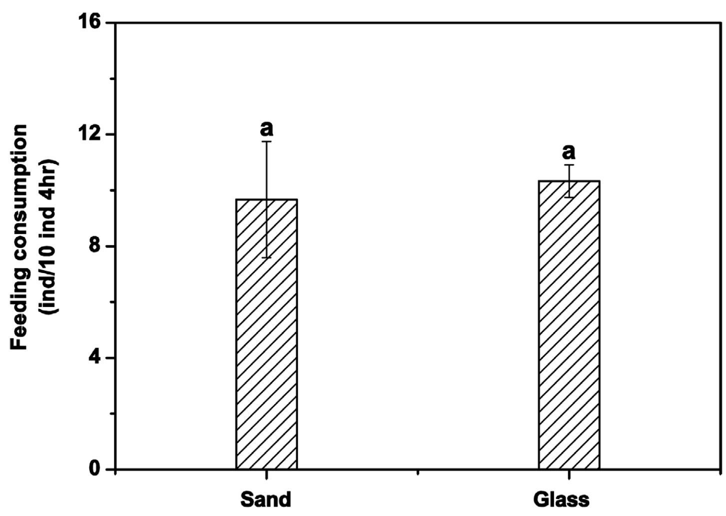

Figure 2. Feeding consumption of juvenile *A. sinensis* on gobies on sand and glass substrate (mean ± SD, n = 3, Mann-Whitney Test, $P = 0.10$). Same letters are not significantly different from each other.

3. Results

The trials showed juvenile Chinese sturgeon feeding consumption on gobies per 4 hours was a mean of 9.67 ± 2.08 gobies eaten on sand and a mean of 10.33 ± 0.58 eaten on glass (mean ± SD, n = 3, **Figure 2**). There was no significant difference in mean number of gobies eaten on sand vs. glass (Mann-Whitney Test, $P = 0.10$).

4. Discussion

There is no field data on density of juveniles during foraging to guide selection of the number of juveniles to test together, nor is there data on density of wild *R. giurinus* that we could use to simulate the natural situation. Historically, there were likely thousand or tens of thousands of juveniles foraging individually on gobies. (No observations indicate *A. sinensis* juveniles forage in groups.) Thus, there were likely many juveniles foraging on many gobies during each high tide, so density of juveniles could have been high, but likely never as great as the number of gobies, which in 2004 was 17.2 g of gobies per 100 m^2 of substrate in the summer and autumn [3] [5]. According to stomach content analysis of juveniles, there were 5 - 6 individual (goby or shrimp) in each stomach of the average *A. sinensis* juvenile in the Estuary [15]. Thus, foraging data suggested experiments should contain 1 juvenile *A. sinensis*: 6 *R. giurinus* or 10 juvenile Chinese sturgeons: 60 Barcheek gobies. This ratio would likely keep the predator: prey density in the correct general proportions of few juveniles to a far greater number of *R. giurinus.*

In a previous study, we found juvenile significantly preferred sand substrate when given a choice of four substrates (numbers indicate particle diameter): sand (<0.02 cm), small gravel (1.0 - 2.0 cm), medium gravel (4.0 - 5.0 cm), and rubble (13.0 - 15.0 cm) [16]. There are several papers that also show a preference for sand substrate by juvenile or adult sturgeons, including *A. fulvescens* [7] [9]; *A. transmontanus* [8]; *A. gueldenstaedtii*; *A. brevirostrum* [10]; and *Scaphirhynchus albus* [16]. Peake tested juvenile *A. fulvescens* preference for sand, gravel, rock or smooth plastic bottom and found a strong preference for sand [7]. He concluded the preference was likely innate, which is consistent with the conclusion of Gu *et al.* [6]. Preference for sand substrate has been inferred with foraging of many sturgeon species [13], but the advantages of foraging on sand (if any) are not documented.

In the laboratory, Peake found that juvenile *A. fulvescens* preferred sand substrate (not gravel, rock, or smooth plastic) regardless of food distribution or availability [7]. The author suggested that preference for sand is an instinctive behavior for *A. fulvescens*, but he provided no evidence on the adaptive significance of the preference for sand. The present results suggest the strong preference for sand substrate by juvenile *A. sinensis* found by Gu *et al.* [6] which is not related to a greater feeding efficiency for *R. giurinus* on sand, which indicates that sand substrate may be a clue for sturgeons of great forage abundance, so juveniles innately seek sandy substrate where forage abundance is greatest. In the Yangtze Estuary, juvenile *A. sinesnsis* are mainly distributed on sandy-bottom shoals [3]. Sandy bottom shoals have a rich benthic biomass with >20.14 g and 16.46 g per 100 m^2 in summer and autumn, respectively, in which Gobies accounted for 86.0% (17.32 g) and 91.3% (15.03 g), respectively [5]. Gobies is the top prey of juvenile *A. sinensis* in the Estuary with the highest index of relative importance (IRI = 1856.99) and percent of weight (50.54%), number (28.13%) and frequency (51.85%) [2] [5].

The effect of substrate type on feeding efficiency of cultured sturgeons is poorly studied. Cultured *A. medirostris* fed a commercial trout pellet on slate-rock, cobble, sand, and glass substrates found feeding on sand did not result in the greatest specific growth rate and swallowed sand was thought to cause a negative effect on growth and survival [13]. Sand is also found in the gut of juvenile *A. sinensis* in the Yangtze Estuary [5]. Swallowing sand during feeding by wild *A. sinensis* might cause a decrease in foraging efficiency slightly, although we did not find a statistical difference between foraging efficiency by *A. sinensis* feeding on sand or fiberglass. For feeding efficiency of cultured *A. sinensis*, we suspect sand or glass or other smooth tank bottoms would make no difference in feeding efficiency on pellet food, but this hypothesis should be tested.

Our report on the effect of sand substrate on feeding efficiency by juvenile sturgeon is the first to demonstrate that, although have a strong preference for sand, the preference is not because foraging on sand gives an advantage during feeding on *R. giurinus*. Instead, the preference for sand seems likely to be an innate habitat preference that predisposes *A. sinensis* to seek sand habitat, where abundance of and other forage is greatest. Because the protected has a strong preference for sand substrate to optimally forage, dredging and disposal of dredged spoils in the Yangtze Estuary should be managed carefully to minimize the impact on critical habitat. The inter-tidal shoreline around Chongming Island used for foraging by juvenile sturgeon should be considered for special designation relative to dredging.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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