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Using drift nets to capture early life stages and monitor spawning of the Yangtze River Chinese sturgeon (*Acipenser sinensis*)

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Summary

A sampling system for capturing sturgeon eggs using a D-shaped bottom anchored drift net was used to capture early life stages (ELS) of Chinese sturgeon, *Acipenser sinensis*, and monitor annual spawning success at Yichang on the Yangtze River, 1996–2004, before and just after the Three Gorges Dam began operation. Captured were 96 875 ELS (early life stages: eggs, yolk-sac larvae = eleuthero embryos, and larvae); most were eggs and only 2477 were yolk-sac larvae. Most ELS were captured in the main river channel and inside the bend at the Yichang spawning reach. Yolk-sac larvae were captured for a maximum of 3 days after hatching began, indicating quick dispersal downstream. The back-calculated day of egg fertilization over the eight years indicated a maximum spawning window of 23 days (20 October–10 November). Spawning in all years was restricted temporally, occurred mostly at night and during one or two spawning periods, each lasting several days. The brief temporal spawning window may reduce egg predation by opportunistic predators by flooding the river bottom with millions of eggs. During 1996–2002, the percentage of fertilized eggs in an annual 20-egg sample was between 63.5 to 94.1%; however, in 2003 the percentage fertilized was only 23.8%. This sudden decline may be related to the altered environmental conditions at Yichang caused by operation of the Three Gorges Dam. Further studies are needed to monitor spawning and changes in egg fertilization in this threatened population.

Introduction

Chinese sturgeon, *Acipenser sinensis*, is a large anadromous sturgeon species with historical natal populations in the Yangtze and Pearl rivers (Bemis and Kynard, 1997; Xiao et al., 2006). Human activities have likely caused extirpation of the Pearl River population. Construction of the Gezhouba Dam, which was completed in 1981 at Yichang on the Yangtze River, caused a great decline in the population by preventing pre-spawning adults from reaching most of the historical spawning habitats (Wei et al., 1997, 2005; Chang and Cao, 1999; Wei, 2003; Yang et al., 2006). Because of the decline in abundance and threats to Chinese sturgeon, the species was listed as a first class protected animal by the Chinese national government in 1989.

Some adults continue annual migration upstream in the Yangtze River and spawn at the Yichang reach just downstream of Gezhouba Dam (Wei et al., 1997; Chang, 1999; Wei, 2003; Xiao et al., 2006). Spawning has been documented for many years by examining the stomachs of copperfish, *Coreius heterodon*, and other bottom fishes that eat sturgeon eggs (Hu et al., 1985; Hu et al., 1992). Examining the stomach contents of predatory fish revealed the periods in which sturgeon spawn; egg predators are likely a strong selective factor with regard to spawning strategy. Although the number of eggs in predatory fish stomachs was used to develop egg abundance indices (Deng et al., 1991; Hu et al., 1992; Chang, 1999) and to estimate the potential number of females on the spawning grounds (Chang, 1999), the results were controversial because the state of digestion caused uncertainties of egg counts. Egg digestion in a predator's stomachs also made it impossible to determine egg quality, developmental stage and the percent of eggs that were fertilized. Tracking annual egg quality and fertilization could be important to evaluate changes in the pre-spawning and spawning environments of sturgeon caused by anthropogenic influences such as the Three Gorges Dam (TGD).

Monitoring the spawning success of Chinese sturgeon before and after TGD operation is important because of the potential of drastic environmental changes created by the dam that may greatly affect adult staging and spawning as well as development of the eggs. The Yichang reach, which is located directly downstream from Gezhouba Dam, is only 45 km downstream from TGD. Operation of TGD will have a major influence on the Yichang reach by reducing discharge by about 41% during October–November (Anonymous, 1996; Chang and Cao, 1999), which is the spawning season of Chinese sturgeon. Further, controlled propagation and release of juveniles since 1983 has increased recruitment <5% (Yang et al., 2005); thus, natural spawning by adults at Yichang is critical to the continuation of the population.

Fertilized eggs of Chinese sturgeon are among the largest of any sturgeon (mean diameter = 4.1 mm). They are highly adhesive, sticking initially to any structure, and typically hatch after about 5 days at ambient river temperatures (YARSG, 1988; Chen, 2004). Laboratory observations found that hatching yolk-sac larvae have a strong downstream dispersal using swim-up and drift behaviour that lasts about 7 days (Zhuang et al., 2002). This behaviour moves young fish many kilometers away from the egg deposition site. Thus, sampling at and just downstream of a spawning site can likely capture two of the early life stages (ELS), e.g., eggs and yolk-sac larvae.

In China, capturing ELS with nets has been used to evaluate natural reproduction of fish with pelagic eggs, but the technique has not been used for fishes such as sturgeon that spawn in swift currents in the main stems of river bottoms. Fish such as black carp *Mylopharyndodon piceus*, grass carp *Ctenopharyngodon idellus*, silver carp *Hypophthalmichthys molitrix*, and bighead carp *Aristichthys nobilis*, spawn pelagic eggs that drift with the current and are easily captured by nets (Yi and Liang, 1964; Yi et al., 1988). During 1981–1983, attempts to collect Chinese sturgeon ELS at Yichang used bottom excavation and drift netting, but the results of this major effort were poor (Yu et al., 1986).

Capturing sturgeon ELS with a variety of drift nets set downstream of spawning sites has been used in several rivers to document spawning, identify the general spawning reach, or to estimate production of ELS of sturgeon species. Taubert (1980) used circular plankton nets (bottom-set and off the bottom) to capture shortnose sturgeon *Acipenser brevirostrum* ELS. Yu et al. (1986) captured a few yolk-sac larvae of Chinese sturgeon with drift nets set about 30 km downstream from the spawning grounds. D-shaped bottom drift nets have been used to capture ELS of lake sturgeon *Acipenser fulvescens* (Kempinger, 1988), white sturgeon *Acipenser transmontanus* (McCabe and Tracy, 1994), and shortnose sturgeon *A. brevirostrum* (Kieffer and Kynard, 1996). However, these studies do not provide guidance on the techniques and effort needed to capture ELS in large main stems like the Yangtze River. We are unaware of any study published in the peer-reviewed literature that presents details of fishing with D-nets in a large, main river artery.

Based on the success of tracking acoustic-tagged pre-spawning Chinese sturgeon at Yichang in 1993 (Kynard et al., 1995; Wei et al., 1998), we subsequently tagged and tracked pre-spawning adults each year from 1996–2004. Telemetry was used to track movements, and determine spawning locations and habitats (Wei, 2003; Yang et al., 2006). In the present paper, we report on techniques used to capture ELS and verify spawning, as well as present new information on spawning timing, the diel pattern, and estimate the percent fertilization and hatching rates of the embryos.

Materials and methods

Study area

Telemetry tracking in previous studies identified the spawning grounds as the 7 km of river downstream from Gezhouba Dam to Yanzhiba Islet (Kynard et al., 1995; Wei et al., 1997; Wei, 2003; Yang et al., 2006; Fig. 1). During the tracking studies, most spawning occurred in the 4-km reach upstream of Miaozui; we therefore sampled for ELS in this reach during the present study. We created a sampling grid for egg capture by dividing the reach into cross-river transects (I, II, III…) and longitudinal transects (A–D). We sampled for ELS in grid cells A–C in transects II–V (Fig. 1).

River discharge decreases in the autumn at Yichang (Jiang et al., 2007; Yang et al., 2007). During the October–November spawning season of Chinese sturgeon, width of the river at Yichang is 560–1660 m and the mean flow is 17 981–9874 $m^3\ s^{-1}$ (Anonymous, 1996). Water depth varies in this area between 15–25 m, depending on bottom topography, except for a 41-m deep depression in transect II-C. The riverbed in the main channel is strewn with rocks and gravel; clusters of large boulders are present at some locations. During October–November of 1996–1999, the average surface velocity (1 m deep) was 2.0 $m\ s^{-1}$ and the average velocity 50 cm above the bottom was 1.42 $m\ s^{-1}$ (Yang et al., 2007).

Drift nets

A single D-shaped net with a 2-mm mesh size was used in 1996. This net had been used previously to capture ELS of shortnose sturgeon, *A. brevirostrum* (Kieffer and Kynard, 1996). Two nets with a smaller mesh (0.75 mm) were used in all subsequent years. These nets were identical in other respects to the net used in 1996. Nets were 5 m long, 0.86 m wide (maximum), and the net mouth area was 0.58 m^2. A D-shaped steel rod (maximum height, 0.5 m) supported the mouth. A second heavy steel bar (80-cm long, 25 kg) was attached to the lowest part of the net mouth to keep it close to the bottom. A mechanical current meter (accuracy 15.0 cm sec^{-1}, Model 2030R, General Oceanics, Inc.) was attached to the center bar in the net mouth to record current speed, enabling us to calculate the water volume sampled. A collection jar (10 cm diameter) was attached to the end of the net.

The overall design of the D-net set with anchor, lead lines, and surface buoy is shown in Fig. 2. A 50-m long line (about 3× water depth) was connected to the buoy. All lines used in the setup were 30 mm in diameter. The lead line from the D-net to the anchor chain was 30 m long. The anchor chain was 50 mm diameter and the anchor weighed 10 kg. Buoy volume was 200 L and the buoy had a flag to warn commercial traffic.

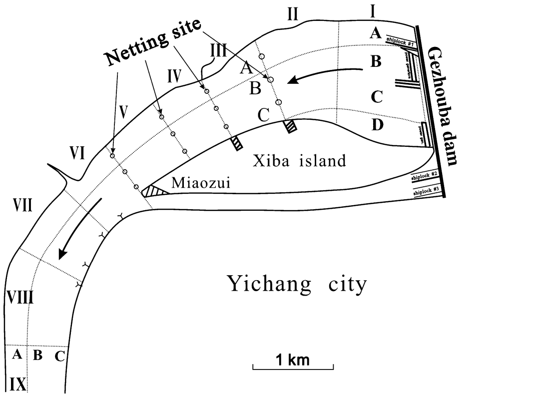

Fig. 1. Map of Yichang spawning reach of Chinese sturgeon (*A. sinensis*), Yangtze River. Sampling Strategy I: variable net setting, depending on monitored fish activity to locate spawning site; Sampling Strategy II: net sets fixed at transects at cross-sections II–V to monitor long-term presence

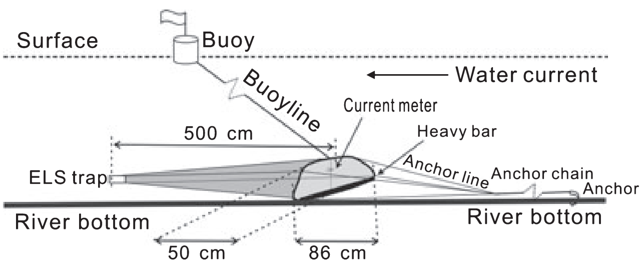

Fig. 2. Drawing of a D-shaped drift net set on riverbed showing anchor, lead lines, float, etc. to capture Chinese sturgeon ELS. Nets were 5 m long with a mouth area of 0.58 m^2

Setting and hauling nets

Two independent types of information signaled that spawning was about to occur and that we should begin setting the nets. The two signals were: 1) a more active behavior by the acoustically-tagged adults in the spawning reach, and 2) a dramatic increase in the catch of predatory fish on and near the spawning grounds (Deng et al., 1991; Wei et al., 1997). As soon as one or both of the signals occurred we started to set the nets.

Two strategies were used for setting the nets. Series I netting used nets set about 50–200 m directly downstream of the estimated location of an acoustically-tagged fish that localized its movements (and, potentially, was spawning). These nets were designed to determine the spawning site of adults. After deployment of these nets, which were usually fished for 2 h, we placed nets in several upstream transects to determine the upstream limit of spawning activity during the first day of possible spawning. After these (Series I) strategically-placed net sets, then Series II nets were set to the left, center and right of the Series I set location, in the center of the transect cell to sample ELS from a wide area and monitor ELS that were present for the long-term. Series II nets were usually set for 2 h, with the nets hauled in to check the catch at 5–15 min intervals. In summary, Series I net sets were in A–C cells of transects I–V; Series II net sets were in the center of cross-river transects II–V. GPS was used to determine the exact location of all nets (latitude and longitude; accuracy at about 15 m) and net locations were plotted on a grid map of the area.

Due to the danger of sampling at night in this commercial reach of the river, nets were only fished from 07.00 to 18.00 hours. The only exception was in 2002, when we set nets at night on the first day of spawning.

The process of setting and hauling the D-nets was as follows: We tracked an acoustically tagged sturgeon from an 80-tonne working boat. The fish tracker directed the boat so that when the boat was directly upstream of the tracked fish, the boat turned sharply to the right or left, slowed its upstream movement, and as the boat ceased upstream movement the D-net anchor was cast off from the bow. The boat slowly drifted downstream to set the anchor. After the D-net anchor was set, a four to six person crew carried the lines and D-net to the bow and lowered the D-net into the water. When the net was oriented correctly, the buoy line and buoy were cast off. We hauled in the nets by approaching the buoy from downstream, catching the buoy line, and pulling the buoy on board. The boat continued upstream with the crew pulling the buoy line and eventually hauling the D-net on board. Lastly, the crew pulled the net anchor on board.

We recorded the set time for each D-net and the reading of the velocity meter reading in the net mouth. After the net was on board, we recorded the velocity meter reading, the haul time, and removed ELS from the net. We only used data from effective netting, i.e. from net sets when the net was fished correctly, the velocity counter operated correctly, etc.

Data collected on ELS

We photographed, counted, and sorted captured ELS by development stage as eggs or yolk-sac larvae Some ELS were stored in formalin or 95% ethanol for future study, the number stored depending on the number caught. To determine spawning time (estimated hour of egg fertilization), we back-calculated the fertilization time using the developmental stage of 10–30 captured ELS and river temperature, employing a timetable of developmental stages developed by Chen (2004). We also calculated the percent of fertilized eggs by randomly examining ≥ 20 eggs from catches on the second day after spawning. When the total number of eggs captured was < 20, we examined all eggs from all samples to obtain as large a sample as possible. The number of net samples was usually three. Unfertilized eggs of most fish stop development after 20 h (Chen, 2004), thus we used only eggs which developed beyond middle gastrulation (20 h old). Captured eggs were reared in tanks on the workboat, and transported the yolk-sac larvae to the Hatchery for Chinese Sturgeon, Yangtze River Fisheries Research Institute. We returned all reared fingerlings to the river. All samplings of ELS and the incubation and rearing of captured ELS were approved by the national government and supervised by local aquatic wildlife management authorities.

Estimate of egg density at sampling sites

We divided the number of ELS captured per 1000 m^3 of water sampled by the total number of nets to obtain a density estimate (number of eggs per net). This density estimate was done for each net and combined with similar values for captures by other net samples to compare values among the various net samples from different transects, days, and years. This was done to gain an understanding of the variability among the number of eggs at different transects, days, and years.

Results

Capture summary

We set 363 effective nets for 52 503 min (875 h 3 min); 201 nets captured a total of 96 875 ELS (Table 1). Most were eggs (n = 94 398; 97.4%) and only 2477 ELS were yolk-sac larvae.

ELS distribution

Spatial distribution of ELS captures (observed density) across transects shows that most were captured in the center and inside transects (Fig. 3). Most ELS were captured in the B and C sections of transects II–V. Capture of many ELS in transect I-B occurred only in 2 years.

Spawning period

ELS were captured as early as 20 October and as late as 12 November (Fig. 4). Thus, females have a maximum spawning window of 23 days.

In 4 years (1996, 1999, 2001, and 2002), there were two spawning periods: first and second (Fig. 4). Spawning may have also occurred twice in 1997 and 2000, but we did not collect ELS in a second period. During a spawning period, the number of days we captured eggs varied from 3 to 10 days. In 50% of the years, the daily observed density of ELS decreased with each additional day of sampling. Typically, after spawning began, we collected eggs for 4–6 days; thereafter we captured the first yolk-sac larvae. We only captured yolk-sac larvae for 1–3 days, showing that they had departed.

In the 4 years when spawning occurred during two periods, there was a large yearly variation for observed density of ELS

Table 1
Summary of Chinese sturgeon (*A. sinensis*) early life stages (ELS = eggs and yolk-sac larvae) captured in bottom D-shaped drift nets, Yichang reach, 1996–2004 during years with one or two spawning periods. Two strategies of net sampling: Strategy I = locate spawning areas; Strategy II = sampling a larger area and monitor long-term presence of ELS. Dates in 1997 and 2000 with no catch data reflect days when no sampling was possible due to adverse river conditions, but spawning occurred (sturgeon eggs were observed in copperfish stomachs)

Year / spawning period	Sampling type	Sampling period Start date	End date	Sampling time (min)	Number of net samples	Number of net samples with ELS	Number of ELS captured
1996 / (1)	I	20 Oct.	21 Oct.	1366	4	2	20
	II	21 Oct.	24 Oct.	2779	6	3	37
1996 / (2)	I	27 Oct.	28 Oct.	1127	4	3	13665
	II	28 Oct.	3 Nov.	1028	6	5	8905
1997 / (1)	I	22 Oct.	22 Oct.	649	4	4	4245
	II	22 Oct.	28 Oct.	2126	24	15	575
1997 / (2)	I	18 Nov.	–	–	–	–	–
	II	–	–	–	–	–	–
1998	I	26 Oct.	26 Oct.	545	4	3	727
	II	27 Oct.	31 Oct.	3441	22	18	22 594
1999 / (1)	I	27 Oct.	28 Oct.	224	4	4	8414
	II	28 Oct.	6 Nov.	3125	34	26	4277
1999 / (2)	I	13 Nov.	13 Nov.	354	4	3	26
	II	13 Nov.	15 Nov.	555	10	4	64
2000 / (1)	I	18 Oct.	–	–	–	–	–
	II	–	–	–	–	–	–
2000 / (2)	I	2 Nov.	2 Nov.	563	4	2	460
	II	2 Nov.	9 Nov.	3709	36	11	538
2001 / (1)	I	21 Oct.	21 Oct.	343	4	2	535
	II	21 Oct.	26 Oct.	2989	6	3	1126
2001 / (2)	I	9 Nov.	9 Nov.	492	4	3	53
	II	9 Nov.	14 Nov.	3420	6	5	73
2002 / (1)	I	27 Oct.	27 Oct.	762	5	4	1343
	II	27 Oct.	5 Nov.	5924	44	21	4552
2002 / (2)	I	9 Nov.	9 Nov.	806	7	3	107
	II	9 Nov.	15 Nov.	5036	36	18	217
2003	I	6 Nov.	6 Nov.	453	4	2	7387
	II	6 Nov.	13 Nov.	7811	57	28	16 897
2004	I	12 Nov.	12 Nov.	358	4	1	1
	II	13 Nov.	16 Nov.	2518	20	8	37
Total				52 503	363	201	96 875

and a weak trend for more spawning in the first period (Table 1, Fig. 4; $F_{[1,11]} = 0.56$, $P > 0.05$). In 1996, we sampled for only a few days and most net sets were of Sampling Strategy I, during the peak of spawning (10 008 eggs were captured in one net in 105 min). The observed densities of ELS in other years were from a few Sampling Strategy I sets and many Sampling Strategy II sets. Thus, data from 1997 and later years sampled egg abundance over many transects when compared to 1996.

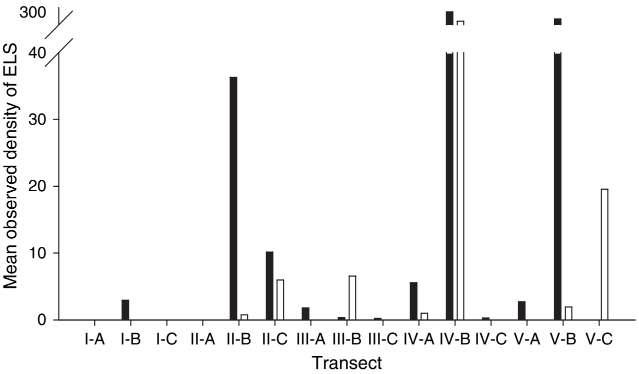

Fig. 3. Spatial distribution and abundance of *A. sinensis* early life stages (ELS) captured at Yichang, 1997–2003. Mean observed density of ELS (number of ELS per 1000 m^3 water sieved) captured in all years in each transect cell shown during both spawning periods. Solid bars = first spawning period; open bars = second spawning period

A summary of observed density of ELS for first vs second spawning periods was: first spawning period mean was 103.1 eggs (range, 0.64–334.4 eggs) for 1996–1999 and 2001–2004; the second period mean was 4.9 eggs (range, 0.048–8.782 eggs) for 1996 and 1999–2002. The first vs second period means were not significantly different (*t*-test, $P > 0.05$), probably because of the great variability among years within both categories.

Diel spawning

Back-calculation of spawning time using fertilization time of eggs showed that most spawning in the first spawning period was at night or early morning (23.00–07.00 hours; Fig. 5). Spawning during the second period peaked at mid-day (11.00–15.00 hours), with weak spawning in the afternoon (17.00 hours) and early night (22.00–24.00 hours).

Percent of fertilized eggs

The mean percent of fertilized eggs during 1996–2004 varied from a high of 94.1% in 1996 to a low of 23.8% in 2003 (Fig. 6). The low percentage of fertilized eggs in 2003 and 2004 suggests a trend toward decreasing fertilization of eggs after 2002.

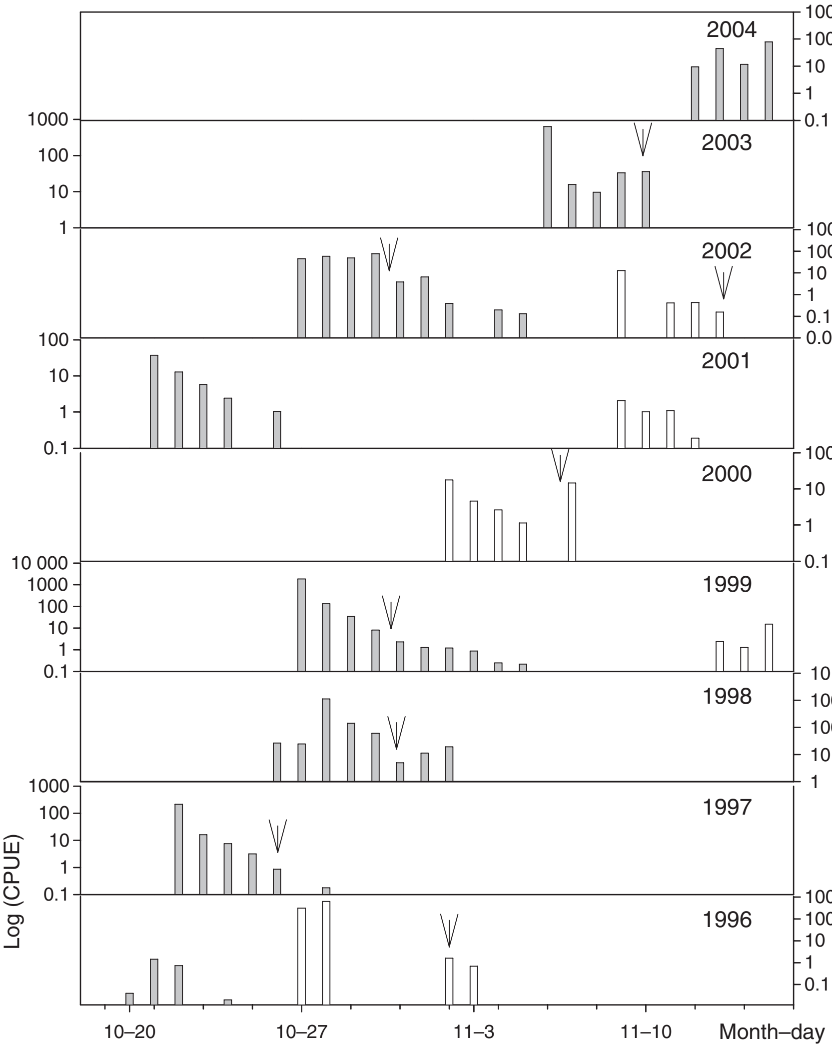

Fig. 4. Daily change of observed density of ELS (number of ELS per 1000 m^3 water sampled) of Chinese sturgeon (*A. sinensis*) eggs during 1st and 2nd spawning periods, 1996–2004. Arrows indicate the day each year when the first yolk-sac larva was captured. Solid bars = first spawning period; open bars = second spawning period

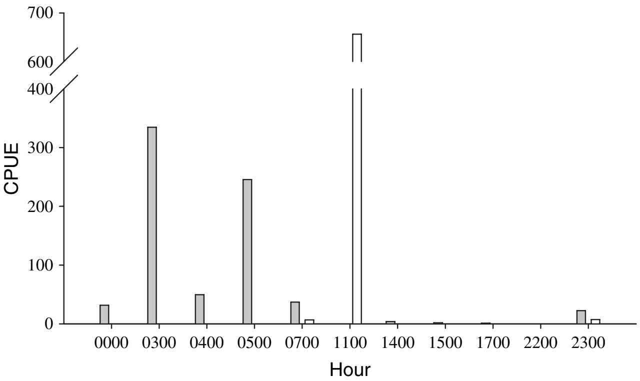

Fig. 5. Diel timing of spawning by Chinese sturgeon (*A. sinensis*) as determined by back-calculation of hour of egg fertilization using captured ELS, 1996–2004. Each vertical bar is the result of all back-calculated samples. Solid bars = first spawning period; open bars = second spawning period

Egg hatchability

Hatchability of eggs was about 70%. For example, in 1997, 74.5%, 68.2% and 68.6% of the eggs from three net sampling periods hatched. In contrast, the percent hatching from samples in 2003 (when the percent of fertilized eggs was the lowest) was only 10.7%. Both egg and total ELS survival was low in 2003 compared to other years.

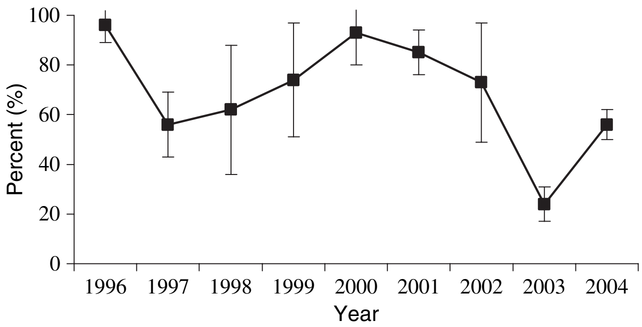

Fig. 6. Percent fertilization of sample of about 20 Chinese sturgeon (*A. sinensis*) eggs collected on estimated second day of spawning each year, 1996–2004. Data points = weighted mean; vertical lines = Standard Deviation

Discussion

For the first time in China fertilized eggs of Chinese sturgeon were successfully captured with drift nets in the main stem of the Yangtze River. The sampling methods used in the present study should be suitable for obtaining a quantitative sample of ELS of other species of Acipenseriformes in the main stem of large rivers. Additionally, the methods are probably suitable for other fishes in China that spawn demersal eggs (adhesive or non-adhesive) on the riverbed, including Chinese sucker *Myxocyprinus asiaticus*, and southern catfish *Silurus meridionalis*.

When direct sampling ELS, a spawning event that was overlooked was discovered by observing the eggs eaten by copperfish. During 1996–2004, in addition to sampling for ELS with D-nets we also collected copperfish and found sturgeon eggs in their stomachs (Wei, 2003). In 1996, D-net sampling noted spawning that was not detected by examining stomachs of egg predators, thus D-net sampling provided a more rigorous detection of sturgeon spawning.

Other sampling methods have been used to capture sturgeon ELS. A wire sieve set at the spawning site was used to capture ELS (Paragamian and Wakkinen, 2002; Paragamian et al., 2002; Caswell et al., 2004). Selecting the location to place the wire sieve was based on telemetry tracking of spawning fish, the same technique that we used. If a less quantitative estimate of spawning is desired (presence vs absence), fiber pads can be laid on the riverbed downstream of likely spawning sites and later examined for eggs (Sulak and Clugston, 1999).

The few days of capturing yolk-sac larvae at the spawning area support the results of laboratory studies that found hatchling yolk-sac larvae (= free embryos) swim up into the water column and disperse downstream (Zhuang et al., 2002). Thus, both laboratory and field studies show that a strong dispersal occurs after hatching.

Based on the maximum spawning window of 23 days, the appropriate conditions for spawning are only needed for about three weeks during any year. Because pre-spawning adults remain downstream of the spawning area for months maturing gametes, this staging environment is also critical to successful maturation of gametes and successful fertilization of mature eggs.

Data on the diel spawning pattern time are scant for most sturgeon species, but all female shortnose sturgeon, *A. brevirostrum*, observed in an artificial spawning stream began spawning at night (Kynard et al., 2009). Most Chinese sturgeon females also initiated spawning at night. Why Chinese sturgeon spawn at night is not clear, because natural light at Yichang does not penetrate more than several centimeters into the water column (B. Kynard, unpubl. data) and thus fish are in any case without light for 24 h a day. Because the great loss of eggs due to predators is well documented, one reason for nocturnal spawning could be related to releasing eggs at an optimal time to avoid egg predation. To test this hypothesis, the diel feeding patterns of egg predators, particularly copperfish, must be understood.

We documented that there were two spawning periods in 4 of 9 years. This phenomenon was also found for Chinese sturgeon using another method, i.e. checking the stomachs of predatory fishes (Wei et al., 1997). Two spawning periods were also found for lake sturgeon using D-shaped drift nets (Kempinger, 1988; Auer and Baker, 2002). Two spawning periods may reduce fish predation on eggs.

The data suggest that fertilization and hatchability of Chinese sturgeon eggs began to decrease after the TGD began operation. Understanding the effects of TGD on temperature regime and reduced turbidity and flow and how these factors affect the sturgeon maturation process and reproductive success is unknown, but could be critical to insuring continued spawning success of adults. Our monitoring of spawning by Chinese sturgeon before operation of the TGD provides important information on spawning, abundance of eggs, fertilization and quality of eggs, during pre-TGD river conditions. These data should help managers evaluate future impacts of the TGD dam on maturation, spawning, and early rearing of Chinese sturgeon. As has been repeatedly suggested in recent years by many sturgeon experts (Rosenthal et al., 2006), monitoring of spawning success in modified river systems of importance for sturgeon spawning should be continued because this is the best way to quickly evaluate the status of the population. These studies should also be augmented with appropriate water quality sampling and laboratory studies to understand the total impact of the altered river environment at Yichang on Chinese sturgeon reproduction (pre-spawning, spawning, and early rearing).

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