

## Article

## Relationship between the Distribution of Broodstock and Vorticity of Spawning Grounds of Four Major Chinese Carps in the Middle Reaches of the Yangtze River during Ecological Operation of the Three Gorges Dam

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Abstract: Hydrodynamic characteristics of spawning grounds are important factors affecting the spawning of four major Chinese carps (Mylopharyngodon piceus, Ctenopharyngodon idella, Hypophthalmichthys molitrix, and Aristichthys nobilis). To investigate the relationship between the preferred hydrodynamic characteristics of spawning sites and the response of fish spawning behavior, we monitored the flow field of spawning sites during ecological operation of the Three Gorges Dam (i.e., man-made flood regulation) in 2014 and 2015. We used the data to explore the correlation between vorticity changes in spawning grounds and the spawning amount. Pearson correlation coefficients of the average vorticity in all cross-sections of the Yidu spawning ground and spawning amount in 2014 and 2015 were 0.730 and 0.822, respectively, indicating a significant positive correlation between vorticity and spawning activity. In some specific regions, this correlation was even stronger (Pearson correlations of the regional vorticity and egg production were >0.95). To further corroborate and analyze the relationship between these specific regions and the distribution of broodstock during the breeding season, an ultrasonic telemetry test of broodstock was conducted in the Yidu spawning ground in 2016. The results showed that the broodstocks were concentrated in the reach near the Quantong Pier (~76 km from the Three Gorges Dam). These regions were consistent with areas of river characterized by highly correlated vorticity and egg production levels, suggesting that these regions are areas preferred by four major Chinese carps for spawning.

**Keywords:** ecological operation; four major Chinese carps; spawning ground; vorticity; hydrodynamics; ultrasonic telemetry; Yangtze River

## 1. Introduction

Black carp (*Mylopharyngodon piceus*), grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*), and bighead carp (*Aristichthys nobilis*) are collectively referred to as



the four major Chinese carps (FMCC) and are economically important freshwater fish in China. The Yangtze River is the main habitat and breeding ground of FMCC. However, since the 1980s, the flow discharge, topographical characteristics, and ecosystem pattern of the Yangtze River have changed, mainly as a result of anthropogenic activities, such as regulation of the Yangtze River, water pollution, and overfishing, especially the construction of large-scale water conservancy projects, such as the Gezhouba Dam and the Three Gorges Dam. This has resulted in a continued decline in the productivity of Yangtze River fisheries, particularly of FMCC following impoundment of the Three Gorges Reservoir in 2003 [1]. In 2009, fewer than 100 million eggs were laid at Jianli [2], compared with 115 billion during the 1960s (of which 40.1 billion were monitored in Jianli [3]. Therefore, the safety of FMCC germplasm resources in China is currently under serious threat.

Spawning is the most critical part of the life history of FMCC, which can only lay eggs in specific areas and under specific conditions. From May to July of each year, when the water temperature is >18 °C, mature fish spawn in curved, sandy, rocky or narrowed reaches of rivers when the river water is at a suitable height. The main factors influencing spawning are the number of broodstock, water temperature, the water level, and the spawning ground [4]. In an attempt to reverse the decline in productivity of FMCC in the Yangtze River, the Ministry of Agriculture has been releasing the parents of each species in the middle reaches of the river since 2010. Since 2011, the Three Gorges Corporation has also carried out ecological operations to promote the breeding of FMCC, preliminarily solving the problem of the reduction in broodstock and the weakening of rising water processes during spawning periods [5,6]. However, less is known quantitatively about how the characteristics of the spawning grounds (e.g., their hydrodynamic characteristics) affect the breeding of these fish [4].

The hydrodynamic characteristics of spawning grounds are important factors in triggering the spawning behavior of FMCC. The hydrodynamic characteristics in the spawning grounds of FMCC have been reported to be qualitatively described by macroscopic vorticity, which could promote the spawning of FMCC [3,7]. Many studies have shown that such turbulence is closely related to the activities of the fish. Some researchers found that, along a hinged concrete bank used for revetment on the lower reaches of the Mississippi River, small vortices generated by trenches on the surface provided a flow-vehicle obstruction zone for fish, resulting in a correlation between the foraging area and surface flow area in terms of the stream structure [8]. Crowder et al. proposed vorticity and circulation as features for quantifying the complexity of habitat flow, reporting that the regional vorticity value was small in a single flow field, but large in a complex flow field. The authors calculated the spawning position of salmon and found that the horizontal vorticity of the spawning ground of the salmon was greater than that of their non-spawning ground [9]. Other researchers calculated and analyzed the average vorticity of the cross-sections and plane average vorticity of the spawning ground of Chinese sturgeon [10,11]. The results revealed that Chinese sturgeon showed a preference in terms of both the cross-section and plane average vorticity levels for spawning. According to previous studies on fish resources in the middle reaches of the Yangtze River [12], the annual ecological operation of the Three Gorges Corporation since 2011 (i.e., in the form of man-made floods) has promoted the spawning behavior of FMCC, although the hydrodynamic characteristics of the spawning ground have changed. However, the relationship between the spawning behavior and the changes in hydrodynamic conditions, as well as the preferences of FMCC for particular hydrodynamic conditions have not been systematically investigated. Therefore, in 2014 and 2015, the Yangtze River Fisheries Research Institute and the China Institute of Water Resources and Hydropower Research conducted joint monitoring of the fish early resources and flow fields in the Yidu spawning site for FMCC during the Three Gorges ecological operation. The cross-section average vorticity, the spawning amount, and the relationship between the two were systematically analyzed. In 2016, the Yangtze River Fisheries Research Institute also carried out ultrasonic telemetry tests to locate the distribution and intensive activity areas of spawning grounds during the breeding period of FMCC. In summary, the goals of this study were to determine: (1) the relationship between mean vorticity and spawning amount; (2) the relationship

between local vorticity and spawning amount; and (3) the relationship between the location of tagged broodstock and spawning and vorticity.

## 2. Materials and Methods

## 2.1. Spawning Site Location Estimation of FMCC

FMCCs are fish that lay drifting eggs. The location and size of spawning grounds were determined by collecting the fish eggs, observing the development period, referring to the water temperature value to calculate the time that the fish eggs are subjected to fertilization, and estimating the drifting process based on the average flow velocity. In this method, if the monitoring site is far from the spawning ground, the estimation error is increased, as is the estimated spawning amount. The Yidu spawning ground, sited below the Gezhouba Dam, is a large, relatively stable, major spawning ground of FMCC in the middle reaches of the Yangtze River. In recent years, the Yangtze River Fisheries Research Institute has monitored early-stage fish resources as close as possible to the core area of the spawning ground to improve the determination of its location. From May to July of 2014, the monitoring section for early resources was established at the Longwo section of Yidu County, Yichang City, in the middle reaches of the Yangtze River (~55 km from Gezhouba). From May to July of 2015, the monitoring section was established in the Weibicun Section (~44 km from Gezhouba), which is 1 km downstream of the convergence with the Qingjiang River. Three fish egg collection points were set up on the left, right and the middle of each monitoring section in the river. At each collection point, fish eggs were collected from the surface, middle and bottom layers of the river via a conical net. Egg collection was carried out for 10 min each time. Dissolved oxygen, water temperature, and other parameters were also recorded. The eggs were identified during developmental stages after collection, followed by fixation and storage in 95% ethanol. The samples were then sent back to the laboratory for DNA tests and extraction and sequencing of cytochrome B using PCR amplification to identify the species.

In 2014 and 2015, a total of 12,209 fish eggs were collected (9782 eggs in 2014 and 2427 eggs in 2015); 1210 eggs were randomly selected for molecular biological identification [13,14]. In total, 27 species were identified, belonging to four families and two orders. Among the eggs identified, 460 eggs were from FMCC (258 in 2014 and 202 in 2015), of which silver carp accounted for 62.4%, followed by grass carp (27.8%). The proportion of black carp and Bighead carp was lower (5.7% and 4.15%, respectively). The above method was then used to estimate the spawning grounds, and the Yidu spawning area was narrowed down to a 12 km-long stretch in the Chenjiahe-Yunchi River section (the uppermost Chenjiahe section is ~21 km downstream of the Gezhouba Dam) (Figure 1).



**Figure 1.** Projection of the spawning ground of FMCC in Yidu and the illustration of flow field (Z indicates the topographic elevation, yd01~yd09 indicate the locations of the ultrasonic signal receivers).

## 2.2. Flow Field Measurement of Spawning Sites of FMCC

From 4 June to 7 June 2014 and 25 to 28 June 2015, the Three Gorges Corporation implemented ecological man-made floods for the breeding of FMCC. From 5 to 8 June 2014 and 26 to 28 June 2015, the Yangtze Fisheries Research Institute and China Institute of Water Resources and Hydropower Research conducted combined monitoring of flow field and early-stage fish resources for the Yidu spawning ground. The measurement was consistent with the Three Gorges ecological operation. Flow field measurements were carried out every 500 m, covering the Chenjiahe-Yunchi section, for a total of 24 sections (Figure 1).

The flow field measurement system comprised a walking acoustic Doppler velocity profiler (ADCP, manufactured by RDI, USA, 600 kHz, depth of measurement range 0.7–75 m, suitable for topography and flow measurement in the middle reaches of the Yangtze River), a GPS satellite positioning system (China CHCNAV Company, i80 GNSS receiver, plane accuracy:  $\pm(8 + 1 \times 10^{-6} \times D)$  mm), a recording computer, ADCP matching Win River (version 1.06) measurement software, and Huace navigation software. During each measurement, the ADCP and GNSS receivers and recording computer were mounted on a boat, which navigated a cross-section of the river. Data, including the profile velocity, bed topography, and GPS position, were collected continuously (Figure 2). The ADCP measurement divides the trajectory section into microsections, each of which is further divided into several bins in a vertical direction; the data, including velocity, depth, and positional coordinates, are stored in each bin.



Figure 2. Flow field measurement results.

## 2.3. Calculation of Mean Vorticity in Spawning Ground Section

Vortex is a special form of velocity gradient. There are various scales and forms of vortices under the combined effects of river terrain and water flow. Here, we only focused on vortices in a cross-section of the river, defining this type of vortex as a section vortex, the axis of which was parallel to the flow direction (Figure 3). In natural rivers, it is difficult to directly measure vorticity. This study used VB.NET to independently program code and read the flow velocity vector, depth, and GPS of each measurement section and bin through the flow field data of ADCP. The coordinates and other data were used to calculate the average vorticity of the cross-section based on the velocity component of the adjacent bin. Details of the calculation method can be found in [9,10].

If the unit vorticity of a region contains both positive and negative vorticity, the simple superposition of the regional vorticity will obscure the complexity of the flow field itself. Therefore, to avoid the cancellation of the positive and negative vorticity in the calculation process, we summed the regional vorticity integrals after taking the absolute value.

The mean vorticity of the section was calculated using Equations (1)–(3):

$$\Omega = \left(\frac{\partial w}{\partial y} - \frac{\partial v}{\partial z}\right)i + \left(\frac{\partial u}{\partial z} - \frac{\partial w}{\partial x}\right)j + \left(\frac{\partial v}{\partial x} - \frac{\partial u}{\partial y}\right)k\tag{1}$$

$$\Omega_x = \left(\frac{\partial w}{\partial y} - \frac{\partial v}{\partial z}\right)i\tag{2}$$

$$\overline{\Omega}_{ABS} = \frac{\Gamma_{ABS}}{A_{TOT}} = \frac{\iint |\Omega_x| dy dz}{A_{TOT}} = \frac{\sum \left| \left( \frac{\Delta w}{\Delta y} - \frac{\Delta v}{\Delta z} \right) \right| \Delta y \Delta z}{\sum \Delta y \Delta z}$$
(3)

where *x* is the flow direction; *y* is the transverse direction; *z* is the vertical direction;  $\Omega$  is the vorticity at a certain position;  $\Omega_x$  is the vorticity along *x*-direction (the vortex axis was parallel to the flow direction);  $\overline{\Omega}_{ABS}$  is the average vorticity of the cross section;  $\Gamma_{ABS}$  is the absolute circulation of cross-section;  $A_{TOT}$  is the area of the cross section;  $\Delta u$ ,  $\Delta v$ ,  $\Delta w$  are the flow rates of discrete cells in *x*, *y*, and *z* directions, respectively;  $\Delta y \Delta z$  is the area of cells in the cross-sectional direction.



Figure 3. The vorticity of the river section.

## 2.4. Positioning of Broodstock during Spawning Based on Ultrasonic Telemetry

Ultrasonic telemetry is an advanced technique for tracking and locating aquatic animals and has been widely used in fish behavior monitoring [15–17], fish habitat studies [18,19], and for determining the relationship between aquatic organisms and their environment [20,21]. The ultrasonic telemetry system comprises a transmitter device and a monitoring device. The launching device is an ultrasound tag that is implanted in, or attached to, an aquatic animal. The ultrasound tag used in this study was V9-2X (77 tags, serial numbers 55127–55203, diameter 9.0 mm, length 29.0 mm, weight 2.9 g, battery life 910 days, nominal delay of 180 s; Mai Vision Technologies Co., Ltd., Shanghai, China) and V9-4X (30 tags, serial numbers 34540–34569, diameter 8.0 mm, length 20.5 mm, weight 0.9 g; battery life 210 days, nominal delay of 120 s; Mai Vision Technologies Co., Ltd., Shanghai, China). The emission signal frequency was 69.0 kHz. The monitoring device is an ultrasonic signal receiver with the animal position and other information (e.g., water depth, temperature, etc.) transmitted from the ultrasonic signale plate. The model of the ultrasonic signal receiver used in this study was VR2W (Vemco Ltd., Shad Bay, NS, Canada). The effective detection distance of the receiver in the Yangtze River was 490 m.

To verify the validity of the calculated spawning grounds of FMCC, the distribution of, and preference for, spawning grounds were further determined. From June to July, 2016, based on the results of resource monitoring during early 2014–2015, ultrasonic telemetry tests of broodstock fish was conducted within the Yidu spawning ground (Figure 1). A total of 107 broodstock of FMCC were marked during the trial (20 *M. piceus*, 29 *C. idella*, 37 *H. molitrix*, and 22 *A. nobilis*). The mean body length of the marked fish was 78.76  $\pm$  6.83 cm, and the average body weight was 12.95  $\pm$  2.46 kg. Ultrasound tags were implanted following the surgical methodology described by [22]. After the operation, the broodstock was kept in the laboratory temporarily (retention time 33–35 days). On 1 June 2016, the broodstock was released in the Zhijiang River section, ~60 km downstream of Yunchi. Nine ultrasonic signal receivers were placed in the Yidu spawning ground (Figure 1).

#### 3. Results and Discussion

#### 3.1. Relationship between Spawning of FMCC and Cross-Section Mean Vorticity of Spawning Ground

According to the monitoring data of the Yangtze River Fisheries Research Institute from 2014 and early 2015, there was a response relationship between the spawning amount of FMCC and discharge of Gezhouba from 1 to 10 June 2014, and 21 to 30 June 2015 (Figures 4 and 5). The water level of the river rise continuously from 4 to 7 June 2014 and from 25 to 28 June 2015, as a result of the Three Gorges ecological operation. Spawning started from day 2 of the increasing water level and peaked on day 3 (Figure 4). On day 4 of the increasing water level, the spawning amount started to decrease, although it remained at a relatively high level. Two days after the ecological operation (8–9 June 2014), the flood dropped, and spawning amount decreased significantly, although it did not stop completely. On 21–22 June 2015, the water volume fluctuated, and small-scale spawning behavior of the fish started

(Figure 5). On 26–27 June, the water level had been increasing for 2 days, and the flood had reached its peak. Large-scale spawning behavior occurred on 27 June.

In total, the vorticities of 24 cross-sections were calculated daily using Equation (3). The mean value was defined as the average vorticity for all cross-sections of the river. During the monitoring periods of the flow field (5–8 June 2014 and 26–28 June 2015), there was a response relationship between the spawning amount of FMCC and the average vorticity of all cross-sections of the river (Figures 6 and 7). Figure 6 shows that, from 5 to 6 June 2014, the average vorticity of all cross-sections of the river increased from 0.39/s to 0.46/s, which significantly increased the corresponding spawning amount of FMCC. From 6 to 8 June, the average vorticity of all cross-sections of the river was stable at a relatively high level (0.45–0.46/s), and the corresponding fish spawned continuously for 2 consecutive days, with a high spawning amount. Figure 7 shows that the average vorticity in 2015 reached the maximum (0.48/s) on 27 June, and the spawning volume of the FMCC also peaked. However, no spawning amount was observed on 26 or 28 June.

Studies have shown that many biological behaviors and parameters are subject to, or approximate to, a normal distribution [23]. Therefore, we assumed that the spawning behavior of FMCC under certain flow conditions was also subject to a normal distribution. On the basis of this hypothesis, the Pearson correlation analysis method was applied to analyze the relationship between the spawning amount of FMCC and vorticity. The Pearson correlation coefficients of the spawning of FMCC from 5 to 8 June 2014 and from 26 to 28 June 2015 and the average vorticity of all cross-sections of the river were 0.730 and 0.822, respectively, indicating a strong positive correlation between the spawning of FMCC and the average vorticity of all cross-section soft the river. The cross-section vorticity also had a stimulating effect on the spawning behavior of the fish. From a biological point of view, a large vorticity of the cross-section is conducive to fertilization of the eggs, maintaining the position of the fertilized eggs in the water so that they do not sink and die. Therefore, it is biologically significant that FMCC chose to continue spawning when the vorticity of cross-section in the spawning ground was relatively large.



Figure 4. The response of FMCC spawning amount to water flow in 2014.



Figure 5. The response of FMCC spawning amount to water flow in 2015.



**Figure 6.** The responsive relationship between spawning of FMCC and the average vorticity of the whole section of the river in 2014.



**Figure 7.** The response of the spawning of FMCC and the average vorticity of the whole section of the river in 2015.

3.2. Relationship between the Local Average Vorticity and Spawning Amount of the Fish at Each Section of the Spawning Ground during Ecological Operation of the Three Gorges Dam

To further study the correlation between the local average vorticity of each section of the Yidu spawning ground and spawning amount of the fish during the ecological operation of the Three Gorges dam, the measured sections were equally divided into three subregions: the left and right bank, and middle of the river (Figure 8). The cross-section average vorticity of each subregion during the

ecological operation of the Three Gorges dam was calculated and Pearson correlation analysis was used to calculate the correlation coefficient between variation in the vertical vorticity and changes in spawning amount in the three subregions (Tables 1 and 2). The data showed that the vorticity of the section and spawning amount of the fish were significantly positively correlated (Pearson correlation coefficient >0.95) in the middle of section 12, 16, 18, 20 and left bank of section 19 in 2014 (as shown in the circle of Figure 8a), and right bank of section 1, 8 and 22, the middle of section 14, 15, and 22, and left bank of section 8 and 19 in 2015 (shown in a circle in Figure 8b). These results show that the special hydrodynamic conditions (increased vorticity) generated during the ecological operation may stimulate spawning and reproduction of the fish, resulting in a corresponding increase in spawning amount. According to the 2-year flow field monitoring data, there are many areas where the local cross-sectional average vorticity is highly related to fish spawning.

Section	<b>Pearson Correlation Coefficient</b>			
	at the Left Bank	in the Middle	at the Right Bank	
1	0.883	0.547	-0.669	
2	-0.136	-0.767	-0.142	
3	0.286	-0.085	0.398	
4	0.147	-0.417	0.402	
5	0.035	0.427	0.831	
6	0.134	-0.315	0.739	
7	0.484	0.543	0.852	
8	0.852	0.717	0.195	
9	-0.881	0.728	0.403	
10	-0.231	0.800	0.385	
11	0.478	0.679	-0.443	
12	0.581	0.961 *	0.230	
13	0.478	0.505	0.811	
14	0.786	0.566	-0.348	
15	0.607	0.920	-0.045	
16	0.708	0.988 *	0.209	
17	-0.263	0.526	0.083	
18	0.402	0.972 *	0.878	
19	0.999 *	0.916	-0.039	
20	0.855	0.976 *	0.798	
21	0.916	0.946	0.037	
22	-0.075	0.564	0.168	
23	0.697	-0.349	0.901	
24	-0.624	0.764	-0.269	

**Table 1.** Correlation between regional average vorticity and spawning of fish during the ecological operation of the Three Gorges Dam in 2014.

Note: \* indicates Pearson correlation. coefficient > 0.95.

**Table 2.** Correlation between regional average vorticity and spawning of fish during the ecological operation of the Three Gorges dam in 2015.

Section	Pearson Correlation Coefficient			
Section	at the Left Bank	in the Middle	at the Right Bank	
1	0.743	0.064	-0.976 *	
2	0.059	0.642	-0.908	
3	-0.617	0.534	-0.618	
4	-0.859	-0.687	-0.325	
5	0.759	-0.383	-0.237	
6	0.462	0.440	-0.813	
7	0.410	-0.621	-0.561	
8	-0.984 *	-0.649	0.992 *	

Section	Pearson Correlation Coefficient			
	at the Left Bank	in the Middle	at the Right Bank	
9	-0.902	-0.288	-0.987	
10	-0.451	0.536	-0.532	
11	0.930	-0.544	0.067	
12	0.513	-0.987	0.686	
13	0.030	-0.998	0.924	
14	-0.835	0.972 *	0.703	
15	-0.669	0.958 *	0.823	
16	0.555	-0.312	0.537	
17	-0.049	-0.708	-0.905	
18	0.778	0.234	-0.433	
19	0.954 *	-0.901	-0.239	
20	-0.768	0.266	-0.496	
21	0.145	0.580	-0.898	
22	0.662	0.998*	0.996 *	
23	-0.928	0.915	0.935	
24	-0.896	-0.641	-0.628	

Table 2. Cont.



**Figure 8.** Areas showing significant correlation between cross-section mean vorticity and fish spawning during the ecological operation of the Three Gorges Dam in 2014 (**a**), 2015 (**b**).

# 3.3. Relationship between the Distribution of Marked Broodstock during the Breeding Period and Areas with High Correlation between Vorticity and Spawning

After the marked broodstocks were released 60 km downstream of the Yidu spawning ground, some were traced back to the Yidu spawning ground. The nine receivers received signals from 30 marked broodstock, the tracking numbers of which were 34545, 34559, 34567, 55135, 55162, 55164,

55185, and 55195, corresponding to 1 *C. idella*, 3 *H. molitrix*, and 4 *A. nobilis*. The number of fish monitored and the number of times they were monitored during the breeding period are shown in Figure 9. The results showed that the receiver at position yd05 captured 13 signals from marked fish, and the number of fixes exceeded 400. The receiver at position yd06 captured 15 signals from marked fish, and the number of fixes was ~ 200.

These results indicated that the broodstock was mainly distributed around areas near receivers yd05 and yd06 during the breeding period, and the corresponding hydrodynamic characteristics were relatively specific. Comprehensive analysis of Figures 1 and 9 showed that yd05 was located on the left bank between sections 16 and 17, and that yd06 was located on the left bank of section 18. Thus, FMCC were active within a 490-m radius of receivers yd05 and yd06. The coverage included the middle of section 16, the left and middle of section 17, the left and middle of section 18, and the left bank of section 19. These locations are largely concentrated in the side of Pier Quantong of the Three Gorges Dam, which is in line with the high correlation between vorticity and the spawning amount calculated from the flow field monitoring in 2014 and 2015. Therefore, it is suggested that the Pier Quantong of the Three Gorges dam in the Yidu spawning ground is the preferred spawning ground for FMCC.



Figure 9. Monitoring data of marked FMCCs in spawning grounds during the2016 breeding season.

#### 3.4. Issues with Small Sample Size and Analysis

According to our field experience over several years, each FMCC spawning event lasts for 1–7 days. From 1900 to 1980 (the Yangtze River was a natural river before the Gezhouba dam was built), the average number of times that the water level increased in the middle reaches of the Yangtze River during the FMCC breeding period (May–July) was 7.1, and the average number of days each increase lasted for was 4.5. From 1981 to 2002 (Gezhouba was operated independently before the closure of the Three Gorges Dam), these figures were 7.5 and 4.5, respectively. From 2003 to 2014 (during the period of the joint operation of Gezhouba Dam and Three Gorges Dam), these figures were 6.2 and 4.2, respectively (figures all based on hydrological data from the Yichang hydrological station from 1900 to 2014). If statistics are based on a single day, regardless of whether causes are natural or have been affected by large-scale water conservancy and hydropower projects, then the sample size of the data showing the amount of spawning and vorticity is no more than 10. The relationship between spawning behavior and water flow suffers from a small sample size. Such small sample problems also occur in other research fields (e.g., pathological analysis of rare diseases, DNA, face recognition, chemical sensors, pattern recognition of incomplete information, etc.), [24–28]. Thus, there might be limitations to our analysis of the results of vorticity and spawning amount, based on our

small sample size. In fact, small samples could increase the randomness of correlation analysis and reduce the repeatability of analysis results. However, our observations provide information about fish behavior and flow conditions, even if this information is incomplete. Actually, the correlation between the local vorticity and the spawning amount, revealed by one survey, might be random. But when a region showing strong correlation, as revealed by multiple survey results, coincides with the frequent activity region of marked parent fish, it is unlikely to be explained simply as a random phenomenon. Our field survey indicates a problem associated with fish spawning behavior and spawning field selection preference, implying that there must be a certain relationship between fish behavior and the flow conditions of the spawning ground. Here, we have also used an analytical method to establish the relationship between the spawning amount and vorticity to reveal the hydrodynamic triggers affecting fish spawning. Although it requires further development, the current method is of use. Given these shortcomings associated with the current study, a clearer relationship between fish behavior and flow conditions would be attainable by: (1) increasing the number of samples by reducing the statistical timescale of each spawning event. The sampling interval of each spawning event on a daily scale could be changed to a sampling interval of 2 or 4 h; and (2) increasing the number of investigations of spawning events and verifying the repeatability of the results.

#### 4. Conclusions

Hydrodynamic changes resulting from the continuous increase in water levels in spawning grounds are important factors that stimulate the spawning behavior of FMCC. 'Bubbling water' is the macroscopic expression of vortex, and is also a previously used qualitative description of the characteristics of water flow in the spawning of FMCC (used in the 1982 survey of spawning grounds of four major Chinese carp in the Yangtze River [29]). Based on previous reports, average cross-section vorticity was selected as a hydrodynamic characteristic affecting the choice of spawning grounds, and the flow field distribution of the Yidu spawning ground was measured during the 2014 and 2015 ecological operation of the Three Gorges dam. In addition, the average cross-section vorticities of the spawning grounds of FMCC were calculated. Simultaneous monitoring of early-stage fish resources was performed downstream to analyze the response relationship between the spawning amount of FMCC and the average vorticity of the spawning ground during increasing water levels. In 2016, ultrasonic wave telemetry of broodstock was conducted to capture the location information of breeding fish and to reveal the hydrodynamic characteristics of the positions that the FMCC preferred, enabling the following conclusions to be reached:

- 1. During the ecological operation of the Three Gorges Dam in 2014, the average vorticity of the cross-section of the Yidu spawning ground varied from 0.39/s to 0.46/s, and the Pearson correlation between the spawning amount of FMCC and the average vorticity of all cross-sections of the Yangtze River was 0.730. During the ecological operation in 2015, the average vorticity of all cross-sections of the spawning ground fluctuated from 0.47/s to 0.48/s. The Pearson correlation coefficient of the spawning amount of the FMCC and the average vorticity of all cross-sections of the river was 0.822, indicating a strong positive correlation between the spawning amount of FMCC and the average vorticity of all cross-sections of the average vorticity of all cross-sections of the river was 0.822, indicating a strong positive correlation between the spawning amount of FMCC and the average vorticity of all cross-sections of the river.
- 2. The Pearson correlation coefficient between the partial vorticity of the section and the spawning of the fish was >0.95 during the ecological operation of the dam in 2014, in the middle of Sections 12, 16, 18, and 20 and the left bank of Section 19 of the Yidu spawning ground; and, during the ecological operation in 2015, on the right bank of Sections 1, 8, and 22, the middle of Sections 14, 15, and 22 and the left bank of Sections 8 and 19. This indicated that the cross-section vorticity changed in these areas. There was a significant positive correlation between the changes in the spawning amount of FMCC and average vorticity of cross-section. The special hydrodynamic conditions (increased vorticity) generated in these areas during the ecological operation of the dam could stimulate the spawning and reproduction of FMCC, resulting in a corresponding increase in spawning amount.

3. Ultrasonic telemetry tests of broodstock fish in 2016 showed that the main distribution region and intense-activity areas of the broodstock fish in the spawning ground of Yidu were within a 490-m radius of receivers yd05 and yd06, mainly around the Quantong Piers of the Three Gorges Dam. In line with the high correlation between vorticity and spawning amount in 2014 and 2015, it is inferred that the river section near the Quantong Piers of the Three Gorges dam is a preferred area for FMCC spawning.

Our analysis of the relationship between fish spawning behavior and water flow in this paper suffers from our small sample size. Although the method of analysis used has some limitations, it can be regarded as beneficial. The results of this research indicate that a continuous increase in water level can effectively stimulate the spawning behavior of fish, such as FMCC. The regulation of, and storage by, the reservoir negatively impacts the natural seasonal increases in water level of the river, reduces the stimulating effect of the flow on the spawning behavior of fish. Therefore, reservoirs should be managed to create multiple artificial flood peaks during the fish breeding period.

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