Patterns of larval fish assemblages along the direction of freshwater input within the southern branch of the Yangtze Estuary, China: implications for conservation

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ABSTRACT
The Yangtze Estuary is the largest estuary in the western Pacific Ocean, and harbors a high diversity of fish fauna. In this study, larval fish were surveyed monthly across three sections within the southern branch of the Yangtze Estuary (SBYE). A total of 49 taxa were collected, with 51.4% of the identified species being freshwater fish. The collected numbers of larval taxa in the upper, middle, and lower sections were 22, 28, and 43, respectively. Species with differing salinity adaptations displayed different patterns along the direction of freshwater input. The collected numbers of larval taxa were 47 and 29 in the near-shore and mid-stream areas, respectively; and the abundances of larval fish in the near-shore areas tended to be higher than in the mid-stream areas in all the three sections. Larval fish occurred year-round with two peaks of abundance. Our results suggest that the freshwater input was the dominant factor shaping larval fish assemblage structure and dynamics within the SBYE. Influences of the Three Gorges Dam and South-to-North Water Transfer Project on larval fish assemblages within the SBYE should be considered with regard to conservation practices.

KEYWORDS
Ichthyoplankton; near-shore habitat; nursery; seasonal pattern; Yangtze River

Introduction
An estuary is generally characterized as a dynamic and heterogeneous aquatic ecosystem, and supports high biodiversity and productivity (Elliott and Dewailly 1995; Whitfield 2005). Interaction between the freshwater input and tidal cycle generates a dynamic and spatially variable estuarine environment (such as salinity distribution) that shapes and alters biotic community structure along a gradient (Whitfield 2015; Human et al. 2016). The rhythm and magnitude of freshwater input can cause major changes in estuarine physical, biogeochemical, and biological attributes (Lez-Ortegon and Drake 2012; Human et al. 2016). Species compositions and abundances vary along the gradient.
in estuarine systems under different temporal and spatial scales (Neves et al. 2011; Lez-Ortegon and Drake 2012; dos Passos et al. 2013).

The Yangtze Estuary is the largest estuary in the western Pacific Ocean (Zhuang et al. 2006). The estuary is divided into areas inside and outside of the mouth (Chen et al. 1988). The area inside of the mouth is composed of a northern branch and a southern branch which are separated by Chongming Island (Figure 1). The southern branch of the Yangtze Estuary (SBYE) is the main channel and is separated into two sub-channels by Changxing Island and Hengsha Island (Figure 1). This estuary is mesotidal with average tidal amplitude of 2.8 m and a current range of 1.0–2.0 m/s (Chai et al. 2006). The estuary serves as important spawning grounds and nurseries for many fish species and is an important fishing ground (Zhuang et al. 2006). In total, 332 fish species have been recorded in the estuary and its adjacent waters from historical literatures, of which more than 50 species are commercially important (Zhuang et al. 2006).

The environment of the Yangtze Estuary has changed over the last several decades. The Three Gorges Dam (TGD), impounded in 2003, has dramatically modified the seasonal rhythm and magnitude of freshwater input and reduced sediment discharge into the estuary (An et al. 2009). Waterway dredging and wharf engineering, typically along the shorelines, have changed the landscape of the estuary (Zhuang et al. 2006). With increasing urbanization and industrial development along the Yangtze River, water quality within the estuary has diminished (Deng et al. 2010). These environmental changes may be affecting fish assemblages in the estuary.

Multiple measures have been applied to mitigate the negative effects of environmental changes on fish resources in the Yangtze Estuary, such as closed fishing seasons, chartered fishing on typical populations, and establishing of nature reserves (Zhuang et al. 2006). However, declines of fish resources in the estuary have not subsided (Zhuang et al. 2006; Jiang et al. 2009). Patterns of larval fish assemblages reflect the spawning and nursery functions of an estuary for fish (Strydom 2015; Teodosio and Garel 2015; Jiang et al. 2016). Various biotic or abiotic factors, such as zooplankton abundance, water temperature, salinity, and water runoff influence larval fish assemblages in estuaries (Cao et al. 2007; Shuai et al. 2016; Zhang et al. 2016). The sensitivity of ichthyoplankton to environmental factors potentially makes

Figure 1. Distribution of sampling sections (upper, middle, and lower) and the location of sampling sites within the southern branch of the Yangtze Estuary.
larval fish assemblages an important resource for understanding the key factors that determine the spatial and temporal patterns of habitat use, which may contribute to efficient conservation practices (Cao et al. 2007; Able et al. 2010; Primo et al. 2011). For instance, knowledge of spatial patterns of larval fish assemblages provides essential information for setting conservation priorities for spawning grounds and nurseries (Zhuang et al. 2006). Understanding temporal patterns of larval fish assemblages is necessary for setting conservation measures, such as closed fishing season and chartered fishing for spawning protection (Zhuang et al. 2006; Able et al. 2010). Meanwhile, understanding the key factors that determine the patterns of larval fish assemblages is also important to explain the mechanisms of spawning and nursery functions of estuaries (Gogola et al. 2010, 2013; Taylor et al. 2016).

Previous studies on larval fish assemblages in the Yangtze Estuary have focused mainly on areas outside of the mouth (Liu et al. 2008; Liu and Xian 2009; Jiang et al. 2016). Detailed information covering the larval fish assemblage inside of the mouth is limited, with few exceptions describing the distribution of larvae and juveniles in the surf zones (Zhong et al. 2005; Jiang et al. 2009). In this study, we investigated larval fish assemblages and environmental factors monthly throughout a year across three sections within the SBYE. Our main objectives were (1) to clarify the spatial and temporal patterns of larval fish assemblage, and (2) to reveal the major environmental factors influencing these patterns. These results will provide essential information about habitat use of larval fish in the Yangtze Estuary. Additionally, these results have important practical significance for the management and conservation of fish in the estuary.

**Methods**

**Sampling locations and protocol**

Sampling was conducted across three sections along the direction of freshwater input within the SBYE: the upper section (121°08’ to 121°12’ E, 31°41’ to 31°44’ N) located at the origin of the SBYE, middle section (121°17’ to 121°22’ E, 31°32’ to 31°37’ N) located at the middle SBYE, and lower section (121°43’ to 121°49’ E, 31°16’ to 31°26’ N) located at the terminus of the SBYE (Figure 1). Five sites were sampled across each of the upper and middle sections, and six sites were sampled across the lower section (Figure 1). Near-shore areas were represented by the sampling sites nearest to the shores, i.e. sampling sites No.1 and No.5 in the upper and middle sections and sampling sites No.1, No.3, No.4, and No.6 in the lower section; and mid-stream areas were represented by the other sampling sites (Figure 1). The sampling sites were located using a Global Position System (GPS) device (GARMIN Ltd., GPS60CSx, Olathe, Kansas, USA).

Sampling was performed monthly from April 2011 through April 2012. Larval fish were collected using a conical net with a 90-cm mouth diameter, 300-cm length, and 0.6-mm mesh size. The net was placed aside a fishing boat, and was towed horizontally upstream parallel to the riverbank at a speed of about 2 km/h for about 10 minutes. A hydro-bios flowmeter (Digital Flow Meter 23090, Silkeborg, Denmark) was placed at the mouth of the net to measure the volumes of filtered water. The collections were immediately preserved in 4% formalin solution buffered with sodium borate. Larval fish were picked out and identified in the laboratory using combined molecular and morphological analyses (Cheng et al. 2013; Ren et al. 2016). For larval fish specimens with mitochondrial cytochrome c oxidase subunit I (COI) gene sequences that were not included in the collection of known COI gene sequences, morphological characteristics were used for identification to the lowest possible taxon according to Okiyama (1988) and Cao et al. (2007). Larvae were counted by species or taxon for each sample. The density of larval fish was then calculated for each sample from the number of larvae and the filtered water volume, and expressed as the number of larvae per 100 m³ water.

During sampling, the salinity and water temperature (°C) were measured using a portable water quality instrument (Thermo Orion Star A329, Thermo Fisher Scientific Inc, Waltham, MA, USA);
water transparency was measured using a Secchi disc (Accurate to 1 cm); and zooplankton from 50 L of water was collected using a self-made 10-L water sampler, filtered through a 64-μm mesh net, and preserved in a 4% formalin solution. Rotifers, cladocerans, and copepods were identified and counted from each zooplankton sample. Densities of zooplankton were expressed as the total number of rotifers, cladocerans, and copepods per liter of water. Larval fish collection and sampling of environmental variables (i.e. salinity, water temperature, water transparency, and zooplankton) were conducted once per sampling site per month.

Water runoff data measured at Datong station (117°37′E, 30°46′N) were acquired from the Shanghai Water Authority (http://www.shanghaiwater.gov.cn/indexZh.html). Monthly precipitation values measured at Baoshan station (121°28′ E, 31°24′ N) were acquired from the China Meteorological Data Sharing Service System (http://cdc.cma.gov.cn/home.do).

Data analysis

Each species was assigned to one of five ecological guilds based on the life history characteristics, i.e. freshwater species, estuarine resident species, anadromous species, marine–estuarine-dependent species, and marine species (Elliott and Dewailly 1995; Pihl et al. 2002). Information of life history characteristics of fish was acquired from previous literatures (IHB 1976; Ni and Wu 2006; Jin 2010).

The densities of each taxon were determined for larval fish assemblage of each sample. The effects of environmental variables (i.e. salinity, water temperature, transparency, zooplankton, runoff, and precipitation) on larval fish assemblages were analyzed by redundancy analysis (RDA) in a general linear model using the software package CANOCO (version 4.5) (Ter-Braak 1995). The statistical significance of the environmental variables against the larval fish assemblages was assessed using a Monte Carlo permutation test (999 permutations) with forward selection. Larval fishes with occurrence frequencies smaller than 3% in all catches were defined as rare species (Barletta-Bergan et al. 2002). Down-weighting of rare species was performed to avoid undue effects. The data of larval fish density and environmental variables were log (x + 1) transformed prior to the analysis.

Results

Species composition of larval fish

A total of 85,765 larval individuals were collected, and were composed of 49 taxa based on COI gene sequence analysis (Table 1). Thirty-five taxa were determined to species. The remaining 14 taxa were determined to the lowest taxonomic level based on morphological characteristics. Among them, three taxa were determined to two genera, two in Rhinogobius and one in Mugilogobius; 11 taxa were determined to 5 families, 4 in Cyprinidae, 3 in Gobiidae, 2 in Sciaenidae (Table 1). The 49 taxa belonged to 14 families, including 16 species of Cyprinidae, 14 of Gobiidae, and 4 of Engraulidae (Table 1). Among the 35 identified species, 18 were freshwater species, 7 estuarine resident species, and 7 marine–estuarine-dependent species (Table 1).

Spatial and temporal patterns of larval fish

The species number of larval fish collected in the upper, middle, and lower sections was 22, 28, and 43, respectively (Table 1). The proportion of freshwater species was higher in the upper section (64.7%) than in the lower section (46.9%); while the proportion of other ecological guilds was lower in the upper section than in the lower section (Table 1). The species number of larval fish collected in the near-shore sites (8 sites, 47 taxa) was higher than in the mid-stream sites (8 sites, 29 taxa) (Table 1; Figure 2). The proportion of different ecological guilds was similar, such as 52.9% and 56.0% of freshwater species, 17.6% and 20.0% of estuarine resident species, between the near-shore and mid-stream areas (Table 1).
Table 1. Larval fish collected within the southern branch of the Yangtze Estuary from April 2011 to April 2012. Each species was assigned to one of the following ecological guilds: freshwater species (F), estuarine resident species (E), anadromous species (A), marine–estuarine-dependent species (ME), and marine species (M). Occurring months are 1 for January, 2 for February, and so on. The "C3" notes the distribution areas of each larval fish.

| Family       | Species              | Code | Ecological guild | Number of individuals | Abundance (%) | Occurring month | Abundance by sections (%) | Distribution areas |
|--------------|----------------------|------|------------------|-----------------------|---------------|------------------|--------------------------|-------------------|
| Cyprinidae   | Hemiculter bleekeri  | Hble | F                | 57,035                | 77.80         | 4–9              | 93.38 86.30 46.91        | *                 |
|              | Parabramis pekinensis| Ppek | F                | 1518                  | 1.96          | 5–9              | 0.69 1.63 2.70          | *                 |
|              | Pseudolaubuca engrais| Pang | F                | 721                   | 0.79          | 5–10             | 0.38 1.17 1.13          | *                 |
|              | Pseudobrama simony   | Psim | F                | 32                    | 0.04          | 5–9              | 0.01 0.01 0.15          | *                 |
|              | Pseudorasbora parva  | Ppar | F                | 60                    | 0.02          | 4                | 0.01 0.03 0.03          | *                 |
|              | Sauragogobio dumerili| Sdum | F                | 16                    | 0.01          | 4                | 0.01 <0.01 0.01        | *                 |
|              | Carassius auratus    | Caur | F                | 15                    | 0.01          | 4–5              | 0.01 0.01 0.15          | *                 |
|              | Hypophthalmichthys molitrix | Hmol | F           | 4                     | <0.01        | 6–7              | 0.01 0.01 0.01         | *                 |
|              | Mylopharyngodon piceus| Mpic | F               | 4                     | <0.01        | 6, 8             | <0.01 <0.01 <0.01      | *                 |
|              | Culterinae sp.       | Csp. | F                | 1                     | <0.01        | 7                | – 0.01 0.01            | *                 |
|              | Cyprinidae sp.2      | Csp.2| F                | 1                     | <0.01        | 6                | – 0.01 <0.01           | *                 |
|              | Cyprinidae sp.1      | Csp.1| F                | 1                     | <0.01        | 6                | – 0.01 <0.01           | *                 |
|              | Cyprinidae sp.3      | Csp.3| F                | 1                     | <0.01        | 10               | – – <0.01             | *                 |
|              | Elopichthys bambusa  | Ebam | F                | 1                     | <0.01        | 8                | – – <0.01             | *                 |
|              | Cyprinus carpio      | Ccar | F                | 1                     | <0.01        | 4                | – <0.01 –             | *                 |
|              | Abbottina rivularis  | Ariv | F                | 1                     | <0.01        | 4                | – – <0.01             | *                 |
|              | Cobitidae sp.        | Cosp.| F                | 1                     | <0.01        | 6                | – – 0.01              | *                 |
|              | Engraulidae sp.1     | Cnol | F                | 4                     | <0.01        | 6, 8             | <0.01 <0.01 <0.01      | *                 |
|              | Engraulidae sp.2     | Cnol | F                | 1                     | <0.01        | 4                | – <0.01 <0.01         | *                 |
|              | Engraulidae sp.3     | Cnol | F                | 1                     | <0.01        | 4                | – <0.01 <0.01         | *                 |
|              | Salangidae sp.1      | Sari | E                | 5796                  | 2.39         | 9–12,1           | 1.61 1.78 4.27         | *                 |
|              | Neosalanx tangkiae   | Ntan | F                | 354                   | 0.14         | 3–5, 10          | 0.09 0.30 0.08         | *                 |
|              | Neosalanx anderssoni | Nand | E                | 1                     | <0.01        | 6                | – <0.01               | *                 |
|              | Gobiidae sp.1        | Gsp.1| F                | 8                     | <0.01        | 5, 8             | <0.01 <0.01 <0.01     | *                 |

(continued)
| Family          | Species                        | Code | Ecological guild | Number of individuals | Abundance (%) | Occurring month | Abundance by sections (%) | Distribution areas |
|-----------------|--------------------------------|------|------------------|-----------------------|---------------|------------------|---------------------------|-------------------|
|                | Scartelaos histophorus         | Shis | E                | 1                     | <0.01         | 8                | –                         | –                 |
|                | Odontamblyopus rubicundus      | Orub | E                | 2                     | <0.01         | 10               | –                         | –                 |
|                | Acanthogobius hasta            | Ahas | E                | 2                     | <0.01         | 4                | –                         | –                 |
|                | Gobiidae sp.2                  | Gsp.2|                  | 1                     | <0.01         | 9                | –                         | <0.01             |
| Mugilidae      | Liza haematocheila             | Lhae | ME               | 212                   | 0.10          | 4–5              | 0.02 0.09 0.23            | –                 |
| Percichthyidae | Sniperca chuatsi               | Schu | F                | 62                    | 0.07          | 6–8              | 0.04 0.14 0.09            | –                 |
| Callionymidae  | Rhamphoculus luidus            | Roli | ME               | 8                     | <0.01         | 5                | –                         | –                 |
| Sciaenidae     | Sciaenidae sp.1                | Ssp.1|                  | 1                     | <0.01         | 5                | <0.01 0.01 0.01           | –                 |
|                | Sciaenidae sp.2                | Ssp.2|                  | 1                     | <0.01         | 5                | –                         | <0.01             |
|                | Hyporhamphus intermedius       | Hint | F                | 1                     | <0.01         | 6                | –                         | <0.01             |
|                | Gambusia affinis               | Gaff | F                | 1                     | <0.01         | 9                | –                         | –                 |
|                | Tachysurus fulvidraco          | Tful | F                | 1                     | <0.01         | 8                | –                         | <0.01             |
|                | Lateolabrax maculatus          | Lmac | ME               | 1                     | <0.01         | 4                | –                         | <0.01             |
|                | Platycephalus indicus          | Pind | ME               | 1                     | <0.01         | 5                | –                         | <0.01             |
|                |                                 |      |                  | 85,765               |               |                  | 23,219 31,730 30,816       |                   |
|                |                                 |      |                  | 49                   |               |                  | 22 28 43 47 29             |                   |

Table 1. (Continued)
Figure 2. Monthly variations and spatial distributions of the number of larval species collected within the southern branch of the Yangtze Estuary during April 2011 through April 2012. Line graphs show the monthly variation in the number of larval species for the three sampling sections, density graphs show the spatial distributions of the number of larval species at different sites in the upper (U), middle (M), and lower (L) sections during April 2011 through April 2012.
Figure 3. Monthly variations and spatial distributions of larval fish abundance (Ind./100 m³) collected within the southern branch of the Yangtze Estuary during April 2011 through April 2012. Line graphs show the monthly variation of larval fish abundance using mean (± SD) of sampling sites in each section, density graphs show the spatial distributions of larval fish abundance at different sites in the upper (U), middle (M), and lower (L) sections during April 2011 through April 2012.
The abundance number of larval fish collected in the upper, middle, and lower sections was 31.5%, 30.9%, and 37.6% of the total catch, respectively (Table 1). The percentage of abundance for freshwater species was higher in the upper section (36.1%) than in the lower section (29.8%); while the percentage of abundance for other ecological guilds was lower in the upper section than in the lower section (Table 1). The abundance number of larval fish collected in the near-shore sites accounted for 79.2% of the total catch, which was higher than in the mid-stream areas (20.8%) (Table 1; Figure 3). Larval fish abundances of all ecological guilds were higher in the near-shore areas than in the mid-stream areas (Table 1).

The number of collected species showed a clear seasonal pattern with 36 species occurring during May through August, 1 species occurring in December, January, and March, and no larval fish occurring in February (Table 1; Figure 2). The abundance of larval fish had two peaks year-round. The peak during May through August was dominated by *Hemiculter bleekeri*, *Coilia nasus*, and *Para-bramis pekinensis*, with 97.3% of the total catch, and the peak during October and November was composed of a single species, *Salanx ariakensis* with 2.4% of the total catch (Table 1; Figure 3).

Larval fish were dominated by *H. bleekeri* (77.8% of the total catch in abundance), followed by *C. nasus* (15.9%), and *S. ariakensis* (2.4%) (Table 1). The abundance of *H. bleekeri*, a freshwater species, decreased along the direction of freshwater input with 36.9%, 34.2%, and 28.9% of the larvae in the upper, middle, and lower sections. Meanwhile, this species concentrated in the near-shore areas with 81.1% of the larvae in these sites. This dominant fish occurred during April through September, with higher abundance during June through August (Figure 4(a)). The abundance of *C. nasus*, an anadromous fish, increased along the direction of freshwater input with 11.1%, 15.3%, and 73.6% of the larvae in the upper, middle, and lower sections. Additionally, this species concentrated in the near-shore areas with 73.9% of the larvae in these sites, especially in the lower section with 91.1% in the northern near-shore areas. This fish occurred during May through September, with higher abundance observed in May and June (Figure 4(b)). The abundance of *S. ariakensis*, an estuarine resident fish, increased along the direction of freshwater input with 19.6%, 23.1%, and 57.3% of the larvae in the upper, middle, and lower sections. Meanwhile, this species distributed somewhat evenly between the near-shore and mid-stream sampling sites, while concentrating with 65.5% of the larvae in the

Figure 4. Monthly variations and spatial distributions of larval fish abundance (Ind./100 m$^3$) for three dominant species, *Hemiculter bleekeri* (a), *Coilia nasus* (b), and *Salanx ariakensis* (c), collected within the southern branch of the Yangtze Estuary during April 2011 through April 2012. Line graphs show the monthly variation of larval fish abundance using mean (± SD) of sampling sites in each section, density graphs show the spatial distributions of larval fish abundance at different sites in the upper (U), middle (M), and lower (L) sections during April 2011 through April 2012.
Figure 5. Monthly variations and spatial distributions of larval fish abundance (Ind./100 m³) collected within the southern branch of the Yangtze Estuary during April 2011 through April 2012 after excluding the three dominant species. Line graphs show the monthly variation of larval fish abundance using mean (± SD) of sampling sites in each section, density graphs show the spatial distributions of larval fish abundance at different sites in the upper (U), middle (M), and lower (L) sections during April 2011 through April 2012.
northern near-shore area. This fish occurred during September through December, with higher abundance during October and November (Figure 4(c)). When the above three dominant species were excluded, the proportion in abundance of larval fish was 14.5%, 35.5%, and 50.0% in the upper, middle, and lower sections, respectively (Table 1; Figure 5). The proportion in abundance of remaining larval fish was 71.8% in the near-shore areas and 28.2% in the mid-stream areas (Figure 5). The assemblages had an abundance peak with 93.8% of remaining larval fish occurring during May through August (Figure 5).

**Environmental variables**

Water runoff values ranged from 13,500 to 36,800 m³/s, and were higher in the summer (e.g. June, July, and August; 30,100–36,800 m³/s) than in the winter (e.g. December, January, and February; 13,500–15,600 m³/s) (Figure 6(a)). Precipitation values ranged from 28 to 319 mm, and showed a seasonal pattern similar to that of water runoff (Figure 6(a)). Water temperature values ranged from 7.0 to 30.0 °C with the lowest value in February and the highest value in August (Figure 6(b)). Transparency values tended to be higher from April through September, lowest in October, and slightly increased during November through next April (Figure 6(c)). Salinity displayed an opposite seasonal pattern compared with water runoff, had lower values (mean ± standard deviation [SD]) in the summer (0.8 ± 0.5‰), and higher values in the winter (4.1 ± 0.4‰). Salinity tended to be lower in the upper section (2.4 ± 1.2‰) than in the middle section (2.8 ± 1.2‰) which in turn was lower than the lower section (3.3 ± 1.5‰); salinity was similar between the near-shore sites and mid-stream sites in each of the three sections (Figure 7(a)). Zooplankton density (mean ± SD) tended to be higher during April through September (6.4 ± 6.1 Ind./L) than that in other months (1.9 ± 2.3 Ind./L). There was no clear pattern in zooplankton density across the three sections; however, it tended to be higher in the near-shore sites (4.8 ± 6.0 Ind./L) than in the mid-stream areas (3.8 ± 4.2 Ind./L) in each of the three sections (Figure 7(b)).

**Relationship between larval fish assemblages and environmental variables**

The RDA analysis showed that environmental parameters explained 59.0% of the total variation of larval fish assemblages. The first and second axes of the RDA explained 88.4% and 5.9% of the variance of species–environment relationship, respectively (Figure 8). The runoff, precipitation, water temperature, and zooplankton density were positively correlated with a group of fish species, e.g. *H. bleekeri*, *Pseudolaubuca engraulis*, *P. pekinensis*, *Rhinogobius giurinus*, and *Siniperca chuatsi*, which occurred mainly during May through August when water runoff was high, water temperature increased. The salinity correlated negatively with the larval fish assemblage (Figure 8).

**Discussion**

This study is the first intensive survey of larval fish assemblages within the SBYE. Our results showed clear spatial and temporal patterns of larval fish assemblages. The distribution of larval fish in the three sections showed patterns depending on ecological guilds: freshwater species were more abundant in the upper section, and decreased along the direction of freshwater input; while the abundance of other ecological guilds generally showed reversed spatial patterns. Larval fish had a peak in abundance and species number during May through August associated with high freshwater input. Our results suggested that the freshwater input dominated over tidal cycle in determining the patterns of larval fish assemblages within the SBYE.

The interaction of freshwater input and tidal cycle generates a gradient and dynamic estuarine environment (Whitfield 2015; Human et al. 2016). Freshwater input restricts upstream saline penetration and creates a salinity gradient in estuaries, which consequently determines and alters the
spatial patterns of larval fish assemblages along the salinity gradient (Whitfield 2015). Associating with these heterogeneous and fluctuating environmental patterns, our results demonstrated a clear pattern of larval fish assemblages along the direction of freshwater input within the SBYE. First, the proportion of freshwater species was highest in the upper section, and lowest in the lower section, while the proportion of other ecological guilds was typically lowest in the upper section, and highest.
in the lower section. For instance, four of the seven marine–estuarine-dependent species (Repomucenus olidus, Collichthys lucidus, Lateolabrax maculatus, and Platycephalus indicus), four of the seven estuarine resident species (Neosalanx anderssoni, Scartelaos histophorus, Odontamblyopus rubicundus, and Acanthogobius hasta), and one of the two anadromous species (Colilia mystus), and marine species (Engraulis japonicus) occurred in the lower section, but were absent in the upper section. Meanwhile, for the species that occurred in all three sampling sections, the species with differing salinity adaptations displayed different patterns of abundance among the upper, middle, and lower sections. For example, the abundance of the most dominant species (H. bleekeri), together with many of the other freshwater species (e.g. P. engraulis, R. giurinus, and S. chuatsi) tended to be higher in the upper section and decreased downstream. In contrast, the abundance of the other two dominant species, i.e. C. nasus (anadromous species) and S. ariakensis (estuarine resident species), showed an inverse pattern with the highest abundance in the lower section. These spatial patterns reveal the importance of freshwater input in determining the composition and distribution of larval fish assemblages, and showed the dominance of freshwater species in larval fish assemblages within the SBYE. The magnitude of freshwater input into the SBYE has been modified due to the operation of the TGD and the South-to-North Water Transfer Project (SNWTP, under trial operation), which may consequently alter the patterns of larval fish assemblage within the SBYE.

Seasonal patterns of larval fish assemblages are usually determined by temporal fluctuations of environmental factors (Ramos et al. 2006; Primo et al. 2011; Arévalo-Frías and Mendoza-Carranza 2015). Within the SBYE, larval fish occurred year-round with a major abundance and the number of larval species peaked during May through August, which was associated with temporal
fluctuations of water runoff and temperature. The water runoff was high (>30,000 m³/s) during May through August. Meanwhile, many fishes in the Yangtze River spawn at water temperatures above 18 °C (Cao et al. 2007). The water temperature in the Yangtze Estuary increased to approximately 18 °C at the end of April, and continued to increase through July. Such seasonal peaks in abundance and number of species of larval fish were also observed in the lower Yangtze River (Ren et al. 2016). The timing of reproduction is critical for a fish species to obtain optimal environmental conditions for offspring survival (Yin 2003; Shuai et al. 2016). Zooplankton is the major food for most fish species during the larval stage as observed in fishes of the Yangtze River (Zhu 1987; Zhang et al. 2002; Li et al. 2014) and other water systems (Barletta-Bergan et al. 2002; Koutrakis et al. 2004; Winder and Jassby 2011). We observed an increased abundance of zooplankton associated with this peak in larval fish abundance. A temporal match between the occurrence of larval fish and the bloom of zooplankton has been suggested as being critical for successful recruitment of fish populations (Lara-lopez and Neira 2008; Miller and Kendall 2009; Hsieh et al. 2012).

Species in a community usually occupy different niches to partition resource utility, and consequently delimit potential competition (Yin 2003). We observed clear variations in both the time of occurrence and the spatial distribution among different species of larval fish within the SBYE. Larval fish occurred year-round with variability in the dominant species in different seasons. We observed two peaks of larval fish abundance that were dominated by different species. The peak during May through August was dominated by H. bleekeri, C. nasus, and P. pekinensis, and the peak during October and November was basically composed of a single species, S. ariakensis. Even within the major peak during May through August, fine temporal separations among the dominant species were also observed. For example, the highest dominant species (H. bleekeri) was observed with higher abundance during June through August, while the second dominant species (C. nasus) had higher abundance in May and June. A separation of spawning periods in fish assemblages has been reported in other estuaries (Barletta-Bergan et al. 2002; Koutrakis et al. 2004). Meanwhile, our

![Figure 8. Biplots of the first two axes of the redundancy analyses (RDA) ordination of larval fish assemblages within the southern branch of the Yangtze Estuary during April 2011 through April 2012. Scores of environmental variables are represented by arrows and species by triangles, see Table 1 for species codes, the degree of interpretation of the axes for the variance of species–environment relationship is shown in brackets.](image)
results demonstrated that species of different ecological guilds showed different distribution patterns in the three sampling sections along the direction of freshwater input. Further research should be conducted to investigate the growth and recruitment processes during the early life stages for fish occurring in different periods and habitats in the Yangtze Estuary to clarify potential evolutionary segregation through separated spawning periods and spatial distribution.

**Implications for management**

The environment and fish resources in the Yangtze Estuary have been seriously threatened by multiple anthropogenic disturbances. Knowledge about the spatial and temporal patterns of larval fish assemblages in the Yangtze Estuary revealed in this study provides essential information for guiding the efficient management and conservation of fish resources. The management implications that we proposed were based on our results showing the concentration of larval fish within the near-shore areas, seasonal fluctuation of larval fish, and the dominant influence of freshwater input in determining these patterns. Similar spatial distributions and seasonal patterns of larval fish had been recorded in the lower Yangtze River (Ren et al. 2016), in the Yangtze Estuary (Zhong et al. 2005; Liu and Xian 2009), and in other estuaries (Pattrick and Strydom 2014; Strydom 2015). The crucial effect of freshwater input on larval fish assemblages also has been demonstrated in other estuaries (Ramos et al. 2006; Primo et al. 2011; Whitfield 2015).

Our results showed a concentrated distribution of larval fish within the near-shore areas in the estuary. Our results, together with several previous studies (Zhong et al. 2005; Liu and Xian 2009), demonstrated that the near-shore areas are critical for larval fish (potentially for feeding and nursery), and may play an important role in enhancing recruitment success of fish populations in the Yangtze Estuary. However, a large part of these areas have been reclaimed and destroyed with urbanization and harbor construction in the Yangtze Estuary (Zhuang et al. 2006). Designating near-shore areas as protected zones to avoid further deterioration would benefit recruitment success of fish populations in the Yangtze Estuary.

Appointment of a closed fishing season has been the major practice of fish resources conservation in the Yangtze Estuary. The closed fishing season has been set from 1 April through 30 June to protect the spawning stock and larval fish assemblages in the Yangtze Estuary since 2003. Our results showed that larval fish peak during May through August, and the current closed fishing season cover only a portion of the peak of larval fish assemblages in the Yangtze Estuary. We suggest to adjust the closed fishing season in the Yangtze Estuary by extending it to at least include July.

In summary, our research identified a total of 49 taxa of larval fish, and demonstrated a clear pattern of larval fish assemblages within the SBYE. Briefly, the distribution of species with various salinity adaptations showed different patterns along the direction of freshwater input; larval fish tended to concentrate in the near-shore areas compared to the mid-stream areas; there was a major peak of larval occurrence during May through August associated with high water runoff, proper water temperature, and high zooplankton abundance. Our results provide important baseline information of larval fish assemblages within the SBYE. Our results suggested that the freshwater input is a major factor shaping the patterns of larval fish assemblages within the SBYE. The operation of the TGD and SNWTP has been dramatically modifying the patterns of freshwater input into the Yangtze Estuary. While the TGD has dramatically modified the seasonal rhythm of freshwater input (An et al. 2009), the operation of the SNWTP has led to a decreased magnitude of freshwater input into the SBYE (Huang et al. 2010). It is important to further investigate the influences of such freshwater input modification on larval fish assemblages in the Yangtze Estuary, thus to provide fundamental information guiding the operation of the TGD and the SNWTP for fish resources conservation. Anthropogenic alteration of habitat is common in most temperate estuary systems (Zhuang et al. 2006; Verdonschot et al. 2013; Lee et al. 2016). It will be crucial to investigate the patterns of larval fish assemblages through focusing on anthropogenic alterations of freshwater input and its determinant effects for considering protection plans for fishes in such estuarine systems.
Disclosure statement

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