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Temperature drives reproductive activity in a rare trioecy population of *Corbicula* clams

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Abstract

Trioecy systems are generally considered to be less stable and less widely distributed. Recently, a rare and mostly hermaphrodite (>50%) trioecy system was detected in a single indigenous population of *Corbicula fluminea*. While hermaphrodite specimens are common in the invaded range, dioecy systems dominate *C. fluminea* populations in native regions, with trioecy populations being rare and transient in both ranges. To date, the stability of this trioecy system, as well as how environmental conditions effect sex allocation and reproductive activities remains unknown. To address this, the population sex ratio and brooding characteristics were analyzed through morphological and histological examination of *C. fluminea* specimens. Trioecy was sustained over the assessment period, with an approximate sex ratio of 1:1:6 (male: female: hermaphrodite). Greater water temperature significantly increased the population ratio in favour of males, while lower water temperatures were significantly associated shift towards females. Gametogenesis and brooding occurred throughout the year, but asynchronously. The incubation rate substantially increased between June and December, and had a significant and positive correlation with water temperature. Overall, these data suggest that a *C. fluminea* trioecy sexual system can be a stable phenomenon, and water temperature is both a driver of population sex ratio and reproductive activities.

Keywords:

Corbicula fluminea, sexual system, trioecy, hermaphrodite, water temperature, brooding

Declarations

Competing interests

The authors declare that this research have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Introduction

A sexual system is the pattern of resource allocation for male versus female reproduction in sexually reproducing species (Leonard 2019). In animals, dioecy is the most common and stable sexual system, other mixed mating sexual systems, such as hermaphroditism (co-occurrence of functional male and female reproductive organs in each individual), androdioecy (males and hermaphrodites), gynodioecy (females and hermaphrodites), and trioecy (males, females and hermaphrodites) are less stable and less widely distributed (Bello et al. 2013; Collin 2013; Heller 1993; Leonard 2019; Weeks 2012). Trioecy has rarely been detected in animals, although has been found in some species, e.g., nematode (Kanzaki et al. 2017; Tandonnet et al. 2019), *Aiptasia diaphana* (Armoza-Zvuloni et al. 2014)*, Semimytilus algosus* (Oyarzún et al. 2020) and *Galatea paradoxa* (Adjei-Boateng and Wilson 2016). The conditions under which mixed mating sexual systems exist and whether such sexual systems are stable remains poorly understood (Armoza-Zvuloni et al. 2014; Leonard 2019; Oyarzún et al. 2020; Weeks et al. 2006). Nevertheless, it appears that the emergence or stable existence of these sexual systems is likely underpinned by multiple factors (Matsumoto and Crews 2012; Teaniniuraitemoana et al. 2016; Wedekind and Stelkens 2010). Sex determination is a developmental process, which is divided into two major types: genotypic sex determination (GSD) systems and environment sex determination (ESD) system (Breton et al. 2017; Broquard et al. 2021; Dalpé et al. 2022; Matsumoto and Crews 2012; Shi et al. 2015). In organisms with ESD, the environment factors, such as temperature, water flow, can influence the expression of genes involved in sex determination (Chang 2007; Dalpé et al. 2022; Xu et al. 2022), which may induce sex differentiation toward male, female and hermaphrodite development (Shi et al. 2015; Zeng et al. 2022b).

Native to areas of Asia, Africa, Australia and the Middle East (Araujo et al. 1993; Sousa et al. 2008), *Corbicula* clams have achieved an almost global distribution and frequently display highly invasive tendencies through the formation of dense and expansive populations (Crespo et al. 2015; Haponski and Foighil 2019; Lopez-Soriano et al. 2018; Soares and Martins-Silva 2021; Tos et al. 2021). *Corbicula* clams also tend to exhibit sexual polymorphism in different aquatic habitats (Morton 1991). As a non-indigenous invasive species, it predominantly displays hermaphroditism in invaded areas, with only occasional occurrence of male and/or female only specimens (Cao et al. 2017; Darrigran 2002; Denton et al. 2012; Sousa et al. 2008). However, most populations of *Corbicula* clams in indigenous areas are dioecious, with only the occasional occurrence of hermaphrodites (Gong and Wang 2001; Morton 1977; Morton 1991; Tejima et al. 2020). Additionally, *Corbicula*

clams with an androdioecy sexual system have been observed in irrigation ditches in Japan (Houki et al. 2011; Tejima et al. 2020). However, a single indigenous population inhabiting the Yuangjiang River in China, has recently been confirmed as a trioecy sexual system that is mainly composed of hermaphrodites (> 50%) (Pei et al. 2020; Wang et al. 2018; Zhan et al. 2021). Nonetheless, the stability of this trioecy population over time remains unknown (i.e., stable or transient).

The reproduction activities of *C. fluminea* tends to be synchronized (Cataldo and Boltovskoy 1999), and can be susceptible to influence from external environmental factors such as, amongst others, water temperature and water velocity (Bespalaya et al. 2021; Doherty et al. 1987; Henricksen and Bollens 2022; Morton 1983). For most bivalves, including *Corbicula* clams, reproduction is directly linked to temperature with spawning typically taking place within a narrow range of temperatures, which is often species specific (Chelyadina et al. 2021; Denton et al. 2012; Morton 1977; Teaniniuraitemoana et al. 2016). In general, elevated temperature stimulates an increase in the fecundity of *Corbicula* clams (Penk and Williams 2019; Petter et al. 2014). Water velocity can also prevent a females' eggs from becoming fertilized and/or inhibit female filtration of sperm (Galbraith 2009). Fertilization occurs inside the paleal cavity, and fertilized eggs develop from embryos to larvae while incubated within the inner demibranchs water tubes (Darrigran 2002). In general, environmental conditions are not thought to effect *Corbicula* brooding behavior (Morton 1977; Zhao et al. 2012), especially for populations primarily comprised of hermaphrodite specimens (Morton 1983). However, the relationship between the brooding behavior of hermaphrodite specimen and environmental conditions requires confirmation. Knowledge of reproductive dynamics of problematic or invasive species is also considered a key tool for planning and implementing control strategies (Giglio et al. 2016).

In the present study, we assess the prevalence and stability of trioecy over a 12-month period. As this system has appeared to remain intact with a high proportion of hermaphrodite specimens being detected through preliminary incidental sampling, we hypothesize that the trioecy sexual system of *C. fluminea* in the lower reaches of Yuanjiang River is relatively stable. However, we anticipate that environmental factors will govern reproductive activities. For example, we expect that water temperature and water velocity will influence reproductive behavior.

Materials and Methods

Field sampling

From September 2019 to August 2020, specimens of *C. fluminea* were collected from the lower Yuanjiang River, Changde, Hunan, China (111°41′57.66″E, 28°59′34.11″N). The Yuanjiang River is 1,033 km length from its source in the Miao Mountains to where it enters Dongting Lake, with a drainage area of about 89,000 km^2, and the annual runoff can reach 60 billion cubic meters. The Yuanjiang River is located in the middle reaches of the Yangtze River Basin, and is an important tributary of the Yangtze River, which exits Dongting Lake and flows to Yangtze River estuary. Specimens were collected once per month for a 12-month period, using a Petersen-type grab sampler (0.0625m^2). When sampling, water temperature was measured with a thermometer (BLG100, Shenzhen Anseny Electronic Technology Co., Ltd. China). Water level (based on sea level) and river velocity data for sampling days were sourced from the hydrological monitoring station in the lower reaches of the Yuanjiang River, Hunan Hydrology and Water Resources Survey Center (http://slt.hunan.gov.cn/hnsw/).

Specimens were transported in source water to a laboratory at Hunan Agricultural University Changsha, Hunan, China. In the laboratory, specimens were kept in dechlorinated and aerated tap water for a 24 hr period. Before analyses, the external surfaces of the specimens were cleaned thoroughly with a brush. Specimens were then blotted dry using absorbent paper, and the wet weight biomass was captured to the nearest 0.01 g using a high precision electronic balance (T30002NF, WANT Balance Instrument Co., Ltd. Changzhou, China). Shell length (horizontal axis) was measured to 0.01 mm using a digital vernier caliper (IP54, AIRAJ, Qingdao Meijite Precision Tools Co., LTD. China).

Gonad histology

Each month, between 30–40 *C. fluminea* specimens of different shell sizes were selected for histological assessment of gonads (total n = 380 specimens: 8.88–13.98 g min.-max. wet weight; 20.72–34.80 mm min.-max. shell length). Following the protocol outlined by Pei et al. (2020), the integrated gonads of *C. fluminea* were immobilized in Bouin's solution, isolated from the viscera, and then extracted for hematoxylin-eosin (H&E) staining. Prior to staining, the gonadal tissue was first dehydrated using ethanol and made transparent with xylene. Following this, the gonadal tissue was embedded in paraffin and dyed with H&E dye and sealed. When making the gonadal tissue sections, to avoid the influence of different section positions on sex identification,

each specimen was serially sectioned, with a final thickness of 4-6 μm. Sections were mounted on slides and inspected with the use of an optical microscope (ML31, MSHOT, Guangzhou, China). In the sex identification through gonad tissue sections, tissue displaying only the testicular follicles were considered male, while those with only the ovarian follicles were female. Tissue sections displaying testicular follicles, ovarian follicles and ovotestes were considered hermaphrodite.

Incubation observation

A total of 1580 specimens were used for incubation observations. These included specimens used for gonadal sectioning (total n = 380), for demibranchs gill sectioning (total n =120: 3.67–13.78 g wet weight; 22.36–33.96 mm shell length) and for condition index determination (total n = 1,080 specimens: 2.93–13.57 g wet weight; 19.13–35.48 mm shell length). Following inspection, other than for specimens retained for gonad or demibranch histology, the soft tissues were returned to the original specimen to facilitate the determination of a condition index. Fertilized eggs with and without shells were considered larvae and embryo stages, respectively. The presence of larvae and embryo stages was assessed with the use of an optical microscope (ML31, MSHOT, Guangzhou, China). The demibranch gills were prepared for H&E staining following the same protocol as outlined above for gonadal tissue. For each specimen, the entire surface area of the demibranchs was systematically inspected with ten microscope fields of view (eyepiece ×10, objective ×10). In incubating individuals, the presence of larvae and embryo stages were separately categorized as being present in groups of <10, between 10–80, and >80 larvae or embryo stages.

Condition index determination

For each month, 90 specimens were selected for the determination of condition index (CI). The soft internal tissues were carefully dissected from the shells. For each specimen, with the use of a precision laboratory balance (FA2004A, Shanghai Jingtian Electronic Instrument Co., Ltd. China), wet weight of the whole specimens was separately determined for the soft tissues and the shell. In all cases, soft tissues and shells were rinsed with distilled water and blotted dry using highly absorbent paper towels prior to weighing. Subsequently, dry weight was determined for soft tissues and shells by drying all soft and hard tissues in a thermostatic oven for over 24 hr (70°C) until a constant weight at reached. All samples were cooled to room temperature in a

desiccator before final weighing for the determination of dry weight (Coughlan et al. 2021). A CI was then calculated for each specimen using a straightforward and commonly accepted equation for *Corbicula* clams (Wang and Chang 2010):

$$CI = (W_1 / W_2) \times 100\% \quad (1)$$

where W_1 is the dry weight of soft tissues (g) and W_2 is the dry weight of the shell (g)

Statistics analysis

All comparative and correlation analyses were carried out using SPSS 22.0 software (IBM, USA). In all cases $\alpha = 0.05$. Initially, data were tested for normality using Shapiro-Wilk test. To assess the population sex ratio, as data were normally distributed, a Pearson's chi-square test was used. A one-way ANOVA was used to assess the proportional change in sex allocation within the population over the duration of the study. For correlation analysis, Pearson's two-tailed significance tests were used for normally distributed data, otherwise the Spearman's two-tailed significance test was used. Figures were created using Origin 2022 software (OriginLab Corp., Northampton, MA, USA).

Results

Over the sampling period, mean (± SE) water temperature was 20.8°C ± 1.83 (min.-max. range:11.0–30.0°C: Table 1). Mean water level was 32.7 m ± 0.75 (29.9–37.7 m), while mean water velocity was 1990.2 m^3/s ± 664.2 (300.0–7299.8 m^3/s) (Table 1, Fig. 1).

Sexual system

Gonad dissections and histological assessment revealed that advanced gametes were present in the sampled *C. fluminea* population over the duration of the 12-month sampling period. Further, the population displayed a relatively stable trioecy sexual system, which included males (n = 49: 12.89%), females (n = 43: 11.32%) and hermaphrodites (n = 288: 75.79%), with an approximate sex ratio of 1:1:6 (Pearson's chi-square: n = 380, χ^2 = 760.00, $p < 0.01$, Fig. 2). However, no males were detected in March and no females were recovered in May. In addition, the proportion of males increased from September to December 2019 and April to August 2020, while the proportion of females and hermaphrodites decreased, and the opposite was true from January to March 2020.

This caused a significant shift in the population sex ratio between these time periods (Pearson's chi-square: n = 380, $\chi^2 = 14.40$, $p < 0.01$, Fig. 2). The proportion of hermaphrodites was significantly higher than that of males and females (One-way ANOVA: n = 380, F (2, 33) = 105.345, $p < 0.05$), and there was no significant difference between males and females ($p > 0.05$) over the duration of the study. Specimens displayed three types of gonadal follicles: testis follicle (male only), ovarian follicle (female only) and amphipathic follicle (i.e., presence of ovotestes), with hermaphrodite specimens displaying all three follicles (Fig. 3). Population gonadal development was not synchronized, as follicles of specimens for all three of the detected sexes displayed different developmental phases at any given time.

Reproductive characteristics

From September to December 2019, and again from June to August 2020, a large number of larvae and embryos were found in the demibranch gills of *C. fluminea* specimens (Fig. 4). From January to May 2020, only a small number of embryos and larvae were observed in the demibranch gills. No larvae or embryos were observed in gonad sections, although larvae and embryos were detected in the demibranch gills of 13 female and 114 hermaphrodites used for the gonadal assessment (total n = 380). When taken together, these data indicate that *C. fluminea* in the lower reaches of Yuanjiang River have only one breeding season (from June to December, Fig. 4). Asynchrony of population reproduction was also detected, with usually only a minor proportion of the sampled specimens found to be brooding at any one time (Fig. 4). The incubation rate for both larvae and embryos was highest in September (53.08%), and lowest in February (10.00%) (Fig. 4). The brooding of larvae and embryos within the inner demibranchs was only observed in female and hermaphrodite *C. fluminea* specimens, and this brooding did not show a seasonal patten (Fig. 4). The mean larvae shell length (n = 106, horizontal axis) of brooding in inner demibranchs was 257.2 μm, (range: 203.4 –278.8 μm) (Fig. 5).

From September to December, the condition index (CI) gradually reduced from 4.32 % ± 0.14 to 2.76 % ± 0.09, and then gradually increased to a maximum of 4.98 % ± 0.17 in May (Table 1). Although slightly reduced from the May peak, the CI remained relatively stable from June to August.

Factors affecting reproduction

The number of embryos and larvae increased as water temperature increased, with these reproductive

activities lasting from September to December 2019 and again from June to August 2020 (Table 1, Fig. 4, Fig. 5). Higher water temperatures of 20.5–30℃ (specimens n = 1060) were associated with increased incubation rates (from 26.92% to 53.08%, for larvae and embryos combined), while lower water temperatures were (11.0℃–17.5℃, specimen n = 520), corresponded to a reduction of the incubation rate (from 10.00% to 39.23%). Overall, incubation rate was significant positive correlated with increasing water temperature (p < 0.01, R^2 = 0.71: Fig. 6). As water temperature increased (from 20.5℃ to 30.0℃), the number of males present in the population increased (from 10.00 % to 40.00 %), while the number of females decreased. However, as water temperature decreased (from 21.0℃ to 11.0℃) between November 2019 to March 2020, the number of males was also observed to decrease (from 16.67 % to 0), and the number of females increased (from 16.67 % to 23.33 %) (Table 1; Fig. 2). The number of males was positively correlated with water temperature (p < 0.05, R^2 = 0.66), with the number of females being negatively correlated with increased temperature (p < 0.05, R^2 = -0.59: Fig. 6).

Discussion

The population of *C. fluminea* residing in the lower reaches of the Yuanjiang River is highly unique. Trioecy populations of *Corbicula* clams are rare and transient, with usually only a small proportion of hermaphrodites (i.e., < 15%) (Morton 1982; Morton 1987; Wang and Chang 2010; Xu et al. 1988). Through consistent sampling, results confirm the hypothesis that this trioecy sexual system is relatively stable and non-transient. Although *C. fluminea* is a hermaphroditic species in invaded areas, which may be functional (Kraemer 1978) or protandric hermaphrodite (Pigneur et al. 2012), or potentially linked to environmental conditions (Morton 1983; Pei et al. 2020; Wang 2018; Wang and Chang 2010; Zhan et al. 2021; Zhao et al. 2012), while the occurrence of hermaphrodites in the native range is usually extremely low, at approximately 0.5–2.0% (Xu et al. 1988). In the present study, however, hermaphrodites accounted for 75.8% of a native population. Further, within its native range, *C. fluminea* tends to have two reproduction peaks per year, in spring and autumn (Li et al. 2018; Zhao et al. 2012), with usually one or two peaks being the norm in the invaded range (Sousa et al. 2008). The present study recorded one breeding seasons, lasting from June until December. As observed by a variety of studies rising temperatures promoted *C. fluminea* breeding activities (Bespalaya et al. 2021; Cataldo and Boltovskoy 1999; Denton et al. 2012; Ilarri and Sousa 2012; Morton 1977). Although mature *C. fluminea* clams

can continuously spawn under favorable conditions (Hadiana et al. 2020; Kraemer and Galloway 1986), this process is normally synchronous (Cataldo and Boltovskoy 1999; Korniushin and Glaubrecht 2003), and might be a breeding strategy to deal with complex environments. Nonetheless, the present study indicates asynchronous reproductive activities occur within the trioecy population inhabiting the lower reaches of the Yuanjiang River. This is underpinned by asynchronous maturation of gametes (Rybalkina et al. 2013), which is likely caused by environmental factors such as water temperature (Enríquez-Díaz et al. 2009; Rybalkina et al. 2013).

Brooding is an important reproductive strategy, which enables *C. fluminea* to protect new juveniles from adverse environment conditions. Initially, only female *C. fluminea* were thought to be capable of incubating larvae (Morton 1977), and although hermaphroditic *C. leana* and *C. australis* can brood larvae (Byrne et al. 2000; Houki et al. 2011), the present study is the first to demonstrate that hermaphroditic *C. fluminea* are also capable of brooding year-round. The morphology of spermatozoa tends to be correlated with mode of reproduction including the brooding of larvae, self-fertilization and hermaphroditism (Franzén 1983). The spermatozoa of *Corbicula* clams (including *C. fluminea*) belong to the modified type, and have an elongated head and one or two flagella (Pigneur et al. 2012; Wang and Chang 2010). In particular, the nonreductional bi-flagellate spermatozoa type of *Corbicula* clams are thought to be related to brooding and androgenesis behaviors (Byrne et al. 2000; Pigneur et al. 2012). However, *C. fluminea* in Dongting Lake basin (including Yuanjiang River) has been observed to produce both uniflagellate and bi-flagellate spermatozoa (Wang 2018). To ascertain whether the brooding behaviors of *C. flumiena* in lower reaches of Yuanjiang River are linked to environmental factors (e.g., water temperature) requires further study. There is no comparable literature for this phenomenon of brooding for *C. fluminea* within the native range. Interestingly, in the present study, the number of *C. fluminea* with embryo and larvae were greater in autumn 2019 (25.38–53.08 %) than in spring 2020 (10.00–22.31 %), and this contrasts with records from *Corbicula's* invaded range, which show greater in brooding occurrence in spring rather than in autumn months (Aldridge and McMahon 1978; Kennedy and Huekelem 1985). For survival and reproduction, *C. fluminea* may need to adapt to the local environmental conditions, such as temperature and salinity (Bespalaya et al. 2021; Tos et al. 2021), which may be an adaptive breeding strategy of *C. fluminea* in lower reaches of Yuanjiang River as well as the invaded range. This may also be the reason for its substantial reproductive output in the invaded range (Cao et al. 2017). In addition, it may be because the genetic diversity of *C. fluminea* in the native range is much higher than that observed in invaded areas (Wang et al. 2014; Zeng et al.

2022a; Zhan et al. 2021), which may underpin the observed breeding strategies. Nonetheless, differences among populations of *C. fluminea* across the clams native and the invaded ranges require further research.

CI analysis can provide for an indirect estimate *C. fluminea* reproductive activity, as well as allow for prediction of adult readiness to begin spawning (Hadiana et al. 2020). In general, the maximum CI of *C. fluminea* populations tends to coincide with the peak reproductive period (Wang and Chang 2010) and usually appears in May (Mladineo et al. 2007). The CI pattern documented by the present study strongly aligns with this expected trend. The marked decrease in CI values from September to December 2019, which was the period of lowest water level, and slightly lower values detected for June to August 2020 (when compared with January to May) closely align with other indicators that suggest June to December is the main breeding season. This included the June and July period, which displayed some of the highest larvae numbers, as well as some of the highest water levels. Our data suggest that *C. fluminea* ceased reproductive activities during the cooler winter period, but rapidly increased its CI during this time in advance of initiating reproductive activities through increased nutrient storage (i.e., protein, carbohydrate, lipid) (Ilarri et al. 2021).

Corbicula fluminea entered the breeding season in spring, and rising water temperature may have contributed to this drop in CI through increased metabolic rate (Kesler 2004), yet the rising water temperature and water level (providing a relatively dark environment) gradually may promote increased feeding activity of *C. fluminea* (Pouil et al. 2021), so that the CI is maintained through a dynamic balance. Although the reproductive activity remained at a relatively high level, the water temperature and water level decreases gradually from September to December, the CI weakened gradually. This may be due to changes in environmental factors that reduce feeding activity and energy for reproduction rather than growth (Pouil et al. 2021). Although food availability was not assessed, the food availability tends to be a significant contributor to the timing of *C. fluminea* spawning events as clams will need to meet the energetic demand of breeding (Denton et al. 2012) and larval development (Delgado and Camacho 2005; Teaniniuraitemoana et al. 2016). Further work should consider the effect of food availability on both the allocation of sex and timing of breeding activities for this trioecy population. Although water level and water flow were not significantly correlated with sex allocation or breeding activities, the peak incubation period was also the period when water flow and water level were the maximum in this study and have previously shown a synchronous relationship (Tos et al. 2021). The released D-shaped larvae are not capable of swimming (King et al. 1986; McMahon 1983; Wang and Chang 2010), and increased water

velocity will likely facilitate dispersal (Coughlan et al. 2017; Dubois and Martínez-Ortí 2010; Ernandes-Silva et al. 2016; Pernecker et al. 2021; Ramli et al. 2022; Thomaz et al. 2007).

Water temperature can play a decisive role in the sex allocation of bivalves (Dalpé et al. 2022), with a trend toward a higher proportion of males at higher temperatures having previously been observed in the marine bivalves *Mytilus edulis* (Dalpé et al. 2022) and *Crassostrea gigas* (Santerre et al. 2013). In the present study, water temperature, water velocity and water level tended to peak during the June to September period, along with an increase in the male population of *C. fluminea*. It is possible that increased numbers of males are more advantageous during higher water temperature, water velocity and water level conditions, to ensure successful fertilization of eggs spawned by females and hermaphrodites (Houki et al. 2011), as *C. fluminea* is considered to be an androgenesis species (Pigneur et al. 2012). It has been suggested that *C. fluminea* can avail of the phenomenon of sex switching, to facilitate change from male to female or from female to hermaphrodite during a specimen development (Morton 1977; Tejima et al. 2020). However, the *C. fluminea* specimens obtained from the lower reaches of Yuanjiang River had reached sexual maturity (20.72-34.80 mm shell length), with the monthly change of male and female sex ratio having a significant correlation with water temperature. Whether sex conversion occurs in *C. fluminea* in lower reaches of Yuanjiang River under the influence of temperature needs to be verified by further research. Salinity is a very important environmental factor, which appears to be the main factor shaping the distribution of *COI* haplotype distribution of *Corbicula* lineages within their native range and likely affected the Corbicula's distribution and dispersal along the Yangtze River (Zeng et al. 2022a), as well as the sex distribution and reproductive mode of *Corbicula* (Morton 1982; Wang and Chang 2010). However, as the sampling site for this study was a strictly freshwater habitat located about 1,500 kilometers from the Yangtze River estuary, salinity gradients are unlikely to influence the sex ratios within the assessed population. Overall, several studies have demonstrated that environmental factors may influence reproduction of *Corbicula* clams (Morton 1977; Rajagopal et al. 2000), and that *C. fluminea* can attempt to take corresponding countermeasures as the environment changes. These strategies are adopted to maximize population reproductive success relative to the environment (Breton et al. 2017). However, the extent of this plasticity in indigenous populations remains unknown. This study has provided for a unique insight into an indigenous population of *C. fluminea*. However, many questions concerning the evolutionary, ecological and management implications of a stable trioecy population still need to be explored.

Authors' contributions

Deliang Li conceived the idea. Jie Pi, Yangxin Tang and Linwei Liu have performed the *C. fluminea* sampling, measurement and tissue sections preparation. Jie Pi, Deliang Li, Neil E. Coughlan and Yangxin Tang performed results analysis and writing of the manuscript. Xinhua Liu and Jianguo Xiang took part in manuscript preparation. All co-authors have approved publication of this manuscript.

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Data availability

The datasets generated during and/or analysed during the current study are available from the first author on reasonable request.

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575

576 **Table 1.** Water temperature (WT), water level (WL) and water velocity (WV) data from the sampling
577 site situated in the lower Yuanjiang River, Changde, Hunan, China (111°41′57.66″E, 28°59′34.11″N), as
578 well as mean (± SE) monthly *Corbicula fluminea* biometric measurements from September 2019 to
579 August 2020. The data of water level and water velocity are from the day the *C. fluminea* specimens was
580 collected. W_1 dry weight of shell, W_2 dry weight of soft tissues, CI condition index.
581

Month	WT (°C)	WL (m)	WV (m^3/s)	n	W_1 (g)	W_2 (g)	CI (%)
Sep.	26.0	29.9	300.0	90	0.15 ± 0.01	3.42 ± 0.10	4.32 ± 0.14
Oct.	24.0	30.1	385.0	90	0.14 ± 0.00	3.43 ± 0.09	4.01 ± 0.11
Nov.	21.0	30.0	421.0	90	0.15 ± 0.00	4.14 ± 0.10	3.67 ± 0.08
Dec.	17.5	30.9	429.1	90	0.10 ± 0.00	3.63 ± 0.08	2.76 ± 0.09
Jan.	11.0	31.4	720.3	90	0.17 ± 0.01	3.72 ± 0.10	4.58 ± 0.13
Feb.	11.0	32.7	2343.4	90	0.14 ± 0.00	3.65 ± 0.09	3.90 ± 0.11
Mar.	13.5	33.0	1994.5	90	0.15 ± 0.01	3.47 ± 0.09	4.32 ± 0.15
Apr.	20.5	32.6	1696.9	90	0.12 ± 0.00	3.02 ± 0.07	4.01 ± 0.10
May.	23.5	32.5	1595.2	90	0.17 ± 0.01	3.36 ± 0.08	4.98 ± 0.17
Jun.	24.5	37.4	7299.8	90	0.12 ± 0.01	2.98 ± 0.06	4.12 ± 0.15
Jul.	27.5	37.7	5997.3	90	0.11 ± 0.00	2.96 ± 0.11	3.78 ± 0.11
Aug.	30.0	33.7	699.4	90	0.12 ± 0.00	2.92 ± 0.11	4.20 ± 0.11
Mean ± SE	20.8 ± 1.83	32.7 ± 0.75	1990.2 ± 664.2	90	0.14 ± 0.00	3.42 ± 0.03	4.06 ± 0.04

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Figure legends

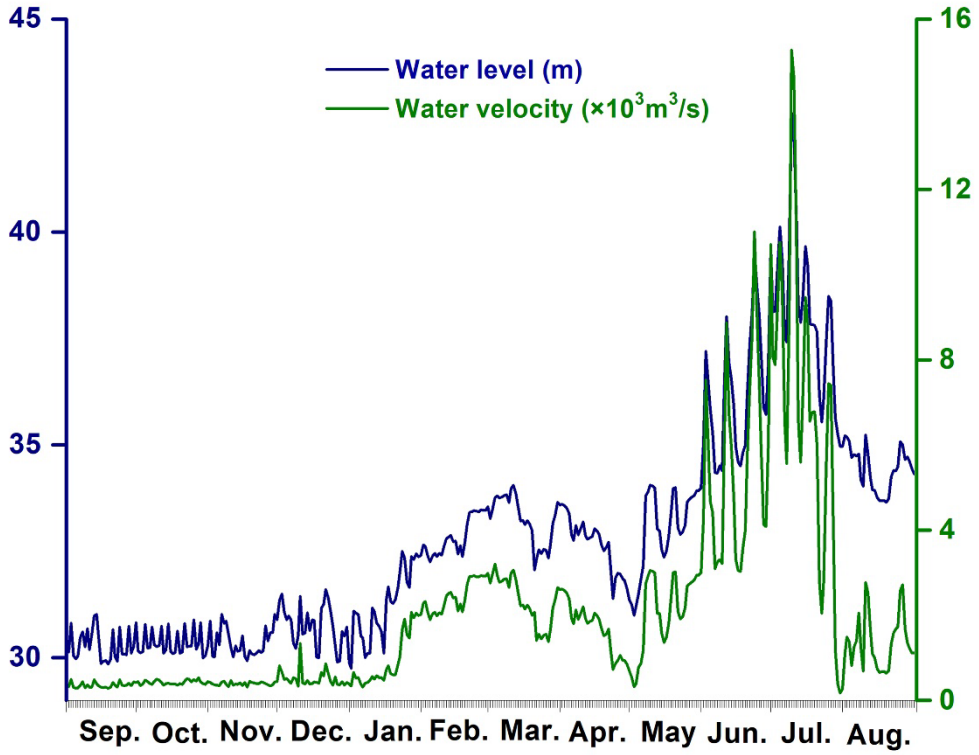


Fig. 1 Daily changes of water level and water velocity in lower reaches of Yuanjiang River, Changde, Hunan, China, between September 2019 to August 2020

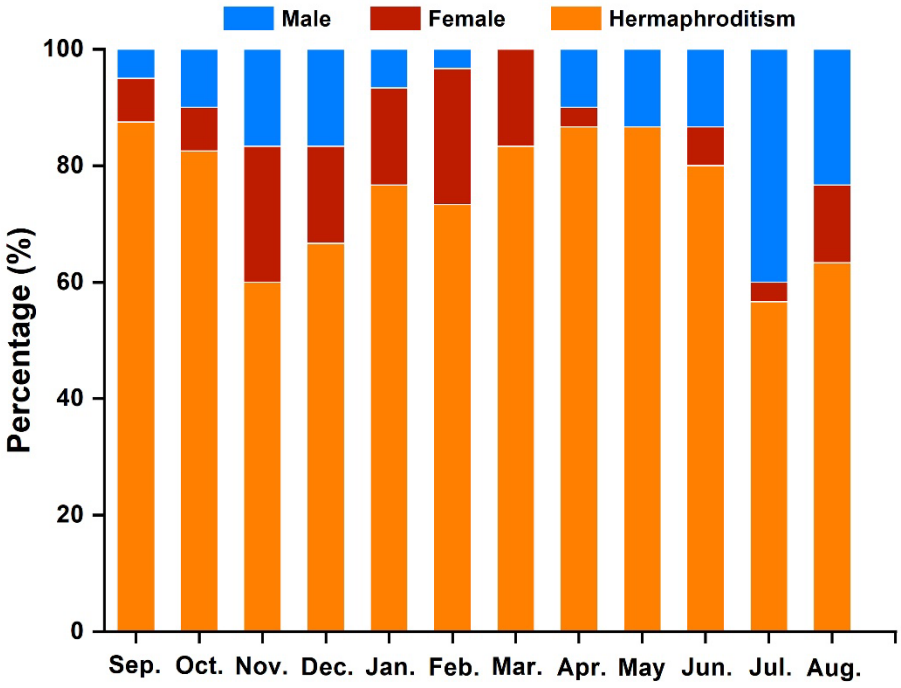
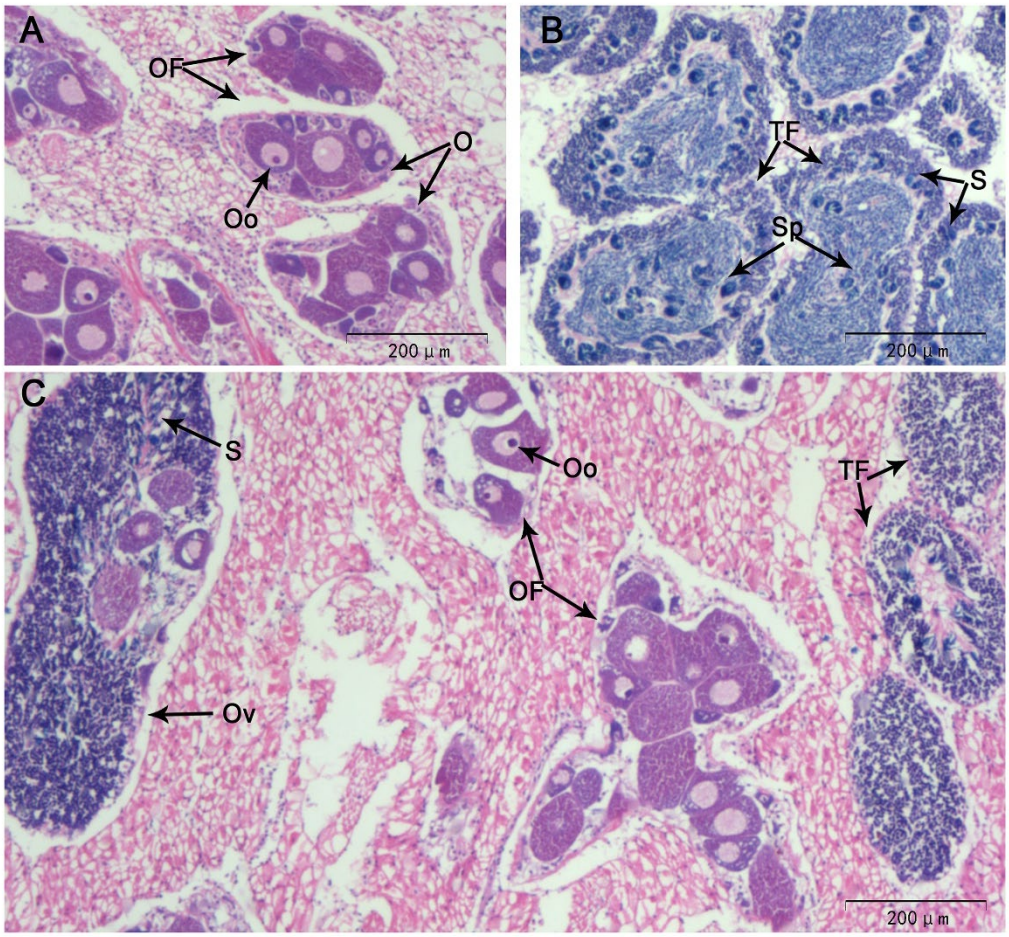


Fig. 2 The monthly percentage of sex allocation in *Corbicula fluminea* inhabiting the lower Yuanjiang River, Changde, Hunan, China, between September 2019 and August 2020

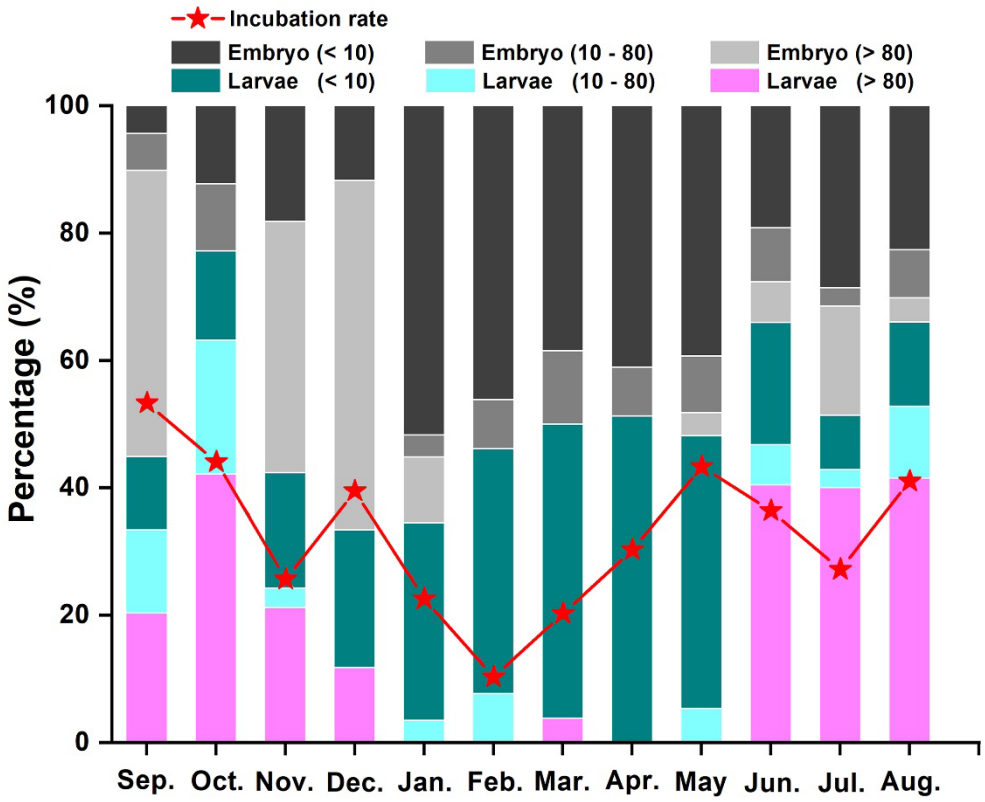


598

599 **Fig. 3** Section observation of difference sex allocation by *Corbicula fluminea*. **A** female; **B** male; **C**

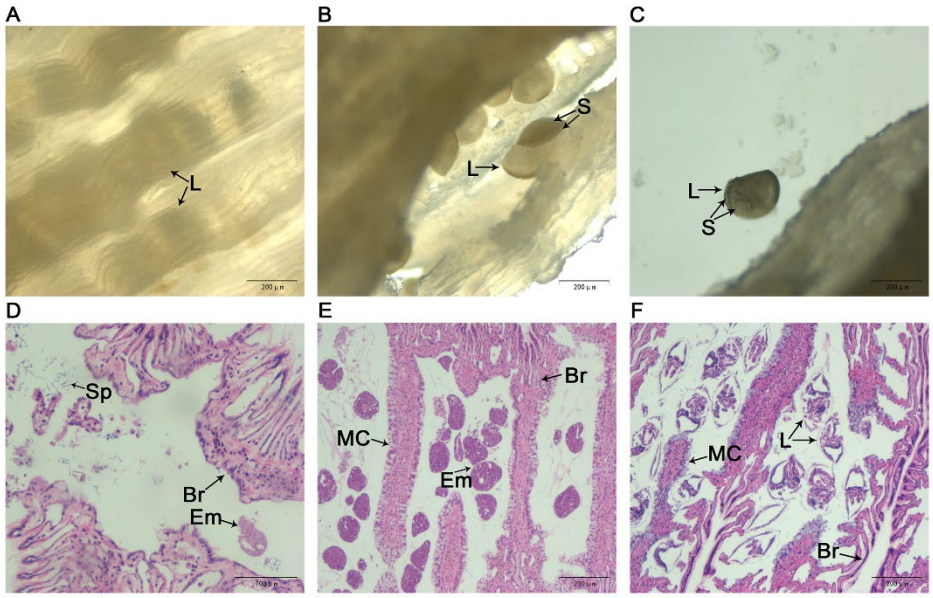
600 hermaphrodite. *OF* ovarian follicle, *O* oocyte; *TF* testis follicle, *S* spermatogonium, *Sp* sperm, *Ov*

601 ovotestes



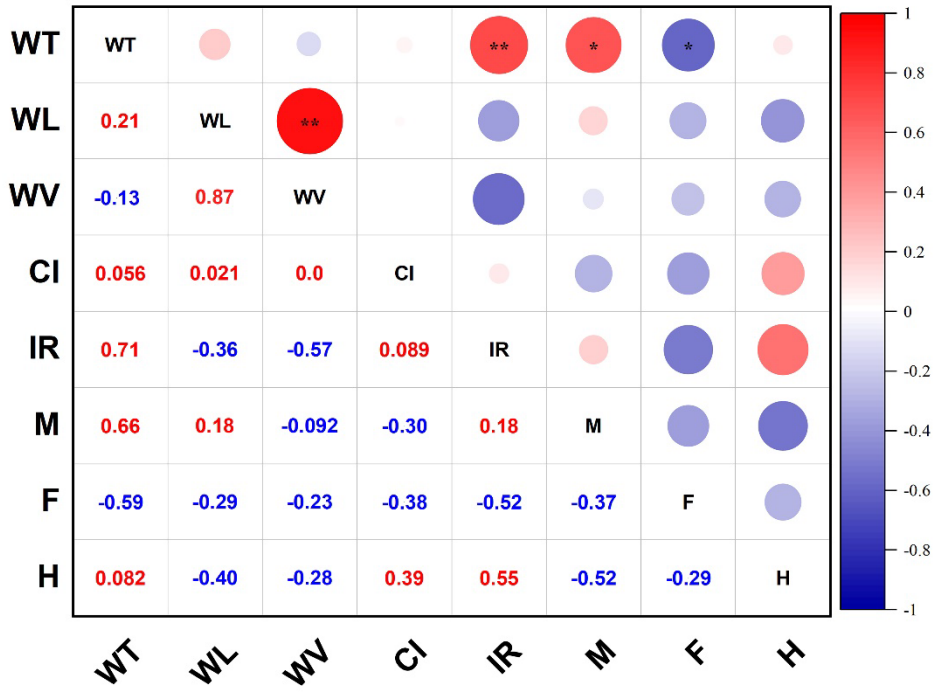
602

603 **Fig. 4** The percentage of incubation type and incubation rate of *Corbicula fluminea* inhabiting the lower
604 Yuanjiang River, Changde, Hunan, China, between September 2019 and August 2020. Fertilized eggs
605 with and without shells were considered larvae and embryo stages, respectively (see Fig. 5). For each
606 specimen, the surface area of the demibranchs was systematically inspected with ten microscope fields
607 of view (eyepiece ×10, objective ×10). The presence of larvae and embryo stages were separately
608 categorized as groups of < 10, between 10–80, and > 80 larvae or embryo stages



609

610 **Figure 5** Incubation observation of *Corbicula fluminea* in demibranch gills. **A-C** observation of the
611 incubation of larvae in the demibranchs. **D-F** observation of embryos in the demibranchs by hematoxylin-
612 eosin staining. *L* larvae, *S* shell, *Sp* sperm, *Br* branchia, *Em* embryo, *MC* mucous cells



613

614 **Figure 6** Correlation analysis of water temperature (WT), water level (WL), water velocity (WV), as
615 well as the number of *Corbicula fluminea* males (M), females (F), hermaphrodites (H), clam condition
616 index (CI), and incubation rate (IR). Red indicates a positive correlation, while blue indicates negative
617 correlation. The larger the circle, the greater the correlation coefficient. * = $p < 0.05$. ** = $p < 0.01$.

618

619