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Research article

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Effects of Microscopic Hydrodynamic Conditions and Exposure Time on the Biomass of Benthic Diatoms

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Abstract:

Background: Benthic algae are at the bottom of the nutrient chain in the river ecosystem and are the basic guarantee to maintain the health of the river ecosystem. Human activities lead to changes in the hydrology and hydrodynamics of many rivers, which affect the growth of benthic diatoms. However, there is a lack of relevant studies on the effects of hydrodynamic conditions and exposure time on the growth of benthic diatoms, so the protection of benthic diatoms and other bait resources is difficult to be realized.

Methods: Based on this, a continuous field observation was carried out to study the growth situation of benthic diatom on pebbles under different flow patterns. The three-dimensional hydrodynamic numerical simulation was used to further analyze the flow field surrounding the pebble. And then, the effects of hydrodynamic conditions and exposure time on the biomass of benthic diatoms were studied.

Result: The research results show that (1) the species composition and dominant species of benthic diatoms are different at different growth stages; (2) there is an appropriate velocity range for the growth of benthic diatoms, and too low and too high flow rates are not conducive to the growth of benthic diatoms; (3) the growth suitability at different parts on the pebble for benthic diatoms from high to low is the rear part of the pebble (without direct erosion), the side parts of the pebble, the upper part of the pebble and the front part of the pebble (direct erosion); (4) most benthic diatoms die if exposed to air for more than four hours.

Conclusion: The effects of hydrodynamic conditions on the biomass of benthic diatoms are obvious on the microscopic scale. The research results in this paper are helpful to further understand the growth status of benthic diatoms in microscopic environment and provide reference and information for the restoration of the hydraulic habitat of benthic diatoms in the reach affected by human activities.

Keywords: benthic diatoms; hydrodynamic conditions; microscopic level; biomass; exposure time

1. Background

The stability of ecosystem in the river is the basis of maintaining its biodiversity. As the bottom of the nutrient chain in the river ecosystems, algae is an important link of material circulation and energy flow in the aquatic ecosystem, and plays an key regulating role in the river ecosystem. Diatoms play an important role in a wide variety of algae, and they dominant in biomass and density in many rivers in China (Hong S and Chen JS 2002). They are widely distributed and have a short life cycle (Dalu T et al. 2017). Many types of fish feed on them, and they are an important bait source in rivers (Mei H et al. 2003). Diatoms can be divided into benthic diatoms and planktonic diatoms. Benthic diatoms account for a large proportion of the primary production of the entire water body, sometimes even exceeding the primary production of planktonic algae. If the coastal area is large and the higher aquatic plants are flourishing, the primary production of the attached algae can account for 40%-50% of the total primary production (Wetzel RG and Pickard D 1996). Compared with planktonic diatoms, benthic diatoms can stabilize the substrate in the faster river flow, providing rich living environment and important habitat for other aquatic organisms (Hill BH et al. 2003; Wang ZY et al. 2013). Therefore, it is of great significance to protect benthic diatoms for the stability of river ecosystem.

Benthic diatoms, because of their fixed growth position, can quickly and accurately reflect the continuous changes of water in a specific region (Yi R et al. 2015), and are considered as a powerful indicator of water quality in freshwater systems (Tatenda D et al. 2016; Cox EJ 2012; Guy B et al. 2004). Because of this, most studies on benthic diatoms focus on the relationship between the growth of benthic diatoms and the water quality indexes such as nutrient concentration and water temperature (Liu L et al. 2019; Sabater S et al. 2010; Ndiritu GG et al. 2003), which has a positive effect on the protection of benthic diatoms. However, the ecological problems facing many rivers in China are not only the changes of water quality, but also the changes of hydrodynamics and hydrologic situation. After decades of water conservancy and hydropower development in China (Zhu ZX et al. 2020; Wang C 2017), the hydrological situation of many rivers has changed and the water reduced river reaches have appeared (Dong ZR 2013). The rapid change of hydrodynamics conditions has caused serious damages to the aquatic habitats and posed a serious threat to the aquatic ecosystems (Li SC et al. 2014; Karr JR 1991; Petts GE 1984). The water reduced river reach downstream of the power station often exposes a large amount of the riverbed substrate to the air, and the operation of the power station also causes some benthic diatoms to be regularly exposed to the air. However, the study on the exposure time of benthic diatoms is lagging behind, which is a challenge for the protection of benthic diatoms. Even in many rivers that are far away from cities and rich in biological resources, changes in hydrodynamic conditions and hydrological conditions have a greater negative impact on river ecosystems than changes in water quality.

Hydraulic disturbance and flow variation are one of the decisive factors affecting the biomass of benthic diatoms (Lohman K et al. 1992). The intermediate transition zone between the river bank and the channel is often the location with the largest biomass of benthic diatoms (Bunn SE et al. 2003), which also indicates that benthic diatoms have obvious selectivity for the growth environment velocity and water depth. The effect of water depth on the reproduction and growth of benthic diatoms is mainly reflected in the population density. At each water depth gradient, the biomass of algae is first increased and then decreased (Wu XD et al. 2011). Within the critical depth range, there is an optimal growth and reproduction point for aquatic organisms. If it is above or below this range, the growth quality of organisms will decrease (Gafny S and Gasith A 1999). This phenomenon also exists in the growth of benthic diatoms (Cai DS et al. 2014). Flow rate is also an important factor affecting the growth of benthic diatoms. The Special Committee on Environmental Issues of The Japanese Ecological Society divided diatoms into five growth types: true still water species, still water preferable species, uncertain species, flowing water preferable species and true flowing water species (JESEIC 1987). The dominant species of diatom community in rivers are uncertain species and flowing water preferable species, while the still water species are dominant in lakes and reservoirs. The biomass of benthic diatoms in the river increases with the increase of river flow rate, but decreases with the increase of river flow rate (Wang CH and Zhang JT 2004). At present, the research on the relationship between the growth of benthic diatoms and the hydrodynamics mainly focuses on the influence of water depth and velocity (Wang H et al. 2017; Wang K et al. 2019), and there are few further studies on the response of benthic diatoms to microscopic hydrodynamic conditions. Furthermore, studies on the effects of exposure time on the biomass of benthic diatoms are lagging behind. The current studies mainly focus on the effects of light intensity and light quality on the photosynthetic rate and the growth of benthic diatoms. The response relationship between the exposure time and the biomass of benthic diatoms under natural light conditions is rarely studied.

In this study, the growth of benthic diatoms on pebbles under different flow patterns was observed continuously in the field. On this basis, a three-dimensional hydrodynamic numerical simulation was used to further analyze the flow field around the pebble, and the effects of the hydrodynamic conditions and the exposure time on the biomass of benthic diatoms were studied on the microscopic scale. The research results in this paper are helpful to further understand the growth status of benthic diatoms in microscopic environment and provide reference and information for the restoration of the hydraulic habitat of benthic diatoms in the reach affected by human activities.

2. Methods

2.1. Study Area

In this study, a relatively stable river section of Baitiao River in Dujiangyan, southwest China, was selected as the research section (Fig. 1). The growth of benthic diatoms on the pebbles was observed continuously in the field by placing the pebble substrate on the bank of the study section and setting the two conditions of still water and high velocity water as control experiments. On this basis, the three-dimensional hydrodynamic numerical simulation was used to further analyze the flow field around the pebble, and the growth pattern of benthic diatom and the influence of microscopic hydrodynamic conditions on its biomass were studied.

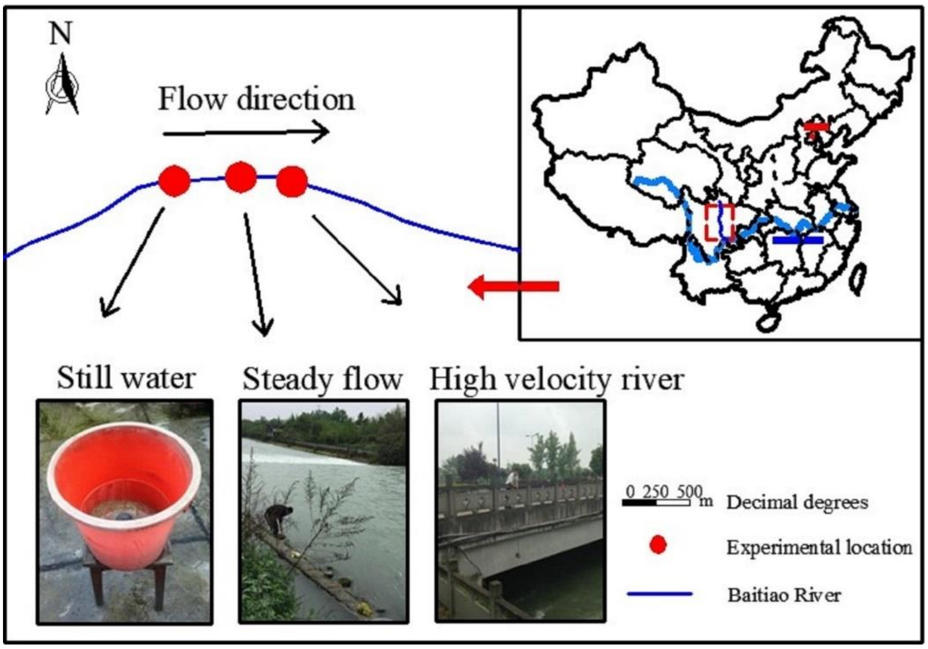


Fig. 1. Study area and experimental setup

2.2. Field Experiment

The experiment on the growth of benthic diatoms on pebble substrate was carried out at the selected experimental site on Baitiao River from April 17 to June 5, 2017. Pebbles of similar shape and size were selected, connected by a wire rope and placed in water, with the space of 0.8m apart (Fig. 2). The water temperature, velocity and depth were measured daily, and the benthic diatoms were brushed from one pebble substrate every six days, and the water samples were collected and taken back to the laboratory for analysis. At the same time, a pebble substrate was suspended from the upstream bridge 380 m from the experimental point to observe the growth of benthic diatoms under the condition of high flow rate. A bucket was selected as the container for the stationary water experiment. Pebbles were placed in the bucket and submerged in the river in the study area. The water in the bucket was changed every three days to observe the growth of benthic diatoms under the stationary water condition.

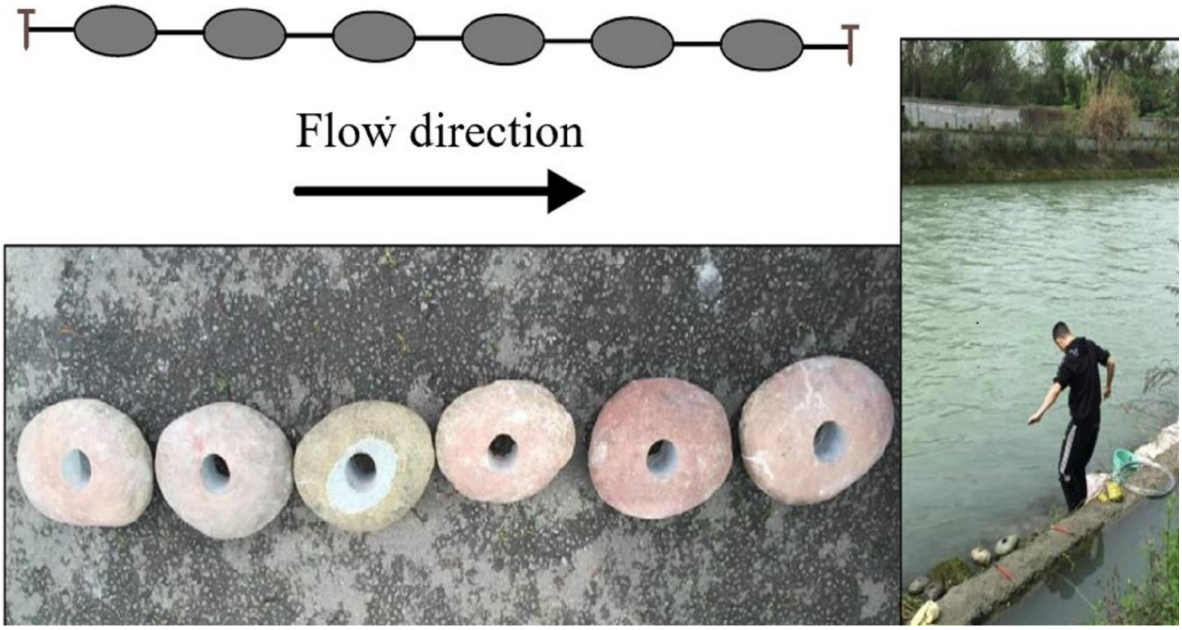


Fig. 2. Setting of pebble substrates

During sampling, the surface of pebble substrate was divided into five sampling points: front, rear, left, right and up (Fig. 3).

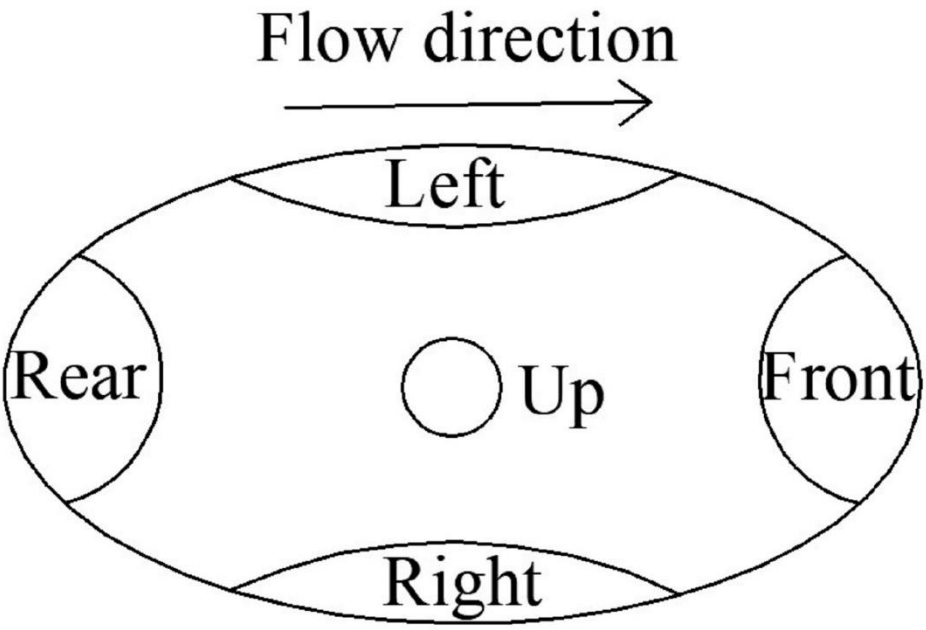


Fig. 3. Zoning of pebble substrate

2.3. Determination Method of Benthic Diatom Biomas

Benthic diatom biomass refers to the mass of organic matter per unit area of algae on the substrate. To ensure the reliability of the results, two methods, Chla (chlorophyll a) and cell density, were used to determine the biomass of benthic diatoms in water samples collected in field experiments.

According to the sampling method recommended by the US Environmental Protection Agency, algae with a specific surface area was brushed on pebbles and mixed into 200mL of pure water. After fully mixing, the algal solution was divided into two 100mL sampling bottles and brought back to the laboratory. One of the samples is fixed with 1% Lugo iodine solution. This sample was used for qualitative and quantitative analysis of benthic diatoms, with the addition of fixed solution samples for species identification and density statistics. The other sample was used for chlorophyll a measurement.

For the identification of benthic diatoms, the samples with the addition of Lugo iodine solution were set for sedimentation under dark conditions for 24 hours, and then the supernatant was extracted with a siphon to a constant volume of 50mL. After fully shaking the 50mL standby qualitative sample, 0.1mL of the sample was absorbed into the counting frame according to the identification method of algae in "Specifications for Investigation of Lake Eutrophication" (Jin XC and Tu QY 1990). After covering by the cover glass, the sample was classified and identified under the microscope. The main reference materials for the classification and identification of benthic diatoms are "Atlas of Common Algae in Inland Waters of China" (Deng J et al. 1990), "Freshwater Algae of China - System, Classification and Ecology" (Hu HJ and Wei YX 2006) and "Color atlas of Diatoms" (Cheng ZD 1996).

For density statistics, 3 to 5 lines were selected to count line by line, and the whole slide was counted if the number is small. At least 2 slides were observed for each sample, and if the difference between the 2 slides and the mean was ±15%, a third slide was counted. Finally, the number of diatoms in each sampling point was calculated, and the diatom density on the pebble per unit area was converted according to the formula (unit: pcs/cm^2).

$$N_i = \frac{C_1 \cdot L \cdot n_i}{C_2 \cdot R \cdot h \cdot S} \tag{1}$$

where, N_i is the number of algae species i per unit area, pcs /cm^2; C_1 is the constant volume of water required by the specimen, mL; C_2 is the actually counted volume, mL; L is the length of each side of the algal count box, μm; R is the number of counted lines; h is the distance of parallel lines in the field of view, μm; n_i is the actually counted number of algae species i; S is the total area of the substrate scraped, cm^2.

When measuring the chlorophyll A, 90% acetone was used to extract the chlorophyll and the absorbance was determined at four wavelengths of 750nm, 665nm, 645nm and 630nm, respectively. And then the chlorophyll A content was calculated by the formula (Ministry of Ecology and Environment of the People's Republic of China 2002).

$$Chl\ \ a = \frac{[11.64 \times (D_{663} - D_{750}) - 2.16 \times (D_{645} - D_{750}) + 0.10 \times (D_{630} - D_{750})] \cdot V_1}{V \cdot \delta} \tag{2}$$

where, V is the volume of water sample, L; D is the absorbance; V_1 is the volume of the extract after constant volume, mL; δ is the colorimetric dish path, cm.

2.4. 3D Hydrodynamic Model and Its Boundary Conditions

2.4.1. 3D Hydrodynamic Model

In order to further analyze the flow field around the pebble and to study the influence of microscopic hydrodynamic conditions on the growth of benthic diatoms, a three-dimensional hydrodynamic model was used to simulate the growth of benthic diatoms. The calculation model was a standard 3D model, and the governing equations were as follows:

1. Continuity equation:

$$\frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} + \frac{\partial w}{\partial z} = 0 \tag{3}$$

2. Momentum equation:

$$\frac{\partial u}{\partial t}+u\frac{\partial u}{\partial x}+v\frac{\partial u}{\partial y}+w\frac{\partial u}{\partial z}=-\frac{1}{\rho}\frac{\partial \rho}{\partial x}+(\nu+\nu_t)\left(\frac{\partial^2 u}{\partial x^2}+\frac{\partial^2 u}{\partial x\partial y}+\frac{\partial^2 u}{\partial x\partial z}\right) \tag{4}$$

$$\frac{\partial v}{\partial t}+u\frac{\partial v}{\partial x}+v\frac{\partial v}{\partial y}+w\frac{\partial v}{\partial z}=-\frac{1}{\rho}\frac{\partial \rho}{\partial y}+(\nu+\nu_t)\left(\frac{\partial^2 v}{\partial x\partial y}+\frac{\partial^2 v}{\partial y^2}+\frac{\partial^2 v}{\partial y\partial z}\right) \tag{5}$$

$$\frac{\partial w}{\partial t}+u\frac{\partial w}{\partial x}+v\frac{\partial w}{\partial y}+w\frac{\partial w}{\partial z}=-\frac{1}{\rho}\frac{\partial \rho}{\partial z}+(\nu+\nu_t)\left(\frac{\partial^2 w}{\partial x\partial z}+\frac{\partial^2 w}{\partial y\partial z}+\frac{\partial^2 w}{\partial w^2}\right)+g \tag{6}$$

3. Turbulent kinetic energy k equation and turbulent dissipation rate equation:

$$\frac{\partial k}{\partial t}+u\frac{\partial k}{\partial x}+v\frac{\partial k}{\partial y}+w\frac{\partial k}{\partial z}=\frac{\partial}{\partial x}\left(\frac{\nu_t}{\sigma_k}\frac{\partial k}{\partial x}\right)+\frac{\partial}{\partial y}\left(\frac{\nu_t}{\sigma_k}\frac{\partial k}{\partial y}\right)+\frac{\partial}{\partial z}\left(\frac{\nu_t}{\sigma_k}\frac{\partial k}{\partial z}\right)+\frac{G_k}{\rho}-\varepsilon \tag{7}$$

$$\frac{\partial \varepsilon}{\partial t}+u\frac{\partial \varepsilon}{\partial x}+v\frac{\partial \varepsilon}{\partial y}+w\frac{\partial \varepsilon}{\partial z}=\frac{\partial}{\partial x}\left(\frac{\nu_t}{\sigma_z}\frac{\partial \varepsilon}{\partial x}\right)+\frac{\partial}{\partial y}\left(\frac{\nu_t}{\sigma_z}\frac{\partial \varepsilon}{\partial y}\right)+\frac{\partial}{\partial z}\left(\frac{\nu_t}{\sigma_z}\frac{\partial \varepsilon}{\partial z}\right)+\frac{\varepsilon}{k}C_{1\varepsilon}\frac{G_k}{\rho}-C_{2\varepsilon}\frac{\varepsilon^2}{k} \tag{8}$$

where,

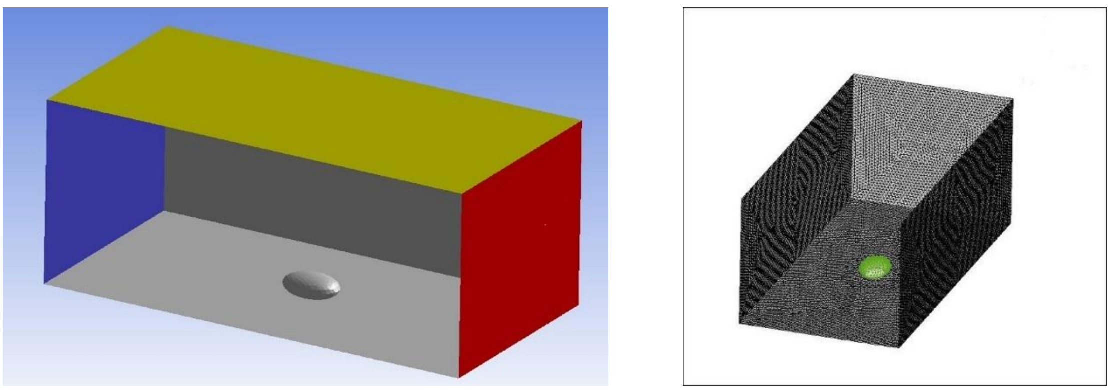
$$G_k=\rho\nu_t\left\{2\left[\left(\frac{\partial u}{\partial x}\right)^2+\left(\frac{\partial v}{\partial y}\right)^2+\left(\frac{\partial w}{\partial z}\right)^2\right]+\left(\frac{\partial u}{\partial y}+\frac{\partial v}{\partial x}\right)^2+\left(\frac{\partial u}{\partial z}+\frac{\partial w}{\partial x}\right)^2+\left(\frac{\partial v}{\partial z}+\frac{\partial w}{\partial y}\right)^2\right\} \tag{9}$$

$$\nu_t=C_\mu\frac{k^2}{\varepsilon} \tag{10}$$

where, u, v and w are velocities in x, y and z directions, separately, m/s; ρ is the water density, kg/m^3; p is the time average pressure, Pa; ν and ν_t are the molecular viscosity coefficient and turbulent vortex viscosity coefficient of water, separately; g is the gravitational acceleration, m^2/s; k (m^2/s^2) and ε (m^2/s^3) are the turbulent energy and turbulent energy dissipation rate, separately; σ_k, σ_ε, $C_{1\varepsilon}$, $C_{2\varepsilon}$ and C_μ are all empirical constants, which are taken as C_μ=0.09, $C_{1\varepsilon}$=1.44, $C_{2\varepsilon}$=1.92, σ_k=1.0 and σ_ε=1.3 in the model.

2.4.2. Boundary Conditions of the Model

In this study, to better extract the hydrodynamic conditions at the microscopic level, the model was generalized into pebbles in the tank, as shown in Fig. 4a. The dimension of the tank is 2 m × 1 m × 0.8 m (L×W×H), and the dimension of the pebble is 0.13 m × 0.09 m × 0.1 m (long axis radius × short axis radius × height). The model was generated with tetrahedral uninstitutionalized meshes, and the number of meshes was 1,138,150 (Fig. 4b). Given the velocity inlet, the measured velocity during the field experiment was mainly concentrated in the range of 0.4 m/s to 0.5 m/s. The velocity was finally set as 0.5 m/s and the outlet was free flow.



(**a**) (**b**)

Fig. 4. (a) A generalized model for microhabitat studies; (b) Mesh generation.

3. Results and Discussion

3.1. Changes of Physical and Chemical Indexes of River Water during Experiment

During the experiment, the flow velocity and the water depth of the river changed steadily (Fig. 5). The flow velocity varied from 0.3 m/s to 0.6 m/s, but the flow velocity mainly concentrated in the range of 0.4 m/s to 0.5 m/s during the experiment. The water depth varied from 0.29 m to 0.62 m, but most of them fluctuated around 0.4 m. The variation ranges of both the velocity and the water depth were not large, which can provide a stable habitat for the formation and growth of benthic diatoms.

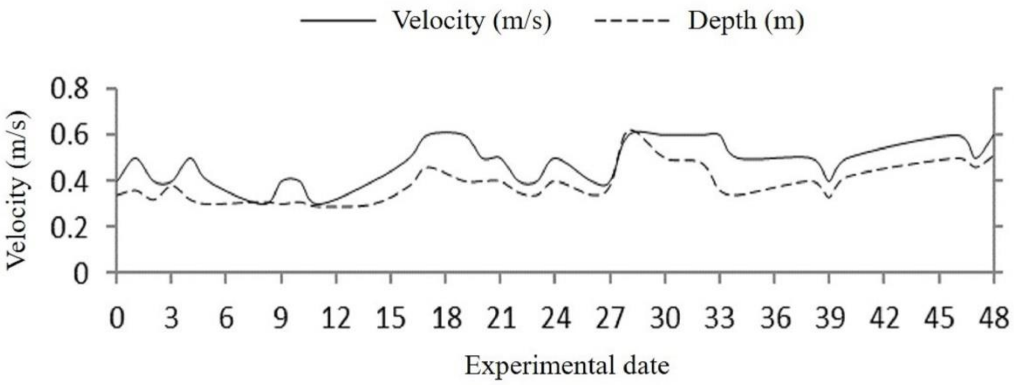


Fig. 5. Variation of velocity and water depth during the experiment.

During the experiment, the changes of physical and chemical indexes such as DO, turbidity, electrical conductivity, pH, COD, TP and TN were also monitored. The changes of environmental factors during the experiment are listed in Table 1. It can be seen from the table that during the experiment, the physical and chemical factors in the water are all within the appropriate range for the growth of benthic diatoms. Generally speaking, the change range of physical and chemical factors is very small, and the visible environmental conditions are basically stable during the growth cycle of benthic diatoms, which can meet the growth needs of benthic diatoms.

Table 1. Changes of environmental factors during the experiment.

Indexes	DO (mg/L)	Turbidity (NTU)	Electrical conductivity (μs/cm)	pH	COD (mg/L)	TP (mg/L)	TN (mg/L)
Range	6.4-7.95	7.43-22.2	257-308	7.41-7.95	11.48-21.94	0.093-0.244	2.14-2.92

3.2. Growth of Benthic Diatoms on Pebble Substrate

3.1.1. Changes of Benthic Diatoms Biomass over Time

Throughout the test period, samples of benthic diatoms on the pebble substrate were taken every 6 days, and then were counted and measured for chlorophyll. Based on the experimental data, the growth curve of benthic diatoms was plotted, as shown in Fig. 6. The growth of microorganisms basically conforms to the Logistic growth curve, and the growth cycle can be divided into four phases: the delay phase, which is a slow grow and multiply phase of algae when inoculated into a new growing environment; the logarithmic phase, during which the cells grow exponentially; the stable phase, which is a phase with decreased rate of cell division, prolonged generation time, and decreased cell activity; the death phase, namely the decline phase, which is the phase of algal population decays. The growth curves of benthic diatoms vary in different environments, which are mainly affected by seasons, temperature, light, water nutrition and other conditions.

From the changes of benthic diatom biomass (diatom density and Chla concentration) on the pebble substrate in each sampling period, the colony building process of benthic diatom in this study can be divided into three stages. The first stage is the early colonization period (from the first to the fourth period). This stage is the delay phase with low biomass and slow increasing speed. The average density of benthic diatoms is 13,375 cells/cm^2 and the average Chla concentration is 24.4 μg/m^2. The second stage is the logarithmic growth phase (from the fifth to the sixth period). The biomass is increased rapidly during this growth cycle and reached to the peak of biomass. At this time, the density of benthic diatoms reaches to 108, 953 cells/cm^2, and the Chla concentration reaches to 89.3 μg/m^2. The third stage is the stable phase (from the sixth period to the end of the experiment). During this period, the fluctuation of algal biomass is small. The average density of benthic diatoms is 109,259 cells/cm^2, and the average Chla concentration is 88.9 μg/m^2.

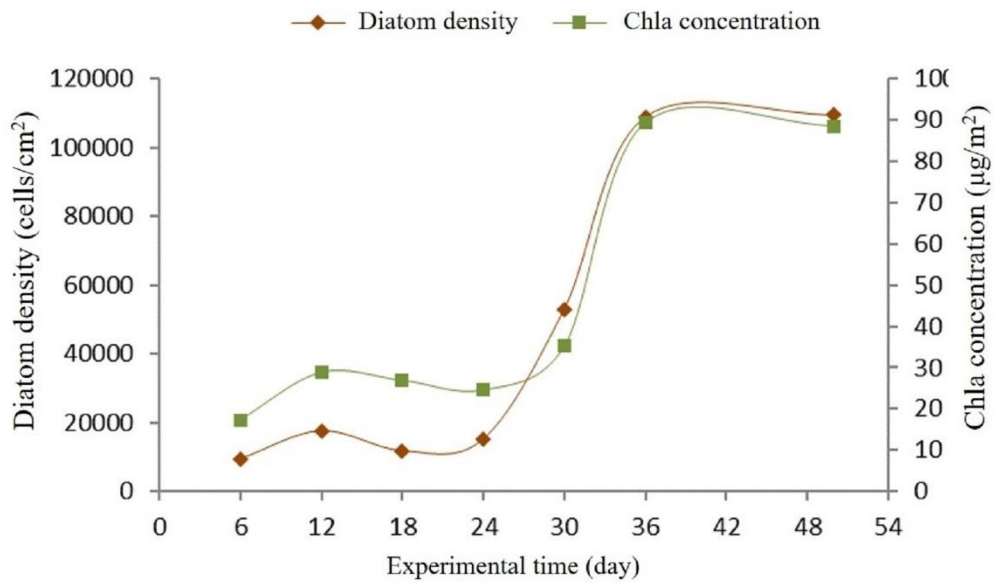
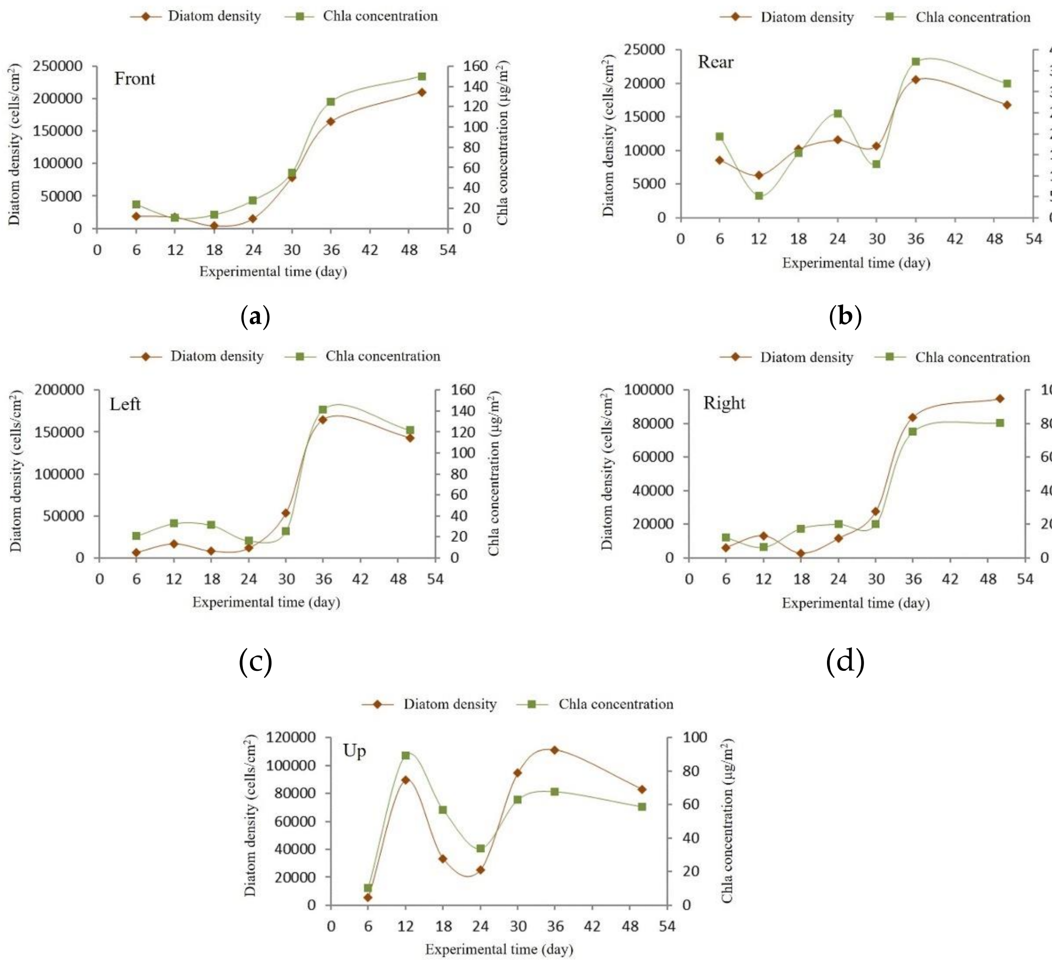


Fig. 6. Changes of benthic diatoms biomass over time

3.1.2. Growth Pattern and Biomass Difference of Benthic Diatoms at Different Parts of the Pebble

The growth of benthic diatoms at different parts of the pebble substrate is obtained by sampling data analysis, as shown in Fig. 7. According to the growth curves of benthic diatoms at different parts on the pebble substrate, the growth rates at the front, left and right parts of the pebble are in line with the Logistic growth curve, showing changes in three stages: the delay phase, the logarithmic growth phase and the stable phase. However, the growth of benthic diatoms at the rear and up parts of the pebbles do not show good pattern, and the biomass increases with the change of the growth cycle as a whole, and begins to decline in the later period.

The rear part of the pebble is directly impacted by the incoming flow, so the change of hydrodynamic conditions has a direct and obvious effect on the biomass of benthic diatoms at this part. The benthic diatom on the top of the pebble shows a trend of increasing - decreasing - increasing - decreasing with the incubation time. The top of the pebble is relatively smooth, which provides a more favorable hydrodynamic condition for the growth of benthic diatom. So in the early days of the experiment, the algal bloomed and the biomass increased dramatically. With the increase of algae metabolites and the competition of diatom community structure for nutrients, the dead diatoms were exfoliated by the water flow, so the biomass began to decline. With the emergence of new propagules on the substrate, and the surrounding environmental factors becoming more favorable to their growth, benthic diatoms began a new round of growth and formation, and so forth.



(e)

Fig. 7. The growth of benthic diatoms at different parts of the pebble (a) front; (b) rear; (c) left; (d) right; (e) up.

At different growth phases, the number of benthic diatoms at different parts of the pebble substrate is different (Fig. 8). It can be concluded that the benthic diatoms at the front of the pebble substrate are more than those at other parts at the beginning of the experiment. This is because the benthic diatoms are more likely to grow and build colonies than those at other parts because the front part is not directly impacted by the incoming flow. According to the sampling data on April 28 and May 4, the biomass of benthic diatoms at the upper part of the pebble increases significantly, while that at other parts is basically the same or slightly decreases as that of the first period. This indicates that the growth rate of benthic diatoms at the upper part of the pebble substrate is higher than that at the other parts, while the biomass at the front part has begun to decline, and the benthic diatoms in the other parts are still in the delay phase. The analysis on May 10 sampling shows that the benthic diatoms at the front part has entered another phase of biomass growth, while the benthic diatoms at the left and right are still in the delay phase. There is little change in the overall biomass. The biomass of benthic diatoms at the rear increases slightly while the biomass of benthic diatoms at the upper part is still in the logarithmic growth stage. The sampling results on May 16 and May 22 show that the biomass of benthic diatoms at the left and right parts of the pebble substrate begin to increase and enter the logarithmic growth phase. The biomass at the front is still very high and in the logarithmic phase. The benthic diatoms at the upper part enters another period of decay. The biomass of benthic diatoms at the rear is relatively lower than that at other parts. In the last period, the benthic diatoms at the front, left, right and rear enter a stable phase, and the community biomass basically remains at a stable level, while the upper biomass begin to decline, and the diatom community is in a declining phase. In general, the growth suitability at different parts of the pebble for benthic diatoms from high to low are the rear part of the pebble (without direct erosion), the side parts of the pebble, the upper part of the pebble and the front part of the pebble (direct erosion).

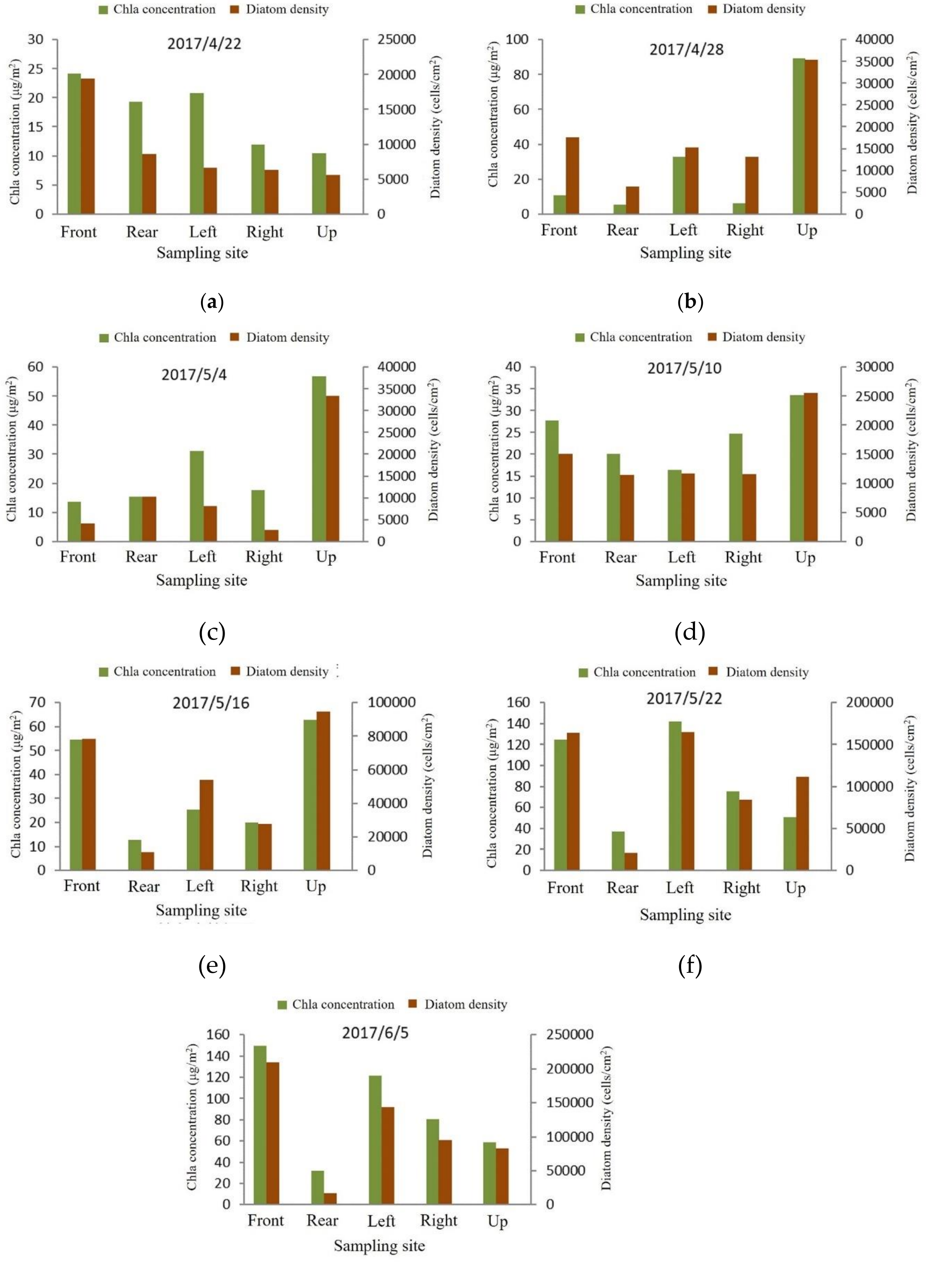


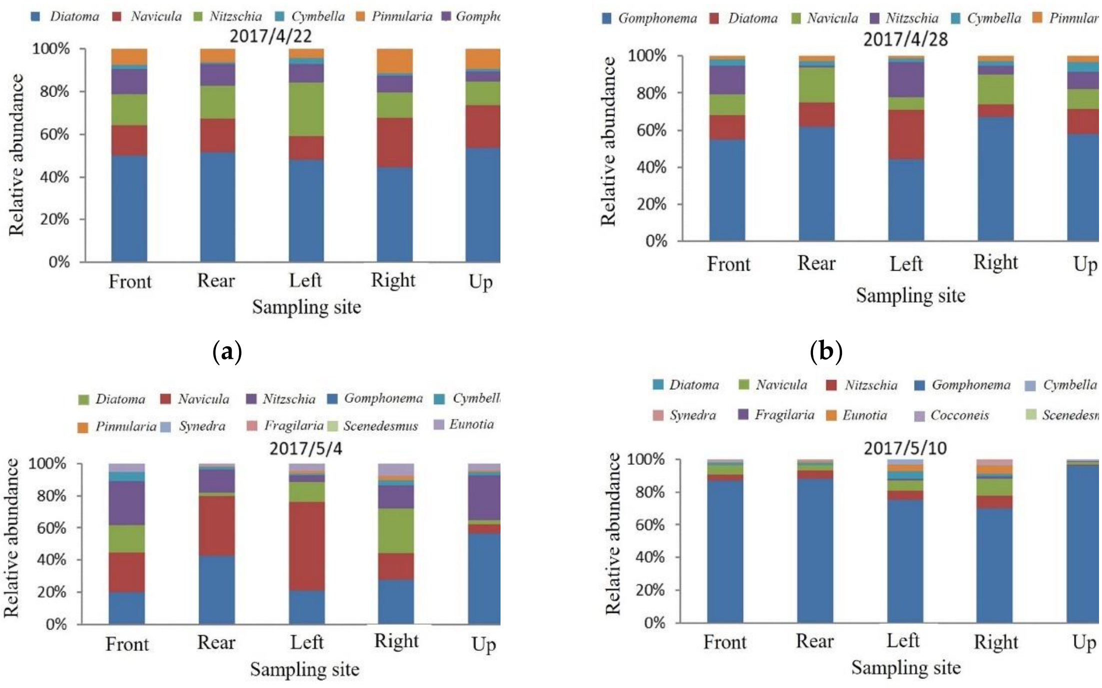
Fig. 8. Comparison of benthic diatom growth at different parts of pebbles in different periods (a) day 6; (b) day 12; (c) day 18; (d) day 24; (e) day 30; (f) day 36; (g) day 49.

3.1.3. Composition and Variation of Benthic Diatoms at Different Parts of the Pebble

At different growth phases, the benthic diatom communities show different community composition and relative abundance (Fig. 9). At the beginning of the colony building, benthic diatoms grow slowly, and there are few species of benthic diatoms. It is not until benthic diatoms built the colony on the pebble substrate that the biomass and species of benthic diatoms increase rapidly, and the species of benthic diatoms also increase gradually.

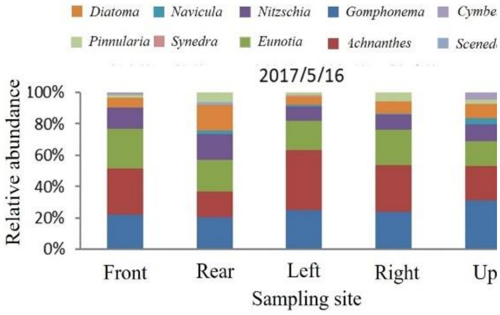
Jones found that although there are many factors that affect the biomass and species of algae, only the environmental conditions during the initial growth period have a fundamental impact on the biomass content (Jones RC 1978). In the experimental rivers, isoflagellates were the dominant species on the pebble substrate at the beginning of the experiment. Due to the small size of isophora and its good adhesion, isophora became a pioneer species due to the small shear force of water. As time went on, heterodytes began to dominate the community structure. The sampling results on May 4 show an increase in the number of scaphoids and a greater variety of benthic diatoms than before. The sampling results on May 10 show that allodips had regained as the dominant algae species and were dominant at all parts of the pebble substrate. The sampling results in the last few periods show that, with the establishment and stabilization of benthic diatom community, benthic diatom species on the pebble substrate were abundant with little change in composition, but on the whole, heterodiatoms still had a dominant position.

The species composition and dominant species of benthic diatoms are different at different growth stages. The species composition and dominant species of benthic diatoms at different parts of the pebbles at the same growth period may also be different, but the difference is small and basically consistent on the whole.

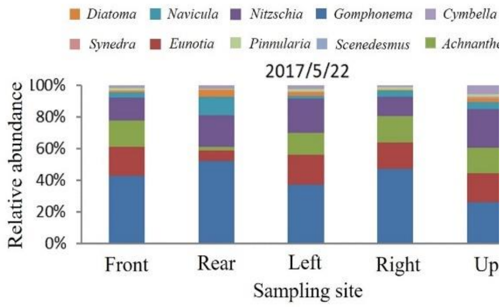


(**a**) (**b**)

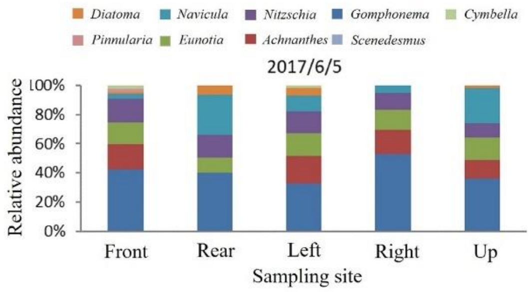
(c) (d)



(e)



(f)

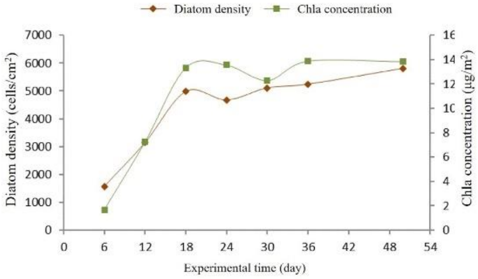


(g)

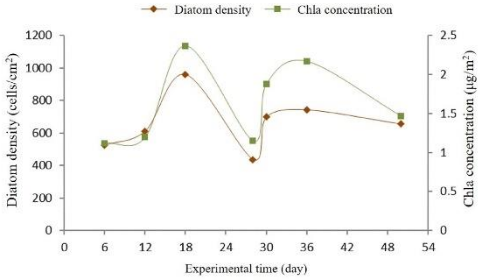
Fig. 9. Comparison of benthic diatoms species at different parts of pebbles in different periods (a) day 6; (b) day 12; (c) day 18; (d) day 24; (e) day 30; (f) day 36; (g) day 49.

3.1.4. Growth of Benthic Diatoms under High Velocity and Still Water Conditions

The growth curve of benthic diatoms on the pebble substrate under high velocity and still water conditions is shown in Fig. 10. Under still water conditions, the growth of benthic diatoms only experiences the logarithmic growth phase and stable phase (Fig. 10a). Thus, in relatively stable environments, benthic diatoms will multiply and build colonies rapidly. However, after the establishment of the diatom community, due to the small variation of nutrients, light, temperature and other environmental factors, the whole diatom community shows relatively stable growth, decline and replacement. Furthermore, the biomass of benthic diatoms is very small under still water conditions, the Chla concentration is only 14 μg/m², and the algae density is only 6000 cells/cm². The biomass of benthic diatoms, both in terms of density and chlorophyll concentration, are very small under high velocity water flow conditions (Fig. 10b), which is a result of the strong shear force brought by the high flow rate, which makes it difficult for benthic diatoms to attach themselves to the pebbles.



(**a**)



(**b**)

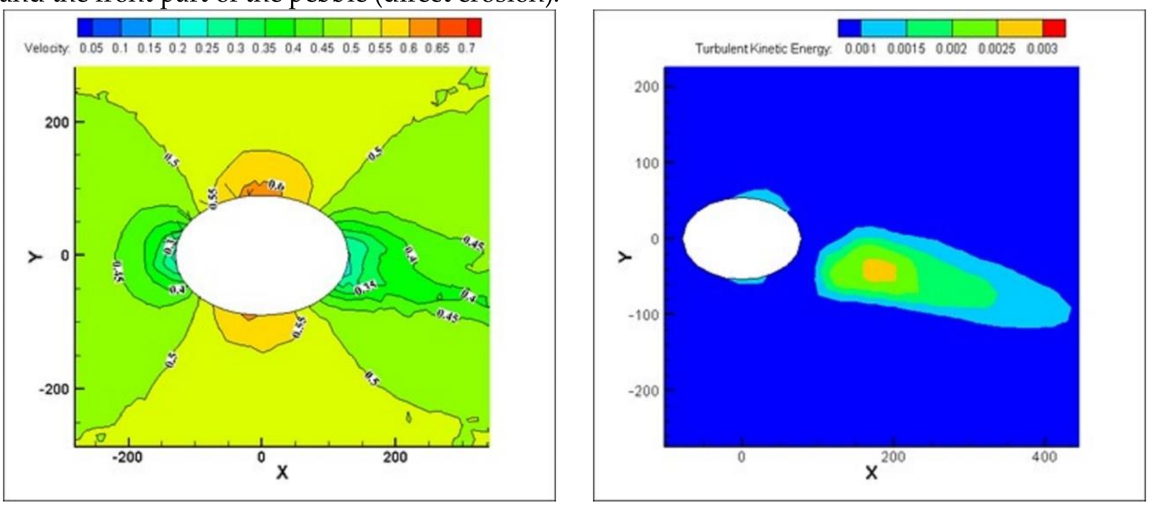
Fig. 10. (**a**) Growth of benthic diatoms under still water conditions; (**b**) Growth of benthic diatoms at high flow rate conditions.

3.3. Micro-scale Hydrodynamic Characteristics of the Flow Field Around Pebbles

After using the standard three dimensional $k\text{-}\varepsilon$ equation to calculate the physical model, the hydrodynamic characteristics around the pebble substrate are obtained (Fig. 11). According to the calculation results, when the water flew around the pebbles, the flow field around the pebbles would change accordingly because of the shape of the pebbles. When the water reached the rear of the pebbles, the flow velocity slowed down to 0.2 m/s near the surface of the pebbles because of the blocking effect (Fig. 11 a and b).

The rear of the pebble was the first to be hit by the current, so the growth of benthic diatoms in the rear was chaotic. With the impact of water flow on this part, the biomass of benthic diatoms would increase or decrease continuously during the growth cycle. The growth of diatoms at this part did not conform to the Logistic growth curve, and the biomass at the back of the pebble was less than that of other parts. The flow fields at the left and right sides of the pebble were relatively uniform and stable, and the velocity near the surface of the pebble was close to 0.6 m/s (Fig. 11 a and b). Compared with the test results, the growth of benthic diatoms at the left and right parts of the pebble was in line with the Logistic growth curve, and the biomass was second only to the front part of the pebble. Therefore, it was proved that at this flow rate, the water can bring more nutrients to the benthic diatoms and carry away the excess metabolites, without causing greater erosion and erosion of algal cells. Turbulent vortices would be formed in front of the pebble due to the flow around it (Fig. 11 a, b, e and f). Vortices were conducive to the transfer and transportation of nutrients and the transfer of metabolites. Therefore, the front part of the pebble substrate was most suitable for the growth and reproduction of benthic diatoms, with more biomass than other parts. It can be seen from Fig. 11 c and d that the flow evenly wrapped the pebbles in the direction perpendicular to the flow, and the flow velocity around the pebbles gradually increased from the outside to the inside. It can be seen from Fig. 11 e and f that most of the upper part of the pebble was in a high velocity zone. Therefore, a higher flow rate might produce scour to the diatom community growing on the upper part of the pebble, and the contact area between the upper part of the pebble and the upper water was large, and the impact and disturbance of the upper water were not conducive to the growth and reproduction of benthic diatom in this part, so the biomass of the upper part of the pebble was also very small.

The results of three-dimensional hydrodynamics simulation reasonably explained the reasons for the differences in the measured growth of benthic diatoms at different parts of the pebble. The growth suitability at different parts of the pebble for benthic diatoms from high to low is the rear part of the pebble (without direct erosion), the side parts of the pebble, the upper part of the pebble and the front part of the pebble (direct erosion).



(**a**) (**b**)

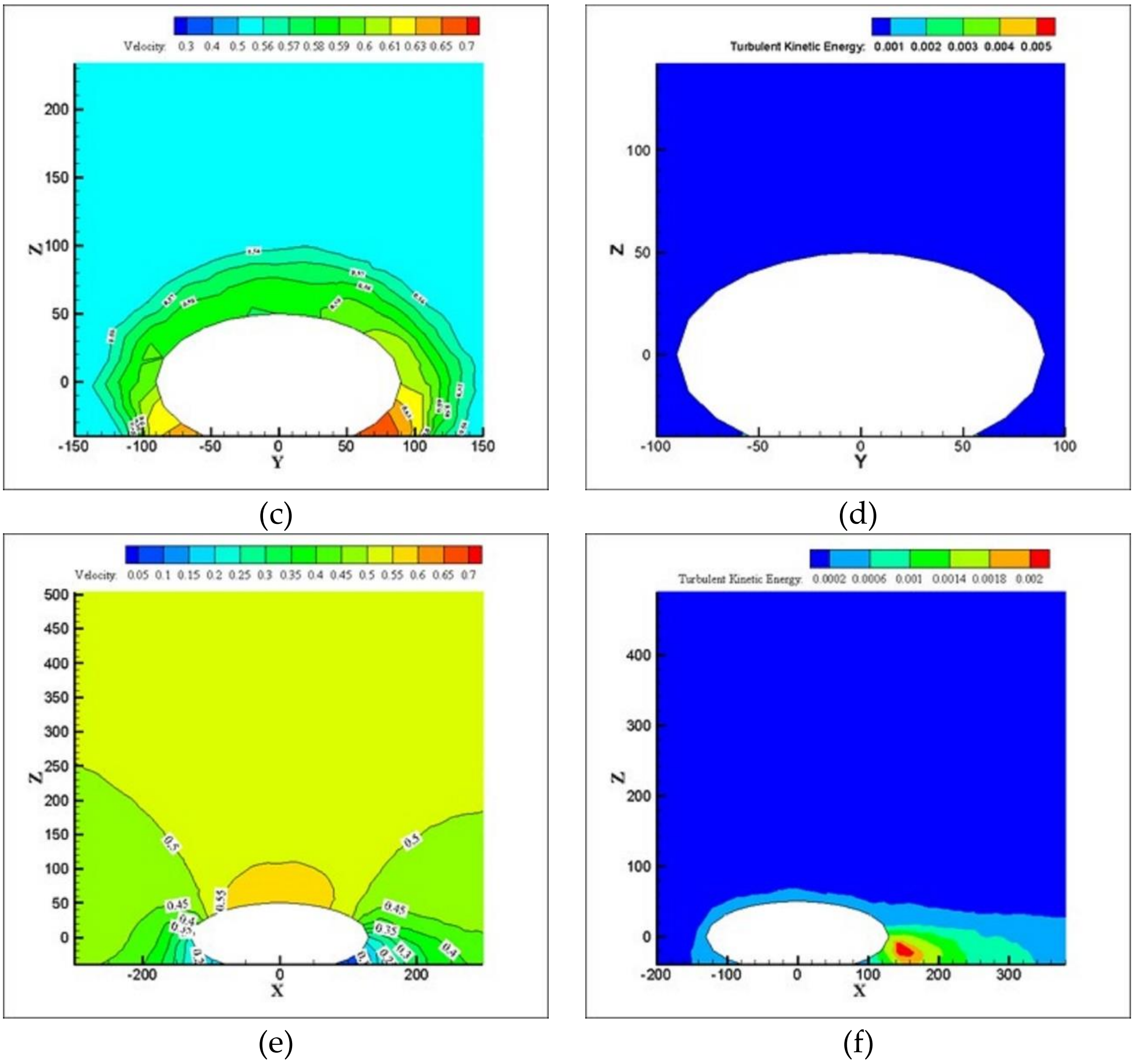


Fig. 11. Three-dimensional hydrodynamic analysis of the flow field around the pebble (a) Velocity (m/s) distribution around the pebble at Z=0; (b) Turbulent kinetic energy (J) distribution around the pebble at Z=0; (c) Velocity (m/s) distribution around the pebble at X=0; (d) Turbulent kinetic energy (J) distribution around the pebble at X=0; (e) Velocity (m/s) distribution around the pebble at Y=0; (f) Turbulent kinetic energy (J) distribution around the pebble at Y=0.

3.4. Time of Death by Exposure to Benthic Diatoms

The water reduced river reach downstream of the power station often exposes a large amount of the riverbed substrate to the air, and the operation of the power station also causes some benthic diatoms to be regularly exposed to the air. However, the study on the exposure time of benthic diatoms is lagging behind, which is a challenge for the protection of benthic diatoms. To verify the damage to diatom cells by strong light when the benthic diatoms on the pebble substrate were exposed to air, the location with similar hydraulic habitats and easy for sampling was selected to dredge some pebbles for the experiment. The pebbles were exposed to the air and sampled at intervals to determine the concentration of Chla and to infer the lethal pattern of benthic diatoms. The changes of Chla concentration of benthic diatom in the whole test period are shown in Fig. 12.

The whole experiment lasted for 240 minutes, and the concentration of Chla in the substrate diatom exposed to the air showed a relatively obvious downward trend in the first 30 minutes, from 7.47μg/m^2 at the beginning to 4.17 μg/m^2. With the time passing by, the downward trend gradually slowed down, and the concentration of Chla decreased to 1.3 μg/m^2 at the end of the experiment. According to experiments, the exposure time of 30-40 minutes can cause the death of about half of the benthic diatoms, while the exposure time of more than four hours will cause the death of most of the benthic diatoms. In the restoration of benthic diatoms, the potential of benthic diatoms can be judged by the exposure time of each river region in combination with the scheduling scheme of power station.

Under natural light conditions, the photosynthetic rate of benthic diatoms generally decreases with the increase of light intensity (Zhuang SH and Sven H 2001). The reason may be that benthic diatoms usually achieve the maximum photosynthetic rate at a low light intensity (Taylor WR 1964), while the activity of chlorophyll enzymes is activated under strong light, resulting in the destruction of chlorophyll A (Zhuang SH and Sven H 1999; Kowallik W 1967). Meanwhile, after being exposed to the air, the chlorophyll in the body of the benthic diatom is illuminated directly by the strong light, which is likely to damage to the II optical system in the photosynthetic reaction center, resulting in the light inhibition phenomenon (Shi PL 2015).

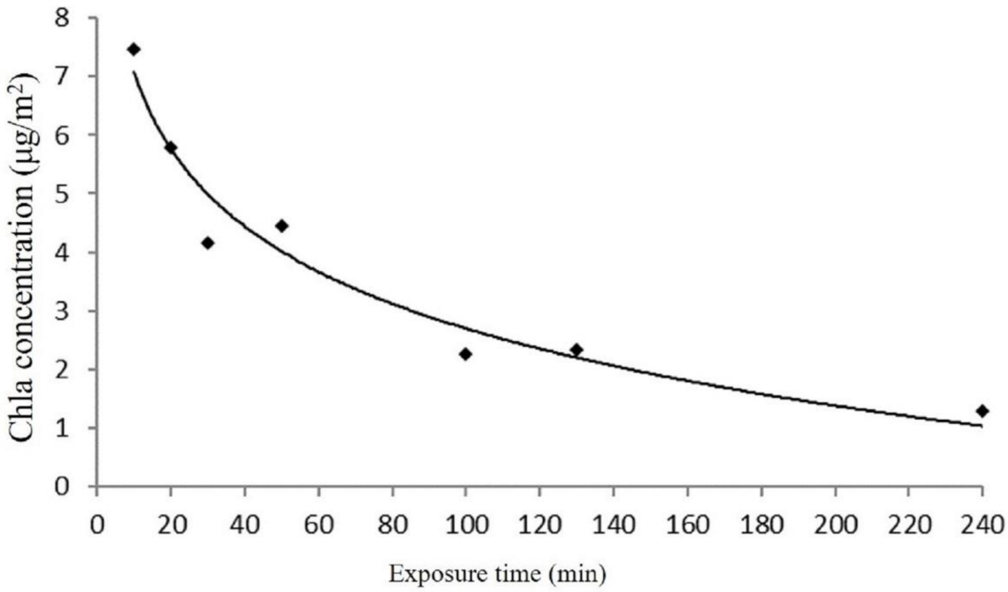


Fig. 12. Changes in chlorophyll content of benthic diatoms exposed to air over time

4. Conclusions

In this paper, the effects of hydrodynamic conditions and exposure time on the biomass of benthic diatoms were studied on the microscopic scale by combining the field experiments and the three-dimensional hydrodynamics simulation. The following conclusions are obtained:

1. The species composition and dominant species of benthic diatoms are different at different growth stages. The biomass and the number of species mainly increase after the formation of the colony. The species composition and dominant species of benthic diatoms at different parts of the pebbles at the same growth stage may also be different, but the difference is small and basically consistent on the whole.
2. There is an appropriate velocity range for the growth of benthic diatoms, and too low and too high flow rates are not conducive to the growth of benthic diatoms.
3. The growth suitability at different parts on the pebble for benthic diatoms from high to low is the rear part of the pebble (without direct erosion), the side parts of the pebble, the upper part of the pebble and the front part of the pebble (direct erosion). This is mainly caused by the difference of the hydrodynamic conditions at the micro level at each part of the pebble.
4. When exposed to the natural light for 30-40 minutes, about half of the benthic diatoms die, and most of them die once exposed for more than four hours. In the restoration of benthic diatoms, the potential of benthic diatoms can be judged by the exposure time of each river region in combination with the scheduling scheme of power station.

The effects of hydrodynamic conditions and exposure time on the biomass of benthic diatoms are obvious on the microscopic scale. The research results in this paper are helpful to further understand the growth status of benthic diatoms in microscopic environment and provide reference and information for the restoration of the hydraulic habitat of benthic diatoms in the reach affected by human activities.

Abbreviations

Chla: Chlorophyll a; DO: Dissolved oxygen; pH: Potential of hydrogen potential of hydrogen; COD: Chemical oxygen demand; TP: Total phosphorus; TN: Total nitrogen.

Declarations

Ethics approval and consent to participate
Not applicable.

Availability of data and material
Not applicable.

Consent for publication
All authors have read and agreed to the published version of the manuscript.

Authors' contributions
Conceptualization, Z.Z.X.; methodology, L.Y.; software, W.H.W.; investigation, L.Q.Y. and W.H.; resources, Y.L.H.; writing—original draft preparation, Z.Z.X.; writing—review and editing, L.Y.; Supervision, W.Z.G.; project administration, L.H.

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Competing interests
The authors declare no conflict of interest.

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Figures

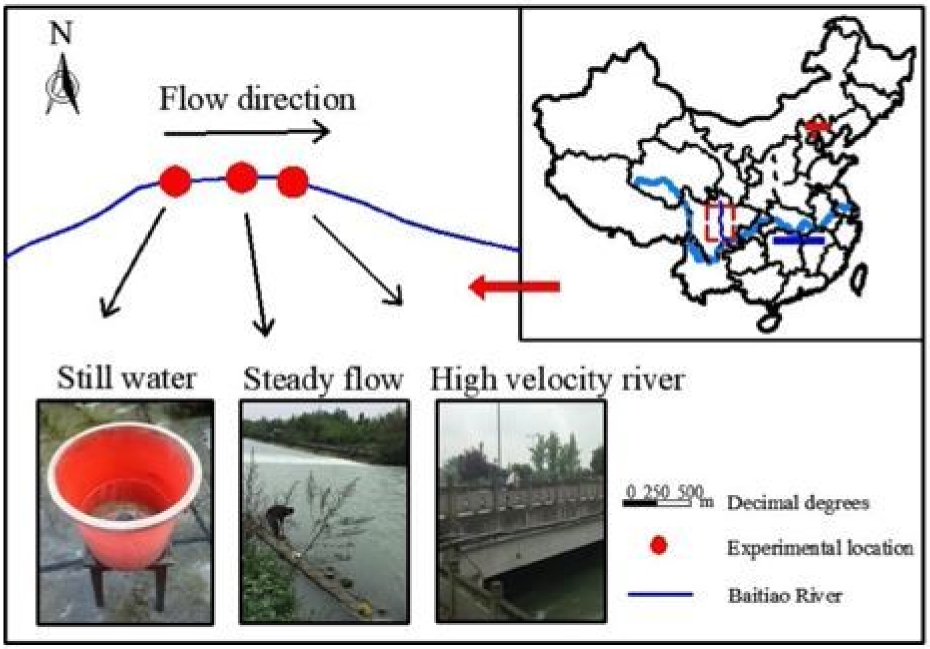

Figure 1

Study area and experimental setup

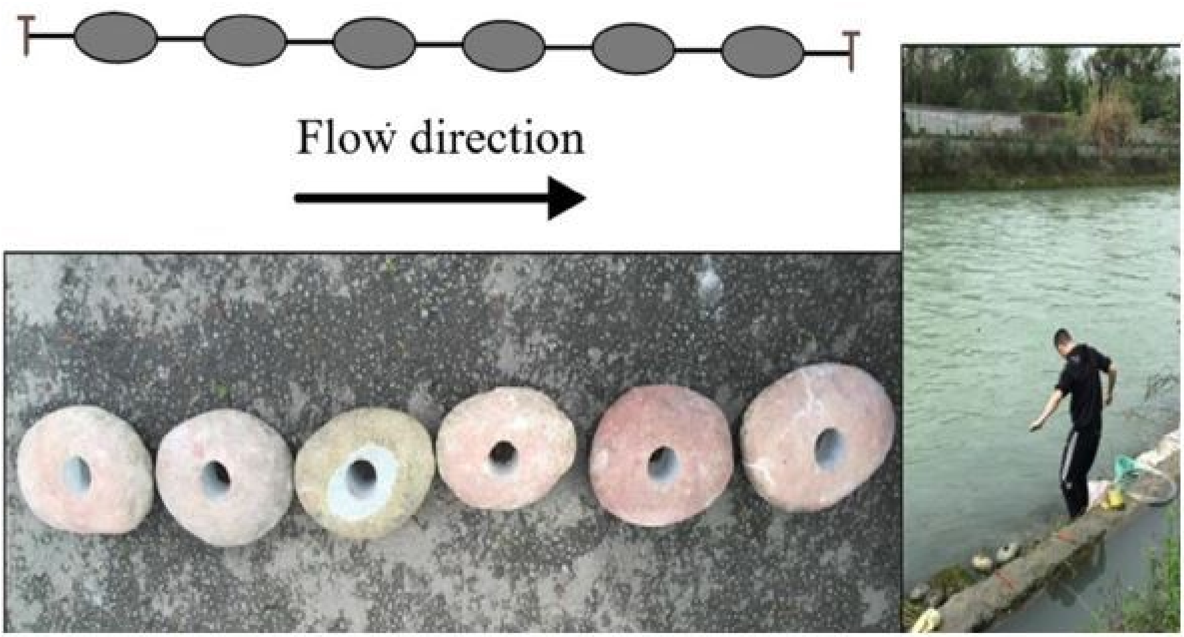

Figure 2

Setting of pebble substrates

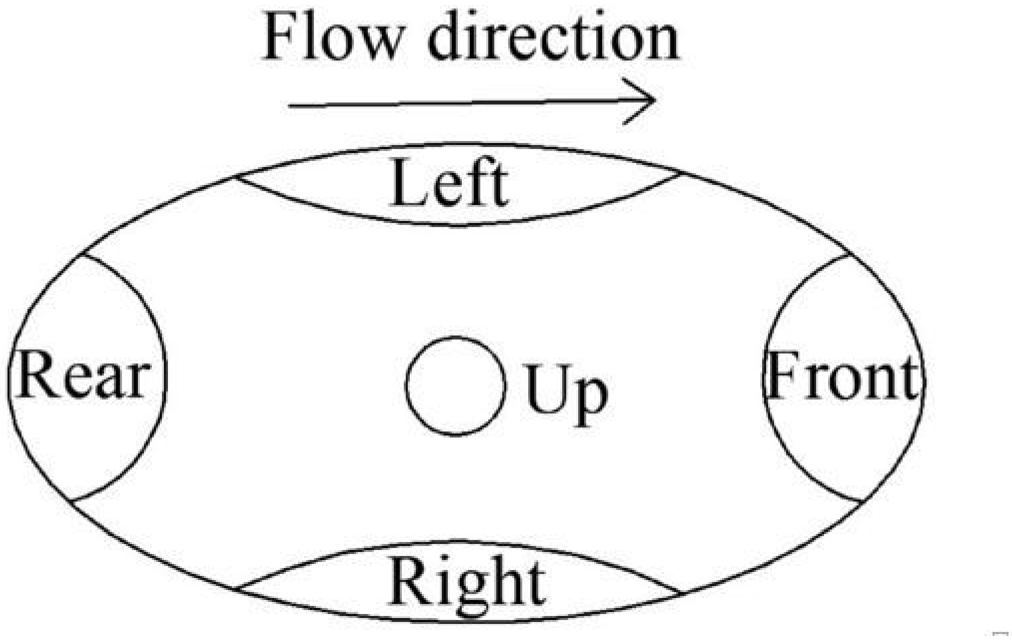

Figure 3

Zoning of pebble substrate

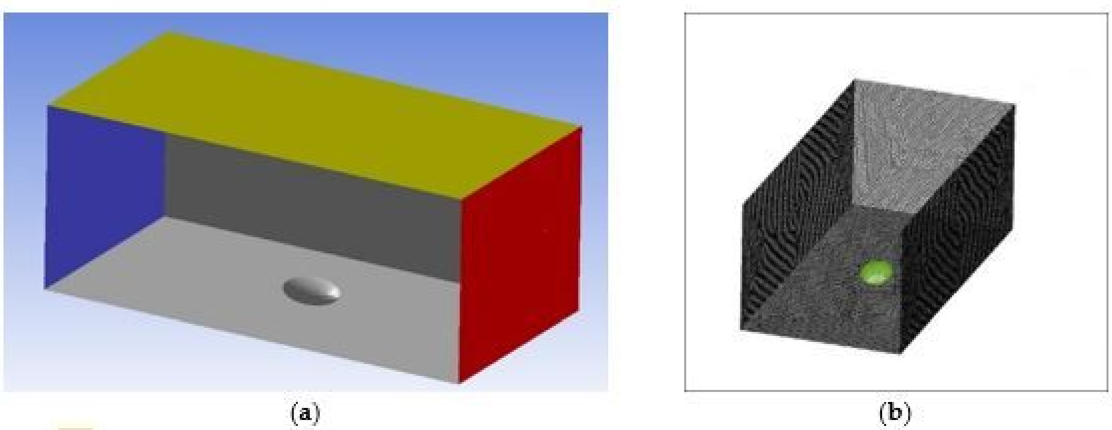

Figure 4

(a) A generalized model for microhabitat studies; (b) Mesh generation.

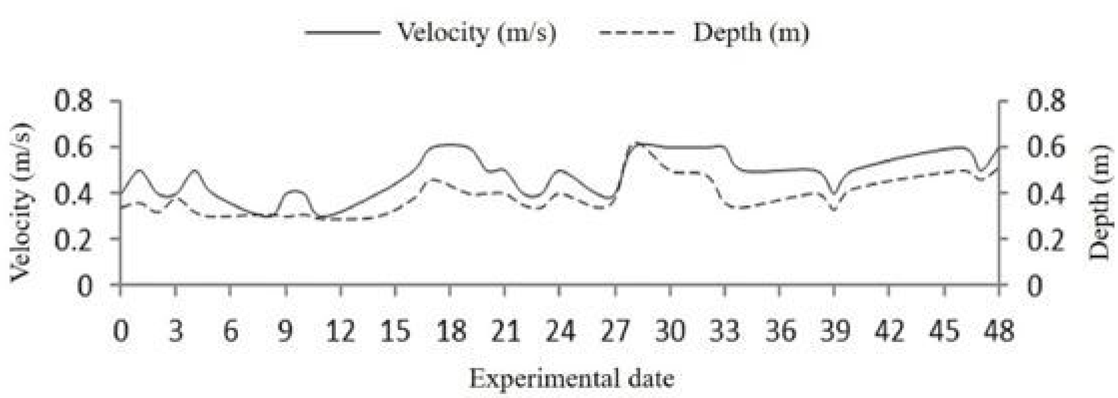

Figure 5

Variation of velocity and water depth during the experiment.

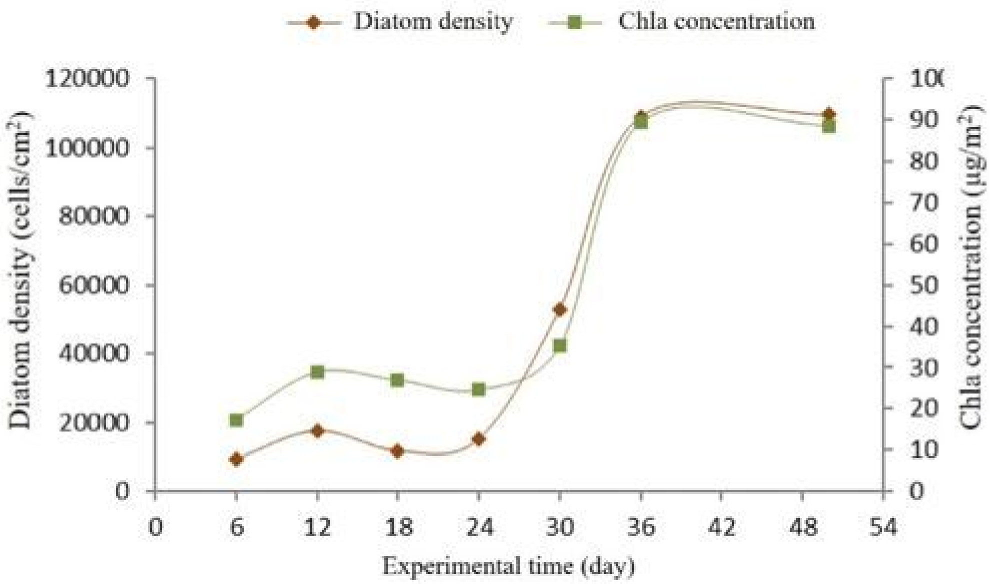

Figure 6

Changes of benthic diatoms biomass over time

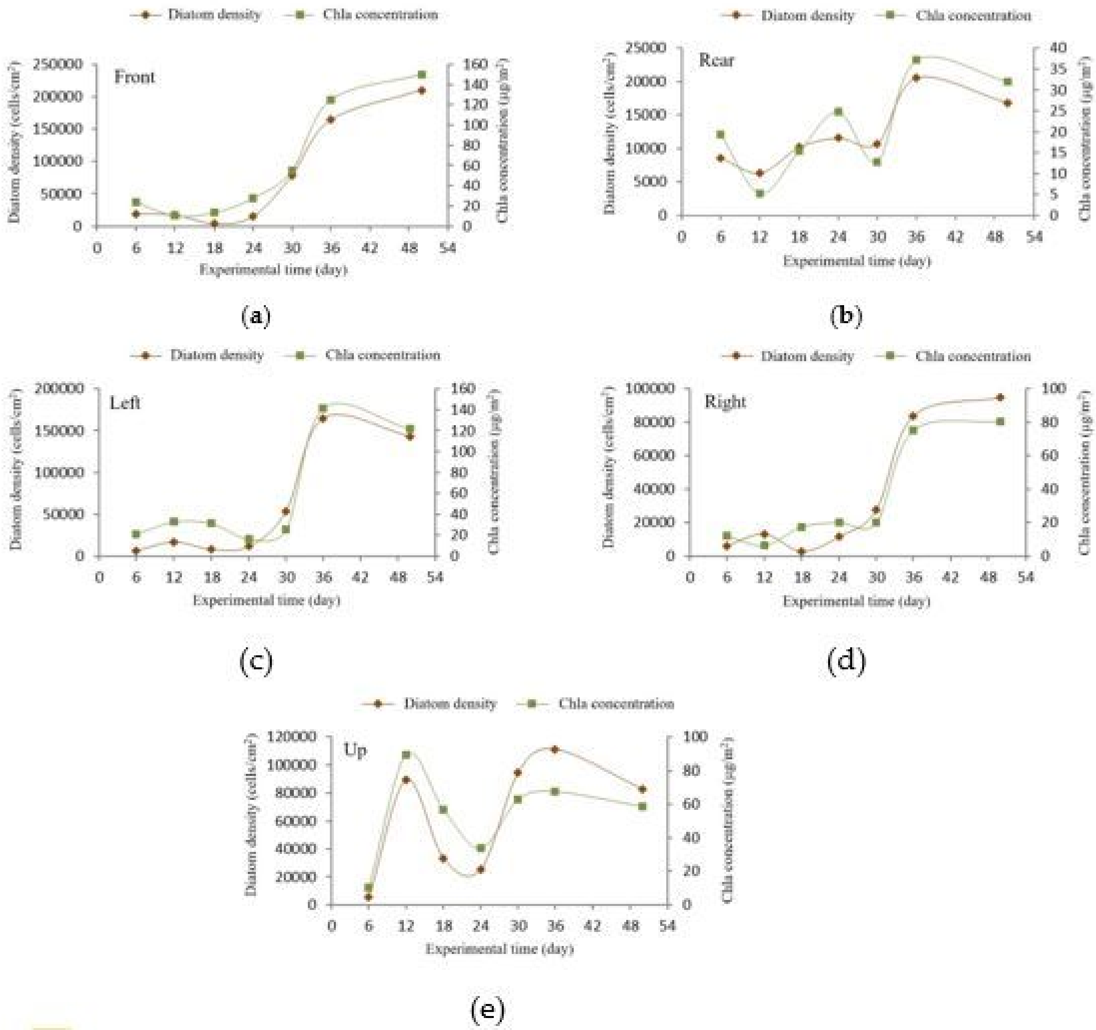

Figure 7

The growth of benthic diatoms at different parts of the pebble (a) front; (b) rear; (c) left; (d) right; (e) up.

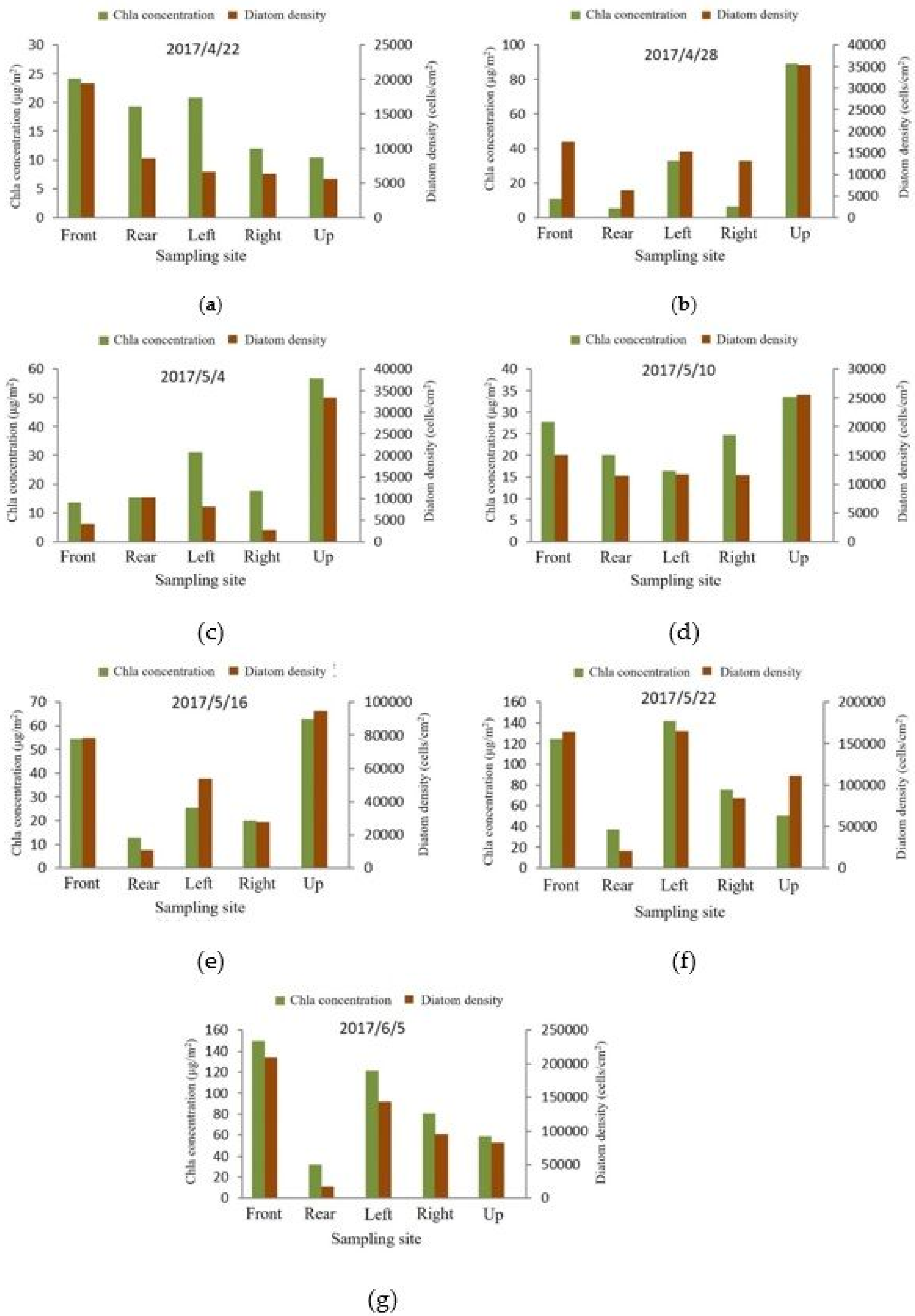

Figure 8

Comparison of benthic diatom growth at different parts of pebbles in different periods (a) day 6; (b) day 12; (c) day 18; (d) day 24; (e) day 30; (f) day 36; (g) day 49.

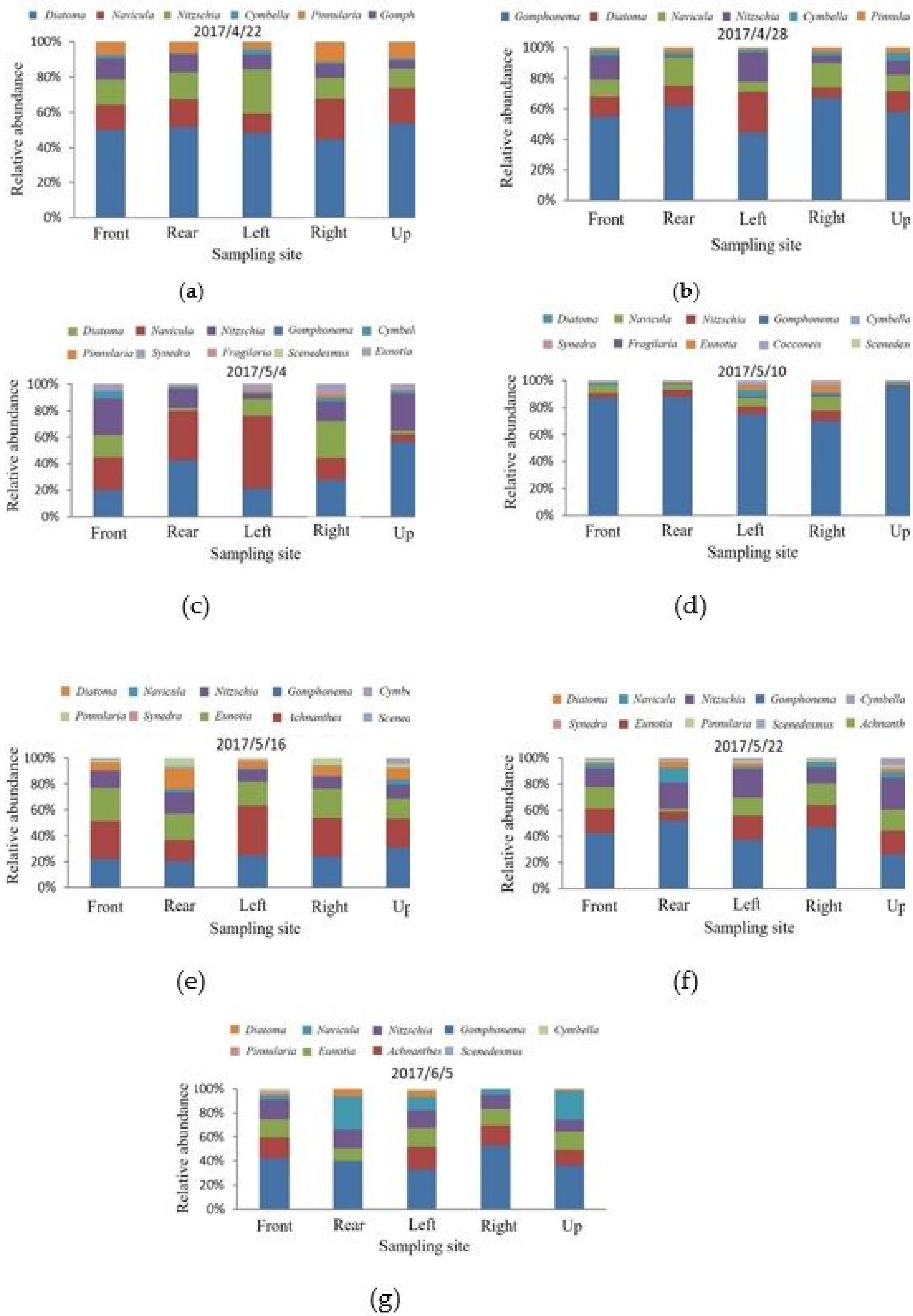

Figure 9

Comparison of benthic diatoms species at different parts of pebbles in different periods (a) day 6; (b) day 12; (c) day 18; (d) day 24; (e) day 30; (f) day 36; (g) day 49.

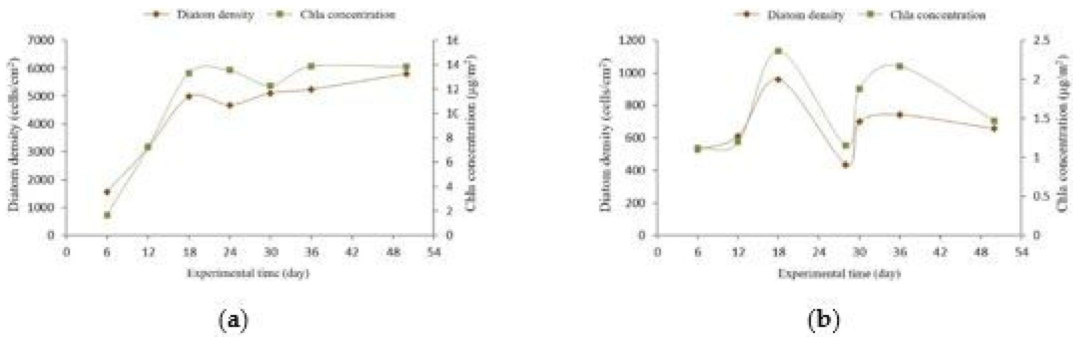

Figure 10

(a) Growth of benthic diatoms under still water conditions; (b) Growth of benthic diatoms at high flow rate conditions.

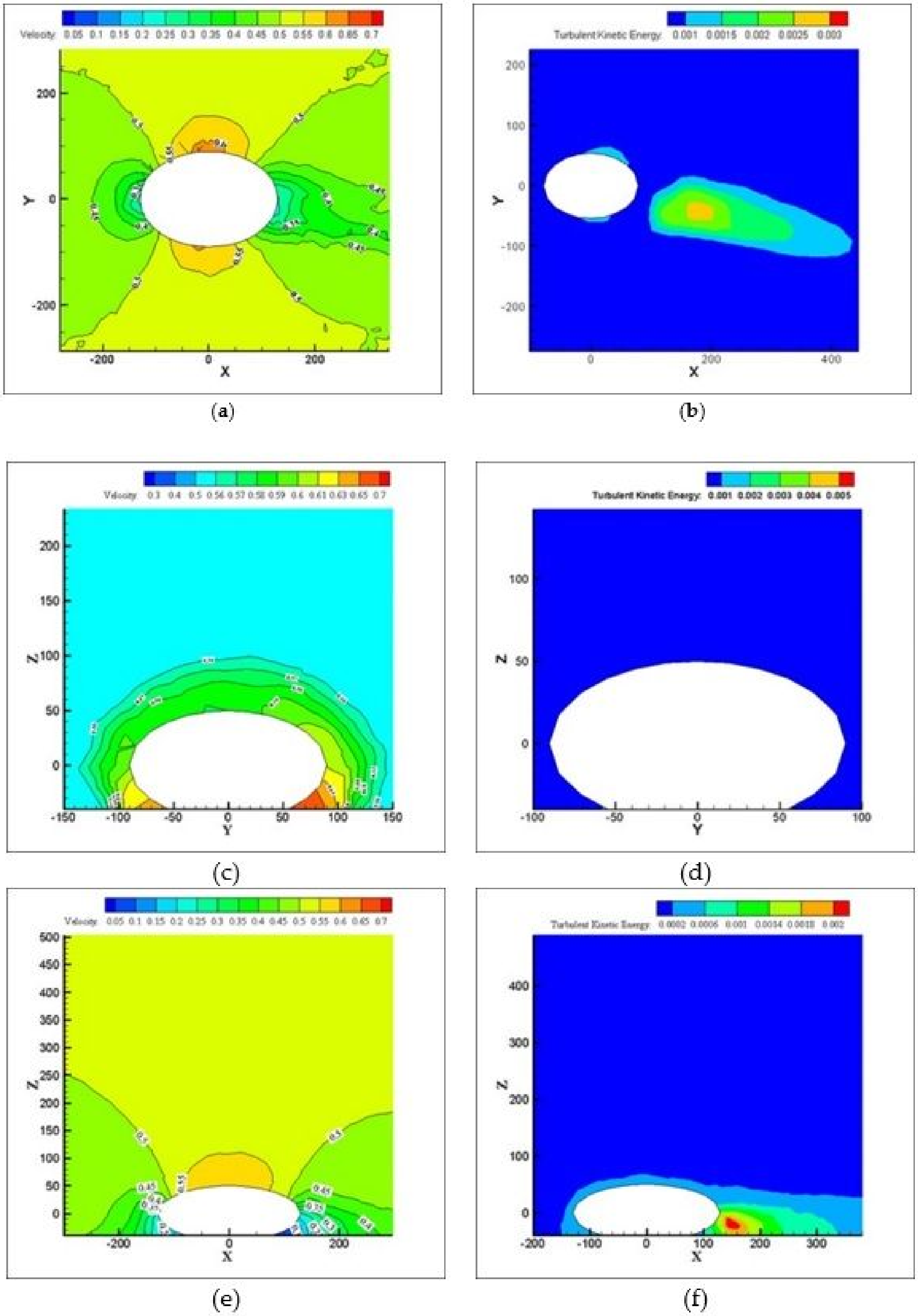

Figure 11

Three-dimensional hydrodynamic analysis of the flow field around the pebble (a) Velocity (m/s) distribution around the pebble at Z=0; (b) Turbulent kinetic energy (J) distribution around the pebble at Z=0; (c) Velocity (m/s) distribution around the pebble at X=0; (d) Turbulent kinetic energy (J) distribution around the pebble at X=0; (e) Velocity (m/s) distribution around the pebble at Y=0; (f) Turbulent kinetic energy (J) distribution around the pebble at Y=0.

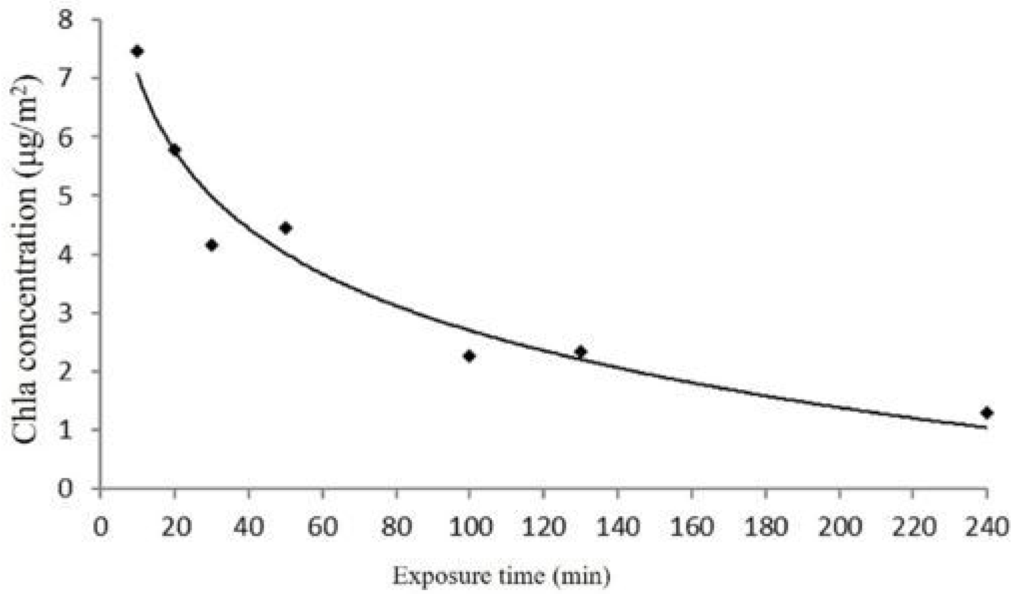

Figure 12

Changes in chlorophyll content of benthic diatoms exposed to air over time