The following supplement accompanies the article

Model-based analysis of the energy fluxes and trophic structure of a *Portunus trituberculatus* polyculture ecosystem

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1 INPUT DATA OF B, P/B, AND Q/B

1.1 Phytoplankton and periphyton

The biomass of phytoplankton was divided into micro-, pico-, and nano-phytoplankton. The mixed water (1L) of the phytoplankton samples were taken from surface layer, middle layer and bottom layer of the enclosure pond using a water sampler. The phytoplankton samples were first filtered through a 100 mesh sieve to remove mesozooplankton, then filtered through a 400 mesh sieve and a 1200 mesh sieve, respectively to separate the phytoplankton with different particle size. The 400 mesh sieve and the 1200 mesh sieve were rinsed with 1L no granular seawater to collect the phytoplankton with the particle size of 10~38um and smaller than 10 um, respectively. Both the filtered water and the rinsed water were fixed with 1% Lugol's solution (Nauwerck 1963). The separated phytoplankton samples were enumerated using the methods of Lund et al. (1958). The volume of the different type of phytoplankton was calculated according to Hillebrand et al. (1999). The volume of phytoplankton was converted to the organic carbon of phytoplankton using following equations (Eppley et al. 1970):

$$\log C = 0.76 \times (\log V) - 0.352 \quad \text{(Diatoms)} \qquad \text{(S1)}$$

$$\log C = 0.94 \times (\log V) - 0.6 \quad \text{(Non-diatomaceous algae)} \qquad \text{(S2)}$$

Where C was the organic carbon content (pg) of phytoplankton. The V was the volume (um^3) of phytoplankton. The biomass of phytoplankton was then converted from organic carbon to energy content. The conversion relationship was: 1g organic carbon = 46 kJ energy (Salonen et al. 1976)

The biomass of periphyton: At the beginning of the experiment, polyvinyl plastic rectangles (25 × 150 cm) were set in each enclosure to collect the periphyton. At each sampling time, the polyvinyl plastic (2 × 20 cm) which was situated 20cm under the water surface was cut and brought back to the lab. The attached algae on the polyvinyl plastic (2 × 20 cm) was scraped and smashed it with the ultrasonic wave. Took the water sample of 50 cm^3, filtered it through the membrane of 0.45 um and determined its chlorophyll value by Spectrophotometry (Jeffrey & Humphrey 1975). The biomass of periphyton was converted from chlorophyll to organic carbon content according to the ratio of chlorophyll to organic carbon as 1:50 (Harris 1986). The biomass of periphyton was then converted from organic carbon to energy content. The conversion relationship was: 1 g organic carbon = 46 kJ energy (Salonen et al. 1976).

The P/B ratios of phytoplankton groups and periphyton were estimated by using "light and dark bottle" oxygen method.

Primary production of phytoplankton was measured using the "light and dark bottle" oxygen method (Diana et al. 1991). Water samples from the upper (25 cm), middle (50 cm) and lower (150 cm) layers were collected on five occasions of the enclosure pond during the culture period. Two light and two dark bottles (250 mL) filled with water samples of each phytoplankton size (>38 um, 10~38 um, <10 um) were incubated at the water depth in each enclosure (Johnson et al. 1981). The dissolved oxygen was measured with Winkler titration method (Griffiths & Jackman 1957). The net primary production of phytoplankton was calculated as:

$$P_G = DO_L - DO_D \quad \text{(S3)}$$

$$R = DO_O - DO_D \quad \text{(S4)}$$

$$P_n = P_G - R \quad \text{(S5)}$$

Where P_G is the gross primary production, R is the respiration, DOo is the amount of initial dissolved oxygen, DO_L is the amount of dissolved oxygen in the light bottles, and DO_D is the amount of dissolved oxygen in the dark bottles, P_n is net primary production. The units of P_n, [(mg O2) L^{-1}], was converted to (mg C) L^{-1} according to Winberg (1 mg O^2 = 0.375 mg C; Winberg 1980).

Primary production of periphyton: At the beginning of the experiment, polyvinyl plastic rectangles (25 × 150 cm) were set in each enclosure to collect periphyton. Sections of these instruments (1 × 1 cm) which positioned 25, 50, and 150 cm below the water surface were scraped into two light and two dark bottles (250 mL) filled with the same column water (Zhang 2011). Meanwhile, one light and one dark bottle (250 mL) without periphyton filled with the same column water was used as the control to adjust the data for the primary production of phytoplankton and the respiration of water. The net primary production of periphyton was calculated as:

$$P_{G1} = DO_{L1} - DO_{D1}$$

$$R_1 = DO_{O1} - DO_{D1}$$

$$P_{G2} = P_{G1} - P_G \quad \text{(S6)}$$

$$R_2 = R_1 - R \quad \text{(S7)}$$

$$P_n = P_{G2} - R_2$$

Where P_{G1} is the gross primary production of phytoplankton and periphyton, R_1 is the respiration of water and periphyton; P_G is the gross primary production of phytoplankton, R is the respiration of water; P_{G2} is the gross primary production of periphyton, R_2 is the respiration of periphyton; P_n is the net primary production of periphyton; DO_{O1} is the amount of initial dissolved oxygen, DO_{L1} is the amount of dissolved oxygen in the light bottles with periphyton, and DO_{D1} is the amount of dissolved oxygen in the dark bottles with periphyton. The units of P_n [(mg O^2 L^{-1}], were converted to (mg C) L^{-1} according to Winberg (1 mg O^2 = 0.375 mg C; Winberg 1980). The production of phytoplankton and periphyton was then converted from organic carbon to energy content. The conversion relation was: 1g organic carbon = 46 kJ energy (Salonen et al. 1976).

1.2 Swimming crab *Portunus trituberculatus*

The biomass of *P. trituberculatus* were calculated as the average value of the initial biomass, harvesting biomass, and the biomass of each sample measurement. The crabs were

sampled at 10 d intervals, which were caught with a cage net in each enclosure. The full carapace width, carapace length, carapace width and body height of crabs were measured by a vernier caliper. The body weight of crabs was calculated following the method of Liu et al. (2009):

$$Y = -54.113 + 0.491X_1 + 0.632X_2 + 0.325X_3 + 0.451X_4 \text{ (0-80d)} \quad \text{(S8)}$$

$$Y = -123.484 + 0.627X_1 + 0.928X_2 + 1.433X_3 + 0.152X_4 \text{ (80-120d)} \quad \text{(S9)}$$

Where Y is the body weight of crab (g), X_1 is the full carapace width (mm), X_2 is the carapace width (mm), X_3 is the carapace length (mm) and X_4 is the body height (mm).

The total mortality of *P. trituberculatus* in the 90 d culturing period was calculated as 51.30% according to our field experiment. The natural mortality (M) of *P. trituberculatus* during the whole culturing period was obtained using empirical formula from (Gislason et al. 2008):

$$\ln M = -2.1104327 + 1.5067827 \ln L_\infty - 1.7023068 \ln L + 0.9664798 \ln K + 763.5074169 \times \left(\frac{1}{T}\right) \quad \text{(S10)}$$

where M is an annual instantaneous rate (y^{-1}), L_∞ is the asymptotic total carapace width of *P. trituberculatus*, which is 16.44 cm (Gao et al. 2016). L (cm) is the total carapace width of *P. trituberculatus* for which the M estimate would apply, K (0.28) is the rate at which the rate of growth of *P. trituberculatus* in the total carapace width declines as the total carapace width approaches L_∞(y-1) (Lee et al. 2006), T is absolute temperature (kelvin).

The average total carapace width of *P. trituberculatus* in the first, second and third month of the 90 d culturing period was measured as 3.04 cm, 5.73 cm, and 10.78 cm, respectively. The mean water temperature of the cultured pond in the first, second and third month of the 90 d culturing period was measured as 303.16 K, 299.66 K, and 296.25 K, respectively. As a result, the natural mortality of *P. trituberculatus* in the first, second and third month of the 90 d culturing period was calculated as 35.23%, 12.29%, and 4.30%, respectively. The total calculated natural mortality was 51.82%. On account of that the stocking of *P. trituberculatus* may lead to a mortality of 8.00% in the polyculture ecosystem, and the actual total mortality of *P. trituberculatus* in this polyculture ecosystem was 51.30%. The natural mortality of *P. trituberculatus* in the first, second, and third month of the 90 d culturing period was decreased to 29.56%, 10.13%, and 3.39%, respectively (The mortalities were decreased according to the size of the estimated national mortality of the first, second, and third month, respectively). According to our sampling data, the average individual wet weight of *P. trituberculatus* in the first, second and third month of the 90 d culturing period was calculated as 7.23 g, 38.27 g, and 77.62 g, respectively.

The biomass of the crabs expressed in wet weight (g m^{-2}) was converted to energy (kJ m^{-2}). The dry weight was measured as 21.05% of the wet weight of the crabs, the energy content of the dry weight of the crabs was measured as 16.63 kJ g^{-1}.

The dry weight of *P. trituberculatus* was obtained by oven-drying specimens for 48 h at 60°C. The energy content of the dried samples was measured by an oxygen bomb calorimeter (PARR-1281, America).

The P/B ratio of *P. trituberculatus* was calculated as the average individual production to the average individual biomass of crabs expressed in energy (kJ m^{-2}) during the 90 d culture period. The average individual production of the crabs was calculated as the difference between the average individual biomass of the harvested crabs and the average

individual biomass of the initial crabs. The Q/B ratios of *P. trituberculatus* was obtained from (Yang et al. 2010).

1.3 White shrimp *Litopenaeus vannamei*

The biomass of *L. vannamei* was calculated as the average value of the initial biomass, harvesting biomass, and the biomass of each sample measurement. The shrimps were sampled at 10 d intervals, which were caught with a cage net in each enclosure. The total length of shrimps was measured by a vernier caliper. The body weight of shrimps was calculated as the method of Xie et al. (2007):

$$W = 0.01955L^{2.784} \quad \text{(S11)}$$

Where W is the body weight of shrimp (g), L is the total length (from the tip of the rostrum to the distal end of the telson; cm). Animals were returned to their enclosure after measuring.

The total mortality of *L. vannamei* in the 90 d culturing period was calculated as 36.43% according to our field experiment. According to Ye et al. (1994), we assumed that the mortality of the shrimps associated with stocking was 10%. The natural mortality (M) of *L. vannamei* during the whole culturing period was obtained using empirical formula from (Gislason et al. 2008):

$$\ln M = -2.1104327 + 1.5067827 \ln L_{\infty} - 1.7023068 \ln L + 0.9664798 \ln K + 763.5074169 \times \left(\frac{1}{T}\right) \quad \text{(S12)}$$

where M is an annual instantaneous rate (y^{-1}), L_{∞} is the asymptotic total length of *L. vannamei*, which is 12.88 cm (Aragón-Noriega & Alberto 2016). L (cm) is the total length of *L. vannamei* for which the M estimate would apply, K (0.21) is the rate at which the rate of growth of *L. vannamei* in length declines as length approaches L_{∞}(y-1) (Li et al. 2015), T is absolute temperature (^{0}Kelvin).

The average total length of *L. vannamei* in the first, second and third month of the 90 d culturing period was measured as 1.63 cm, 5.33 cm, and 7.76 cm, respectively. The mean water temperature of the cultured pond in the first, second and third month of the 90 d culturing period was measured as 303.16 K, 299.66 K, and 296.25 K, respectively. As a result, the natural mortality of *L. vannamei* in the first, second and third month of the 90 d culturing period was estimated as 33.03%, 7.31%, and 3.05%, respectively. The total natural mortality was calculated as 43.39%. On account of that the stocking of *L. vannamei* may lead to a mortality of 10.00% in the polyculture ecosystem, and the actual total mortality of *L. vannamei* in this polyculture ecosystem was 36.43%. The natural mortality of *L. vannamei* in the first, second, and third month of the 90 d culturing period was decreased to 20.10%, 4.45%, and 1.86%, respectively (The mortalities were decreased according to the size of the estimated national mortality of the first, second, and third month, respectively). Based on the sampling data, the average individual wet weight of *L. vannamei* in the first, second and third month of the 90 d culturing period was calculated as 0.16g, 2.06 g, and 5.88 g, respectively.

The biomass of the shrimps expressed in wet weight (g m^{-2}) was converted to energy (kJ m^{-2}). The dry weight was measured as 22.08% of the wet weight of the shrimps, the energy content of the dry weight of the shrimps was measured as 19.81 kJ g^{-1}.

The dry weight of *L. vannamei* was obtained by oven-drying specimens for 48 h at 60°C. The energy content of the dried samples was measured by an oxygen bomb calorimeter (PARR-1281, America).

The P/B ratio of *L. vannamei* was calculated as the average individual production to the average individual biomass of the shrimps expressed in energy (kJ m^{-2}) during the 90 d culture period. The average individual production of the shrimps was calculated as the difference between the average individual biomass of the harvested shrimps and the average individual biomass of the initial shrimps. The Q/B ratios of *L. vannamei* was obtained from (Qi et al. 2010).

1.4 Short-necked clam *Ruditapes philippinarum* and redlip mullet *Liza haematochila*

The biomass of *R. philippinarum* and *L. haematochila* was calculated as the average value of their initial biomass and harvesting biomass. The biomass of the clams and redlip mullets expressed in wet weight (g m^{-2}) were converted to energy. The conversion equation of the *R. philippinarum* was:

$$\text{Biomass (kJ m}^{-2}\text{)} = \text{Biomass (g m}^{-2}\text{)} \times \text{meat weight to total clam weight (\%)} \times \text{dry matter content of meat (\%)} \times \text{energy content of the dry matter of the meat (kJ g}^{-1}\text{)} \quad \text{(S13)}$$

where the ratio of meat weight to total clam weight (%) was measured as 28.37%, the dry matter content of the meat (%) was 20.48%, the energy content of the dry matter of the meat was 20.53 kJ g^{-1}.

The dry weight of *L. haematochila* was measured as 25.99% of the wet weight, the energy content of the dry weight of the *L. haematochila* was measured as 21.40 kJ g^{-1}. The dry weight of the *R. philippinarum* and *L. haematochila* were obtained by oven-drying specimens for 48 h at 60°C. The energy content of the dried samples was measured by an oxygen bomb calorimeter (PARR-1281, America).

P/B ratios of *R. philippinarum* and *L. haematochila* were calculated as their average individual production to their average individual biomass during the 90 d culture period, respectively. The average individual production of *R. philippinarum* and *L. haematochila* was calculated as the difference between their harvested average individual biomass and their initial average individual biomass, respectively. The average individual biomass of *R. philippinarum* and *L. haematochila* during the 90 d culture period was calculated as the average value of their harvested average individual biomass and their initial average individual biomass, respectively. The Q/B ratios of *R. philippinarum* and *L. haematochila* were obtained from Zhang & Yan (2010) and Li et al. (1995), respectively.

1.5 Macrobenthos and microbenthos

The biomass of macrobenthos and microbenthos were measured by collecting the sediment of the pond enclosure with a sediment sampler. The collected sediment samples were filtered through a 0.5 mm mesh screen. The benthos with the size larger than 0.5 mm was collected as macrobenthos. The sampling of macrobenthos was conducted following the specification for oceanographic survey of China (State oceanic administration People's Republic of China 2007). The energy content of the biomass of macrobenthos was measured by an oxygen bomb calorimeter (PARR-1281, America). The benthos with the size smaller than 0.5 mm was collected as microbenthos. The sampling of microbenthos was conducted following Liu et al. (2014). The biomass of the microbenthos expressed in organic carbon was measured by Vario ELIII Elemental Analyzer (Elementar, Dortmund, Germany). The biomass of the microbenthos expressed in organic carbon was converted to energy content. The conversion relationship was: 1g organic carbon = 46 kJ energy (Salonen et al. 1976).

The P/B ratio of macrobenthos was obtained from Zhou & Xie (1995), the P/B ratio of microbenthos was obtained from Schwinghamer et al. (1986). The Q/B ratios macrobenthos, and microbenthos were both adopted from Lin (2012).

1.6 Macrozooplankton and microzooplankton

The biomass of macrozooplankton and microzooplankton were obtained from the field experiment. A total of 30 L of the surface layer, middle layer and bottom layer water samples were filtered through a 150 μm plankton net to collect the macrozooplankton. The water samples of macrozoopalnkton were condensed to 30 ml, preserved with 5% buffered formalin and examined in the laboratory (Nauwerck 1963). The macrozooplankton samples were enumerated and length measured with a stereo dissecting microscope. The wet weight of cladocera and copepods were calculated according to the body length-body weight regression equation from Zhang & Huang (1991). The volume of planktonic mollusks was calculated according to its geometric shape, and the volumes were converted to wet weight assuming that 1 mm^3 of the volume was equivalent to 1 mg of wet weight biomass (Zhang 2014). The microzooplankton was collected by taking the mixed water 1 L of the surface layer, middle layer and bottom layer water samples of the enclosure pond. The water samples were filtered through a 100 mesh sieve. The filtrates were fixed with 5% buffered formalin (Nauwerck 1963). The biomass of the microzooplankton was measured under 100×microscope. The volume of protozoa and rotifers were calculated according to their geometric shape, the volumes were converted to wet weight assuming that 1 mm^3 of volume was equivalent to 1 mg of wet weight biomass (Zhang 2014).

Biomass of macrozooplankton and microzooplankton expressed in wet weight was converted to energy content using the following relationships: the dry weight of the macrozooplankton and microzooplankton were about 20% of their wet weight, and the carbon content were about 40% of their dry weight (Omori 1969). Meanwhile, it was assumed that 33% of the dry weight of the macrozoopalnkton and microzooplankton were lost due to fixation (Giguere et al. 1989). The organic carbon of the macozooplankton and microzooplankton were then converted to energy content as 1 g organic carbon = 46 kJ energy (Salonen et al. 1976).

The P/B and Q/B ratios of macrozooplankton and microzooplankton were calculated according to the P/Q ratios from Straile (1997) and the measurement of zooplankton respiration. The respiration of mcrozooplankton and microzooplankton was measured following Williams (1981). When the P/Q ratio, respiration, and unassimilated consumption (Winberg 1960) were known, the value of P and Q of macrozooplankton and microzooplankton, can be calculated according to the equation:

$$Q/B = P/B + R/B + U/B \quad \text{(S14)},$$

respectively.

1.7 Bacterioplankton and benthic bacteria

The biomass of bacterioplankton and benthic bacteria was analyzed using acridine orange direct count method (AODC). The volume of bacteria was calculated with the measured length of the long and short axes of the bacteria by fluorescence microscopy software Image Pro-Plus 5.1. The relationship between bacterial volume and biomass of organic carbon was: B (C) =5.6×10^{-13} g of C um^{-3} (Bratbak 1985). The relation of organic carbon and energy of the biomass of bacterioplankton and benthic bacteria were: 1 g organic carbon = 46 kJ energy (Salonen et al. 1976).

The P/B value of baterioplankton was obtained from the field experiment by following the method of Schwaerter et al. (1988).

The P/B value of benthic bacteria was calculated according to P/Q ratios of benthic bacteria from Moriarty (1986) and the measurement of benthic bacteria respiration. According to Hargrave (1972), the respiration of benthic bacteria accounted for 0.64 of the sediment respiration (Hargrave 1972). The respiration of sediment was determined by a sediment respirator which was made of Plexiglas with a diameter of 7.0 cm. The structure of the sediment respirator was described in detail by Zheng et al. (2011). Surface sediment (0–5 cm) was collected by the sediment respirator, and then brought to the laboratory within an hour. In the laboratory, the sediment was put aside for 2 h to recover equilibrium. Overlying water in each respirator was carefully replaced by the bottom water using rubber pipes. Incubations were conducted in water baths for 4 h in darkness at in-situ temperature. Initial and final water samples were taken from respirators and measured for dissolved oxygen with Winkler titration method. Respirators filled with water (without sediment) were used as the control to adjust the data for the respiration of the overlying water. Sediment oxygen demand [SOD, (mg O^2)/ (m^2·d)] was calculated as:

$$SOD = \frac{(DO_O - DO_t) \times V}{A \times t} \times 24 \quad \text{(S15)}$$

Where DO_O (mg O^2 L^{-1}) is the initial dissolved oxygen. DO_t (mg O^2 L^{-1}) is the final dissolved oxygen. V (liters) is the volume of the overlying water. A (m^2) is the area of the respirator cross-section and t (d) is the duration of the sediment respiration experiment. The unit of SOD, [(mg O^2)/ (m^2·d)], was converted to (mg C)/ (m^2·d) according to Winberg (1 mg O^2 = 0.375 mg C; Winberg 1980), and then converted to kJ/ (m^2·d) according to Salonen (1g organic carbon = 46 kJ energy; Salonen et al. 1976). When the P/Q ratio, respiration, unassimilated consumption (Winberg 1960) of benthic bacteria were known, the P value can be calculated according to equation:

$$Q/B = P/B + R/B + U/B \quad \text{(S16)}$$

The Q/B ratios of bacterioplankton, benthic bacteria were calculated according to their P/B values and the measurement of their respiration. When the P/B value, respiration and unassimilated consumption of bacterioplankton and benthic bacteria were known, the Q/B values of bacterioplankton and benthic bacteria can be calculated according to equation:

$$Q/B = P/B + R/B + U/B \quad \text{(S17)},$$

respectively.

1.8 Detritus groups (detritus in the water, detritus in the sediment, *Aloidis laevis* and shrimp feed)

The biomass of detritus: the biomass of detritus in the water was obtained by determining the total organic carbon of the mixed water samples taken from the surface layer, middle layer and bottom layer of the enclosure pond using a water sampler. The total organic carbon of the mixed water samples was measured by TOC automatic analyser (Multi N/C 2100S, Analytik Jena AG, Germany). The organic carbon of detritus in water was converted to energy content according to Salonen (1g organic carbon = 46 kJ energy; Salonen et al. 1976).

The surface layer of 5 cm of the benthic sediment was sampled by a bottom sampler. After drying and grinding the sediment samples, the energy content of the sediment samples was measured by an oxygen bomb calorimeter (PARR-1281, America). The inputs of artificial feeds of shrimp feed and blue clam *A. laevis* were recorded every day. After drying

and grinding the energy content of the samples of shrimp feed and *A. laevis*,the energy content of shrimp feed and *A. laevis* were measured by an oxygen bomb calorimeter (PARR-1281, America).

2 DIET COMPOSITION (THE DIETS OF CONSUMERS WHEN WE FIRST CONSTRUCT THE MODEL)

The original input data of the diet composition of *P. trituberculatus*, *L. vannamei*, *R. philippinarum*, *L. haematochila*, macrobenthos, microbenthos, macrozooplankton, microzooplankton was mainly revealed by iso-source mixing model analyses of carbon stable isotope ratios. The carbon stable isotope analysis indicated that the *A. laevis* and macrozooplankton contributed about 0.940 and 0.060 to the diets of *P. trituberculatus*, respectively. In addition, the macrobenthos mainly composed of polychetes contributed about 0.004 to the diets of *P. trituberculatus* (Yang 2001). When the prey of *P. trituberculatus* was expanded to include macrobenthos in this model, the percentage of *A. laevis* in the diet of *P. trituberculatus* was reduced to 0.056.

The diets of *L. vannamei* revealed by carbon stable isotope analysis included macrozooplankton (0.060), microzooplankton (0.010), micro-phytoplankton (0.010), nano-phytoplankton (0.010), pico-phytoplankton (0.010), *A. laevis* (0.310), shrimp feed (0.540) and detritus in sediment (0.020).

The diets of *R. philippinarum* revealed by carbon stable isotope analysis included micro-phytoplankton (0.211), nano-phytoplankton (0.223), pico-phytoplankton (0.229), detritus in water (0.275) and detritus in sediment (0.062). As there is no suitable method for the determination of $\delta^{13}C$ ratio of bacterioplankton, the percentage (0.140) of bacterioplankton in the diet of *R. philippinarum* was calculated according to Wetzel (1983) and Zhang et al. (2005). When the prey of *R. philippinarum* was expanded to include bacterioplankton, the percentage of micro-phytoplankton, nano-phytoplankton, pico-phytoplankton and detritus in water in the diet of *R. philippinarum* were reduced by 0.040, 0.040, 0.040, and 0.020, respectively.

The diets of *L. haematochila* revealed by carbon stable isotope analysis included macrozooplankton (0.038), microzooplankton (0.054), micro-phytoplankton (0.193), nano-phytoplankton (0.186), pico-phytoplankton (0.204), periphyton (0.034), detritus in water (0.138) and detritus in sediment (0.153). The percentage (0.030) of bacterioplankton in the diet of *L. haematochila* was calculated according to Liu & Li (1999). When the prey of *L. haematochila* was expanded to include bacterioplankton, the percentages of micro-phytoplankton, nano-phytoplankton, and pico-phytoplankton in the diet of *L. haematochila* were reduced by 0.010.

The diets of macrobenthos revealed by carbon stable isotope analysis included microbenthos (0.087), periphyton (0.019), *A. laevis* (0.243) and detritus in the sediment (0.651). As there is also no suitable method for the determination of $\delta^{13}C$ ratio of benthic bacteria, the percentage (0.054) of benthic bacteria in the diet of macrobenthos was calculated according to Wang & Zhang (1998) and Tsuchiya & Kurihara (1979). When the prey of macrobenthos was expanded to include benthic bacteria, the percentages of microbenthos, periphyton, *A. laevis* and detritus in the sediment in the diet of macrobenthos were reduced by 0.010, 0.010, 0.010 and 0.024, respectively.

The diets of microbnthos revealed by carbon stable isotope analysis included *A. laevis* (0.290) and detritus (0.710). The percentage (0.65) of benthic bacteria in the diet of macrobenthos was obtained from Jin (2010). Assuming that the *A. laevis* and detritus in the

sediment collectively accounted for 0.350 of the diet of microbenthos, each of them accounted for 0.100 and 0.250 of the diet of microbenthos, respectively.

The diets of macrozooplankton revealed by carbon stable isotope analysis included microzooplankton (0.130), micro-phytoplankton (0.180), nano-phytoplankton (0.190), pico-phytoplankton (0.190), *A. laevis* (0.271) and detritus in sediment (0.039). The percentage of bacterioplankton (0.270) in the diet of macrozooplankton was calculated according to Li & Lin (1995), when the prey of macrozooplankton were expanded to include bacterioplankton, the percentages of micro-phytoplankton, nano-phytoplankton, and pico-phytoplankton in the diet of macrozooplankton were all reduced by 0.090.

The diets of microzooplankton revealed by carbon stable isotope analysis included micro-phytoplankton (0.206), nano-phytoplankton (0.216), pico-phytoplankton (0.215), periphyton (0.012), shrimp feed (0.126) detritus in water (0.13) and detritus in sediment (0.095). The percentage (0.088) of bacterioplankton in the diet of macrozooplankton was calculated according to Li & Lin (1995), when the prey of macrozooplankton were expanded to include bacterioplankton, the percentage of micro-phytoplankton, nano-phytoplankton, pico-phytoplankton, and shrimp feed in the diet of macrozooplankton were all reduced by 0.022.

The diet composition of benthic bacteria and bacterioplankton were obtained from Zhou (2015).

3 BALANCING THE ECOPATH MODEL

When the input data were made to the model, it was unbalanced and the Table S1 showed the ecotrophic efficiencies and the unbalanced groups.

The *EE* values of macrobenthos, macrozooplankton and microzooplankton were higher than 1. As the macrobenthos was consumed by *P. trituberculatus* in this ecosystem, the consumption of macrobenthos by *P. trituberculatus* was reduced to 0.001, and the consumption of macrozooplankton by *P. trituberculatus* was increased to 0.059.

The macrozooplankton was consumed by *P. trituberculatus*, *L. vannamei* and *L. haematochila*. For reducing the *EE* value of macrozooplankton to less than 1, the diet of *P. trituberculatus* was revised by reducing the percentage of macrozooplankton to 0.029, and increasing the percentage of *A. laevis* to 0.970. The diet of *L. vannamei* was revised by reducing the percentage of macrozooplankton to 0.010, and increasing the percentage of micro-phytoplankton, nano-phytoplankton, pico-phytoplankton, and *A. laevis* to 0.02, 0.02, 0.02, and 0.33, respectively.

The microzooplankton was consumed by *L. vannamei*, *L. haematochila* and macrozooplankton. For reducing the *EE* value of microzooplankton to less than 1, the diet of *L. vannamei* was revised by reducing the percentage of microzooplankton to 0, and increasing the percentage of shrimp feed to 0.580. The diet of *L. haematochila* was revised by reducing the percentage of microzooplankton to 0.034, and increasing the percentage of bacterioplankton to 0.050.

When the P/Q values of macrozooplankton and microzooplankton were set as 0.30 (Straile 1997)), the P/R values of macrozooplankton and microzooplankton was estimated equal to 1. Therefore, we reduced the P/Q values of macrozooplankton and microzooplankton to 0.29. The P/R values of macrozooplankton and microzooplankton were slightly lower than 1, which were acceptable (Link 2016).

Table S1 The inputs and outputs of the first constructed Ecopath model (Values in bold were the parameters estimated by model, the values in green representing the unbalanced parameters. Pot: *Portunus trituberculatus*; Liv: *Litopenaeus vannamei*; Rup: *Ruditapes philippinarum*; Lih: *Liza haematocheli*; Mab: macrobenthos; Mib: microbenthos; Maz: macrozooplankton; Miz: microzooplankton; Beb: benthic bacteria; Bab: bacterioplankton; Mip: micro-phytoplankton; Nap: nano-phytoplankton; Pip: pico-phytoplankton; Pep: periphyte; All: *Aloidis laevis*; Shf: shrimp feeds; Des: detritus in sediment; Dew: detritus in water. *B*: biomass; *P/B*: production/biomass; *Q/B*: consumption/biomass; *EE*: ecotrophic efficiency *P/Q*: production/consumption; *R/A*: respiration/assimilation; *P/R*: production/respiration)

Group name	*B* (kJ m^{-2})	*P/B* ($90days^{-1}$)	*Q/B* ($90days^{-1}$)	*EE*	*P/Q*	*R/A*	*P/R*	Detritus import (kJ m^{-2} $90days^{-1}$)
Pot	525.78	1.99	6.03	0.83	**0.33**	**0.59**	**0.70**	
Liv	531.78	1.99	13.49	0.96	**0.15**	**0.82**	**0.23**	
Rup	40.73	1.53	27.60	0.90	**0.06**	**0.92**	**0.08**	
Lih	138.35	1.86	18.38	0.97	**0.10**	**0.87**	**0.14**	
Mab	0.57	6.44	21.47	**3.46**	**0.30**	**0.63**	**0.60**	
Mib	2.08	9.23	30.76	**0.05**	**0.30**	**0.62**	**0.60**	
Maz	7.09	40.50	139.65	**1.95**	0.30	**0.49**	**1.00**	
Miz	2.73	108.00	372.41	**2.34**	0.30	**0.50**	**1.00**	
Beb	144.50	28.80	95.40	**0.01**	0.30	**0.62**	**0.61**	
Bap	15.53	66.60	197.10	**0.56**	**0.34**	**0.58**	**0.73**	
Mip	10.71	148.50	0.00	**0.63**				
Nap	20.94	171.90	0.00	**0.28**				
Pip	16.83	288.90	0.00	**0.22**				
Pep	11.93	9.90	0.00	**0.83**				
All	64.15			**0.95**				5,773.50
Shf	49.40			**0.90**				4,446.00
Des	5722.01			**0.96**				
Dew	245.80			**0.45**				

References

Aragón-Noriega, Alberto E (2016) Individual growth of white shrimp *Litopenaeus vannamei* (Boone, 1931) and blue shrimp L. stylirostris (Stimpson, 1874) (Crustacea: Penaeidae) by multi-model approach. Lat Am J Aquat Res 44:480–486

Bratbak G (1985) Bacterial biovolume and biomass estimations. Appl Environ Microbiol 49:1488–1493

Diana JS, Lin CK, Schneeberger PJ (1991) Relationships among nutrient inputs, water nutrient concentrations, primary production, and yield of *Oreochromis niloticus* in ponds. Aquaculture 92:323–341 doi:10.1016/0044-8486(91)90038-9

Eppley RW, Reid FMH, Strickland JDH (1970) The ecology of the plankton of La Jolla California, in the period April through September 1967. In: Stickland JDH (ed) Estimates of Phytoplankton Crop Size, Growth Rate and Primary Production Part III. Bull Scripps Inst Oceanogr 17: 33-42

Gao YF, Wang YF, Bao XN, Ren ZM, Mu CK, Wang CL (2016) Study on the characteristic of molting and growth of *Portunus trituberculatus* cultured in single individual basket. J Biol 33:41–46 (in Chinese with English abstract)

Giguere LAJ, Pierre F, Bernier B, Vezina A, Rondeau JG (1989) Can we estimate the true weigh t of zooplankton samples after chemical preservation. Can J Fish Aquat Sci 46:522–527 doi:10.1139/f89-070

Gislason H, Daan N, Rice JC, Pope JG (2008) Does natural mortality depend on individual size? ICES CM 2008/F:16

Griffiths VS, Jackman MI (1957) The winkler method for dissolved oxygen determination [J]. Anal Chim Acta 17:603–605 doi:10.1016/S0003-2670(00)87093-2

Hargrave BT (1972) Aerobic decomposition of sediment and detritus as a function of particle surface area and organic content. Limnol Oceanogr 17:583–586 doi:10.4319/lo.1972.17.4.0583

Harris GP (1986) Phytoplankton ecology, structure, function and fluctuations. Chapman and Hall, London, 36: 221-235.

Hillebrand H, Dürselen CD, Kirschtel D, Pollingher U, Zohary T (1999) Biovolume calculation for pelagic and benthic microalgae. J Phycol 35:403–424 doi:10.1046/j.1529-8817.1999.3520403.x

Jeffrey SW, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls a, b, and c in higher plants, algae and natural phytoplankton. Biochem Physiol Pflanz 167:191–194 doi:10.1016/S0015-3796(17)30778-3

Jin BC (2010) Using stable isotope to evaluate food sources of pond-cultured sea cucumber (*Apostichopus japonicus*). PhD thesis, Ocean University of China, Qingdao (in Chinese with English abstract).

Johnson KM, Burney CM, Sieburth JMN (1981) Enigmatic marine ecosystem metabolism measured by direct diel ΣCO2 and O2 flux in conjunction with DOC release and uptake. Mar Biol 65:49–60 doi:10.1007/BF00397067

Lee JT, Coleman RA, Jones MB (2006) Population dynamics and growth of juveniles of the velvet swimming crab *Necora puber*, (Decapoda: Portunidae) [J]. Mar Biol 148:609–619 doi:10.1007/s00227-005-0107-1

Li CH, Lin WL (1995) Grazing rate of zooplankton on bacterioplankton in Donghu Wuhan. Acta Ecol Sin 15:142–147 (in Chinese with English abstract)

Li J, Yang JM, Dou SZ (1995) Study on the ecological growth efficiency of juvenile Redlip Mullet. Mark Sci 19:68–69 (in Chinese with English abstract)

Li YH, Song QQ, Zhang ZH, Huang H, Zhou HL, Xiang JH (2015) Analysis of growth and development rules and growth curve fitting of *Litopenaeus vannamei.* Nanfang Shuichan Kexue 11:89–95 (in Chinese with English abstract)

Lin Q (2012) Studies on the ecosystem energy transfer and function in the typical waters of Yellow and Bohai Sea. PhD thesis, Ocean University of China, Qingdao (in Chinese with English abstract).

Link JS (2016) Corrigendum to "Adding rigor to ecological network models by evaluating a set of pre-balance diagnostics: A plea for PREBAL" [Ecol. Model. 221 (2010) 1582–1593] Ecol Model 337:348–349 doi:10.1016/j.ecolmodel.2016.06.012

Liu GC, Li DS (1999) A study on bacteria attached to suspended particles in shrimp ponds. Acta Oceanol Sin 21:97–102 (In Chinese with English abstract)

Liu L, Li J, Gao BQ, Liu P, Dai FY, Pan LQ (2009) Correlation of growth traits of *Portunus trituberculatus* at the different ages and its impact on body weight. Shuichan Xuebao 33:964–971 (in Chinese with English abstract)

Liu XS, Xu M, Zhang JH Mou G, Liu D, Li X (2014) Abundance and biomass of deep-sea meiofauna in the northern South China Sea. J Tropic Oceanogr 33(2):52-59 (in Chinese with English abstract).

Lund JWG, Kipling C, LeCren ED (1958) The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting [J]. Hydrobiologia 11:143–170 doi:10.1007/BF00007865

Moriarty DJ (1986) Bacterial productivity in ponds used for culture of Penaeid Prawns. Microb Ecol 12:259–269 doi:10.1007/BF02011169

Nauwerck A (1963) The relation between zooplankton and phytoplankton in Lake Erken. Symb Bot Ups 17:1–163

Omori M (1969) Weight and chemical composition of some important oceanic zooplankton in the North Pacifc Ocean. Mar Biol 3:4–10 doi:10.1007/BF00355587

Qi M, Shen YH, Zhu CH (2010) A primary study on conversion efficiency of *Litopenaeus vannamei* feed on artificial diets. Fish Modern 37:34–37 (In Chinese with English abstract)

Salonen K, Sarvala J, Hakala I, Viljanen ML (1976) The relation of energy and organic carbon in aquatic invertebrates. Limnol Oceanogr 21:724–730 doi:10.4319/lo.1976.21.5.0724

Schwaerter S, Søndergaard M, Bo R, Jensen LM (1988) Respiration in eutrophic lakes: the contribution of bacterioplankton and bacterial growth yield. J Plankton Res 10:515–531 doi:10.1093/plankt/10.3.515

Schwinghamer P, Hargrave B, Peer D, Hawkins CM (1986) Partitioning of production and respiration among size groups of organisms in an intertidal benthic community. Mar Ecol Prog Ser 31:131–142 doi:10.3354/meps031131

State oceanic administration People's Republic of China (2007) GB/T12763.6-2007 Specification for oceanographic survey. Standard Press of China, Beijing (in Chinese)

Straile D (1997) Gross growth efficiencies of protozoan and metazoan zooplankton and their dependence on food concentration, predator-prey weight ratio, and taxonomic group. Limnol Oceanogr 42:1375–1385 doi:10.4319/lo.1997.42.6.1375

Tsuchiya M, Kurihara Y (1979) The feeding habits and food sources of the deposit-feeding polychaete, *Neanthes japonica*, (Izuka). J Exp Mar Biol Ecol 36:79–89 doi:10.1016/0022-0981(79)90101-1

Wang SH, Zhang ZN (1998) Study of *Neanthes japonica* feeding on natural sediment. J Ocean Univ Qingdao 28:587–592 (in Chinese with English abstract)

Wetzel RG (1983) Limnology, 2nd edn. Saunders College Publications, Philadelphia, PA

Williams PJLB (1981) Microbial contribution to overall marine plankton metabolism: direct measurements of respiration. Oceanol Acta 4:359–364

Winberg GG (1960) Rate of metabolism and food requirements of fishes. Fish Res Bd Can Trans Ser 194: 1-253

Winberg GG (1980) General characteristics of freshwater ecosystems based on Soviet IBP studies. The Functioning of Freshwater Ecosystems. Cambridge University Press, London, p 481–491

Xie RZ, Liu JY, Fan CJ, Lin GC, Zhou ZF (2007) Growth of *Litopenaeus vannamei* Cultivated in High-Intensive Ponds. J Guangdong Ocean Univ 27:50–54

Yang JM (2001) A study on food and trophic levels of Bohai Sea invertebrates. Modern Fish Inf 16(9):8-16 (in Chinese with English abstract)

Yang H, Ma S, Su YP (2010) Differences of food consumption, metabolism and growth of the crab (*Portunus trituberculatus*) fed fresh, frozen and oven-dried clam (*Ruditapes philippinarum*) meat. Period Ocean Univ China 40:65–70 (in Chinese with English abstract)

Ye CC, Sun DS, Zhen BT Len LP, Du QL, Wang XH, Song DG (1994) The direction of released shrimp (*Penaeus orientalis*) and their death characteristic in the northern yellow sea. Mar Fish Res (S1):31-39 (in Chinese with English abstract)

Zhang TW (2011) The model of carbon flux in intensive Penaeid shrimp culture and ecological cultivation. PhD thesis, Ocean University of China, Qingdao (in Chinese with English abstract).

Zhang K (2014) Study on total organic carbon budgets and bacterial productivity and metabolism of polyculture system of *Portunus trituberculatus, Litopenaeus vannamei* and *Ruditapes philippinarum*. PhD thesis, Ocean University of China, Qingdao (in Chinese with English abstract)

Zhang ZS, Huang XF (1991) Research Methods for Freshwater Plankton. Science Press, Beijing, p 358-387(In Chinese with English abstract).

Zhang GF, Yan XW (2010) Clam Aquaculture Study. Science Press, Beijing, p 169–176 (in Chinese)

Zhang JH, Feng JX, Sun S, He Y, Zhang F (2005) Clearance rate, ingestion rate and absorption efficiency of cultivated clam *Ruditapes philippinarum* in Jiaozhou Bay, China. Oceanol Limnol Sin 36:548–555 (in Chinese with English abstract)

Zheng ZM, Lv J, Lu KH, Jin CH, Zhu JY, Liu XS (2011) The impact of snail (*Bellamya aeruginosa*) bioturbation on sediment characteristics and organic carbon fluxes in an eutrophic pond. Clean–Soil Air Water 39:566–571

Zhou B (2015) Research on Grass carp (*Ctenopharyngodon idella*) integrated aquaculture pond ecosystem: Based on EwE model. PhD thesis, Ocean University of China, Qingdao (in Chinese with English abstract).

Zhou YB, Xie ZH (1995) Secondary production of *Neanthe japonica* in shrimp ponds. Shuichan Xuebao 19:140–150 (in Chinese with English abstract)