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Evaluate the Biomass of *Fenneropenaeus chinensis* from the Southern Coast of Shandong Peninsula Using eDNA

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Abstract: *Fenneropenaeus chinensis* is an important economic species in the north of China, and plays an important role in both marine fishing and aquaculture. Long-term overfishing has led to the rapid decline of wild *F. chinensis* resources. The traditional trawl survey could not meet the demands of the *F. chinensis* resource survey. In this study, environmental DNA (eDNA) technology was used to evaluate the biomass of *F. chinensis* in the traditional Qinghai (Qingdao Haiyang) fishing ground in the southern sea area of the Shandong Peninsula, with the purpose of verifying whether eDNA technology can provide a new resource assessment method for fisheries resource species such as *F. chinensis*. The eDNA quantitative results of the Qingdao water samples ranged from 1972 copies/L to 6937 copies/L, with an average of 4366 ±1691 copies/L. Those in Haiyang water samples ranged from 4795 copies/L to 8715 copies/L, with an average of 6737 ± 1348 copies/L. The concentration of eDNA in shrimp culture ponds ranged from 1.14×10^6 copies/L to 7.61×10^6 copies/L, with an average of $3.33 \times 10^6 \pm 2.28 \times 10^6$ copies/L. The amount of eDNA released by each gram of *F. chinensis* per 24 h was about 2.91×10^6 copies. According to this calculation, it was estimated that the distribution of *F. chinensis* was about one shrimp in every 300 m^2 sea area. Similarly, it is estimated that one shrimp is distributed every 240 m^2 in the Haiyang sea area. The result of this study confirms the feasibility of using eDNA to evaluate the biomass of shrimps.

Keywords: *Fenneropenaeus chinensis*; biomass; eDNA; fishery resources

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1. Introduction

Fenneropenaeus chinensis is an important economic species in the north of China, and plays an important role in both marine fishing and aquaculture. Long-term overfishing has led to the rapid decline of *F. chinensis* resources, and annual landings dropped from 40,000 t in history (autumn shrimp-fishing) to about 3000–5000 t now. The spring shrimp fishing caused by the breeding migration of shrimp has disappeared since early 1990. At present, the biomass of *F. chinensis* in the Yellow and Bohai Sea mainly relies on artificial propagation and release, and the released shrimp has contributed more than 95% of the autumn yield. The annual migratory gravid female was an important object to evaluate the supplement of resources by propagation and release, which was related to the ecological security of the *F. chinensis* population. At present, due to disorderly fishing, most gravid female shrimp have been caught in the Yellow Sea off the southeast coast of the Shandong Peninsula, and few of them can enter the traditional spawning grounds along the Bohai Sea (Laizhou Bay, Bohai Bay, and Liaodong Bay). Based on traditional bottom trawl survey methods, gravid female shrimp has not been found in these three spawning grounds for many years. In recent years, the newly emerging environmental DNA (eDNA) technology was expected to effectively remedy the defects of trawl survey. eDNA technology refers to a new technology that analyzes the DNA fragments released into the environment

through feces, mucus, blood, skin, decaying tissues, or molting, to determine whether certain organisms exist in a certain environment and their abundance. The most important advantage of this technology in comparison with the traditional methods, is that it can realize the assessment of the existence or even abundance of target species without monitoring live organisms. In aquatic environment ecology, eDNA technology was first used in the monitoring of bullfrogs [1]. After that eDNA technology has also been widely used in invasive species monitoring [2,3], biodiversity, fish diversity, and fishery resources assessment [4–7], population genetic diversity assessment and conservation genetics [8,9], including activity distribution monitoring of large marine mammals and large fish, and population size assessment [10–12]. The eDNA abundance can also be used to predict and evaluate biomass. Baldigo [13] used eDNA technology to evaluate the population density and biomass of Brook trout (*Salvelinus fontinalis*) in streams. The results showed that eDNA could explain the changes of 44% and 24% of the population density and biomass respectively. Stoeckle [5] research found that the seasonal abundance of up to 70% of the catch was highly consistent both in trawling and eDNA. In view of the fact that the number of gravid female shrimp in spawning grounds in the Yellow and Bohai Seas was becoming less and less, and the traditional trawl survey was weak, and the traditional trawl survey was weak, this study used eDNA technology to evaluate the biomass of *F. shrimp* in the traditional Qinghai (Qingdao Haiyang) fishing ground in the southern sea area of Shandong Peninsula, with the purpose of verifying whether eDNA technology can provide a new resource assessment method for species such as *F. chinensis*.

2. Materials and Methods

2.1. Materials

The gravid female *F. chinensis* in Qinghai fishery ground, belonging to the southern coast of the Shandong Peninsula were collected at two time points. Shrimps in Qingdao waters were collected from Qingdao National Central Fishing Port on 15 April 2022 and those in Haiyang were collected from Haiyang Fishing Port on 30 April 2022. All the samples were gravid females with already-developed gonads. After sampling, shrimps were maintained alive till their transport to the laboratory, where they got weighed.

Corresponding to live sample collection, water sample collection was completed in the corresponding time and corresponding sea area. Specifically, water samples from Qingdao were collected from 6 locations on April 17, and those from Haiyang were collected from 5 locations on April 30 (Figure 1).

In addition to the field experiments, water samples were collected from 8 indoor gravid female *F. chinensis* culture ponds (Tianjin Shentang Aquatic Breeding & Culturing Co., LTD., Tianjin, China) during the 20–25th of April. eDNA quantitative analysis was also carried out. The water volume of each shrimp pond was 15 m^3, with 200 shrimp being raised in each of them. The water was changed 100% every day and replenished to the original water level.

2.2. Methods

A 2 L water intake device was used to collect seawater samples. In each sampling site, the bottom seawater was collected and it was repeated three times according to the depth of the sampling point. Additionally, the water of the surface, middle, and bottom layers of each sampling location were collected respectively, and filtered by means of a 0.45 μm glass fiber filter membrane (Xingya, Shanghai). The eDNA-enriched filter membranes were folded and stored at −20 °C for later analysis.

Water samples from the indoor aquaculture ponds were collected both in the middle and around the pond on a daily scale, just before changing the water. The same methods and sampling volume were used as in the wild. Ten individuals were randomly selected from each pond for weight measurement.

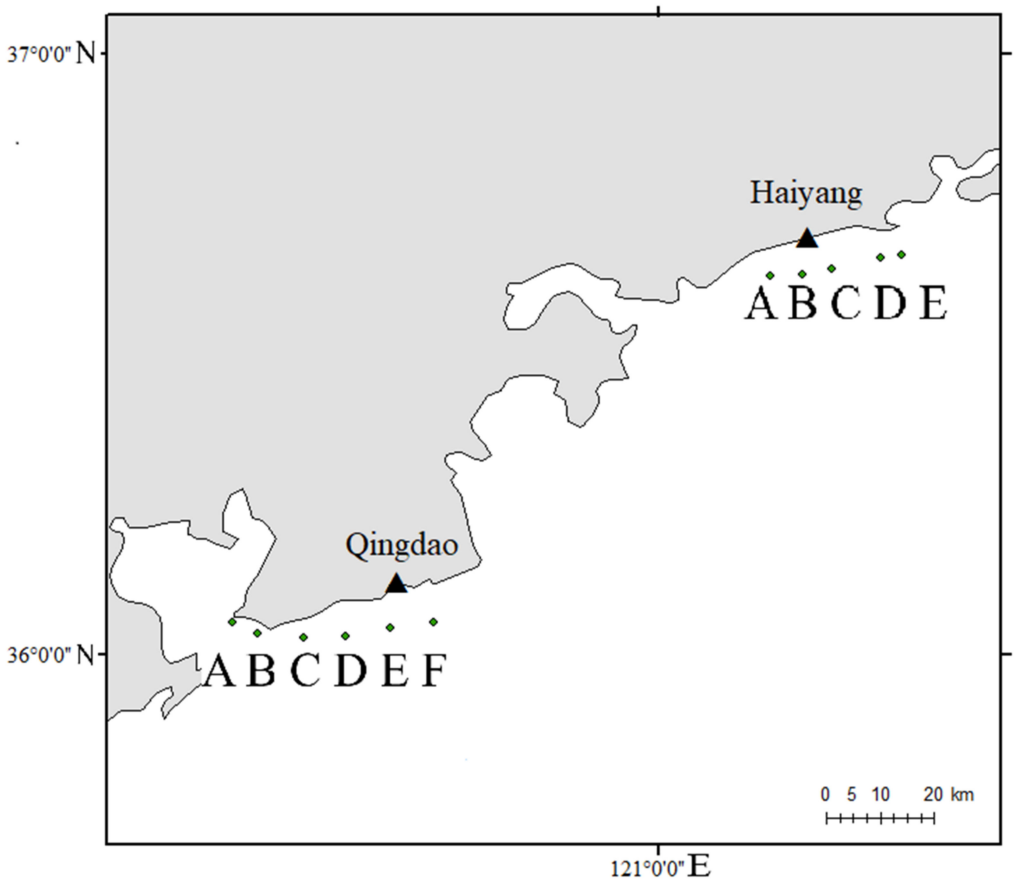

Figure 1. Sampling location distribution in the Qingdao sea area and Haiyang sea area of the South coast of the Shandong Peninsula. ABCDEF indicate each location respectively in the Qingdao sea area and Haiyang sea area.

Based on the eDNA concentrations, the numbers, and the average body weights of shrimp in culture ponds, the eDNA amount released by shrimp per unit body weight (g) was estimated. Based on this standard and the eDNA concentration of the natural seawater, the biomass of gravid female *F. chinensis* in the sampling sea area was estimated. Since the eDNA in natural waters was constantly in the process of accumulation and degradation, the eDNA measured in the experiment included both the current release and the accumulation and degradation of eDNA in a certain period of time before. In the present study, the average temperature of seawater was about 10 °C during sampling. According to the results of our team's previous research, the degradation cycle of eDNA in seawater of *F. chinensis* was about 10 days under this water temperature [14]. The following model was used to correct the concentration of eDNA in seawater [14]:

$$N = \frac{\int_0^{240} 680.71 \times e^{-0.0116x} dx}{\int_0^{24} 680.71 \times e^{-0.0116x}\, dx} \quad (1)$$

N is the concentration multiple of eDNA accumulated in water compared with that at the time of water sample collection; x is the complete degradation time (hour) of eDNA in water, and the maximum value of x is set as 240 h in this experiment.

The eDNA was extracted using a DNeasy Blood and Tissue kit (Qiagen, Germany). The mitochondrial COI gene was the target fragment of eDNA quantification in each water sample, and the quantitative analysis was conducted by absolute quantitative PCR using the probe method. The primer sequence, probe sequence, standard preparation,

and quantitative PCR amplification experiments were all based on the technical system previously established in our laboratory [15].

3. Results

3.1. eDNA Concentration of F. chinensis in Water

A total of 158 and 75 gravid female *F. chinensis* were collected from Qingdao and Haiyang waters, respectively. The body weights of the samples from Qingdao ranged from 46.6 g to 104.4 g, with an average of 74.22 ± 10.23 g. Those from Haiyang ranged from 56.6 g to 134.1 g, with an average of 99.54 ± 16.98 g. The interval between the two samples was half a month, and the average body weight of Haiyang was significantly higher than that of Qingdao ($p < 0.01$). The body weight of *F. chinensis* samples in culture ponds ranged from 55.5 g to 120.3 g, with an average of 85.23 ± 8.17 g.

A total of 9 and 11 samples from different water layers were collected from Qingdao and Haiyang waters, respectively. The eDNA quantitative results of the Qingdao water samples ranged from 1972 copies/L to 6937 copies/L, with an average of 4366 ±1691 copies/L (Table 1). Those in Haiyang water samples ranged from 4795 copies/L to 8715 copies/L, with an average of 6737 ± 1348 copies/L (Table 1). The average eDNA concentration of *F. chinensis* in Haiyang waters was significantly higher than that in Qingdao ($p < 0.01$).

Table 1. The eDNA concentration in the Qingdao sea area and Haiyang sea area.

Locations in the Qingdao Sea Area	eDNA Concentration (Copies/L)	Locations in Haiyang Sea Area	eDNA Concentration (Copies/L)
Surface layer of A	2510.34	Surface layer of A	4795.98
Bottom layer of A	5966.94	Surface layer of B	4908.12
Bottom layer of B	1972.32	Middle layer of B	6297.90
Bottom layer of C	2756.46	Bottom layer of B	5707.80
Surface layer of D	6215.58	Surface layer of C	8715.00
Bottom layer of D	4512.48	Middle layer of C	5672.94
Surface layer of E	6937.56	Bottom layer of C	6105.12
Bottom layer of E	5029.92	Surface layer of D	8687.28
Bottom layer of F	3400.32	Middle layer of D	7574.38
		Surface layer of E	7672.14
		Middle layer of E	8324.40
		Bottom layer of E	6384.42
Mean	4366 ± 1691	Mean	6737 ± 1348

The concentration of eDNA in shrimp culture ponds ranged from 1.14×10^6 copies/L to 7.61×10^6 copies/L, with an average of $3.33 \times 10^6 \pm 2.28 \times 10^6$ copies/L (Table 2). According to this calculation, the amount of eDNA released by each gram of *F. chinensis* per 24 h was about 2.91×10^6 copies.

Table 2. The eDNA concentration in culture ponds.

Number of Culture Ponds	eDNA Concentration (Copies/L)
1	3,487,183
2	4,021,327
3	7,605,872
4	6,015,915
5	1,227,555
6	1,656,314
7	1,138,116
8	1,474,368
Mean	$3.33 \times 10^6 \pm 2.28 \times 10^6$

3.2. Estimation of the Biomass of F. chinensis in Natural Waters

The eDNA concentration in Qingdao waters was 4.3×10^6 copies/m^3, and the average body weight of shrimp samples was 74.22 ± 10.23 g. Therefore, the eDNA concentration per cubic meter of water in Qingdao waters was equivalent to 1.477 g of body weight of shrimp. Equivalently about one *F. chinensis* is distributed in every 50 m^3 of natural water. Since the natural seawater eDNA would have different degradation cycles according to the ambient temperature after being released into the water [14]. The seawater temperature during sampling was about 10 °C, and the degradation cycle was about 10 days [14]. According to Model (1), the accumulated eDNA concentration in water was estimated to be 6 times the concentration of eDNA in the water at the time of sampling. After data correction, it was estimated that the distribution of *F. chinensis* was about one shrimp in every 300 m^3 of water. For the convenience of data comparison, it was roughly converted to one shrimp in every 300 m^2 of water.

Similarly, according to the eDNA concentration of 6.7×10^6 copy/m^3 and the average weight of 99.54 ± 16.98 g in Haiyang, and the biomass density was about one shrimp in every 240 m^3 of water. As above, it is estimated that one shrimp was distributed every 240 m^2. Compared with Qingdao waters, the distribution density increased slightly.

4. Discussion

Compared to nuclear genes, mitochondrial DNA has far more copies than nuclear DNA in cells and sequence is more conserved, which makes it easy to detect. Therefore, the mitochondrial COI gene was adapted in eDNA research. A variety of factors, including different species, physiological status, growth rate, feeding, and nutritional status, could affect the amount of eDNA released by organisms into the environment [16–18]. It was found that during the molting period, *F. chinensis* would release 40–200 times more eDNA than the normal state (to be published). In this study, the sampling time of water and shrimp was spring, and the *F. chinensis* had already mated and completed winter migration. They would not molt before spawning. Therefore, at this stage, the main physiological activities of the shrimp were feeding and supplementing the body nutrients to prepare for spawning, for the concrete manifestation of gonads developing rapidly and body weight increasing significantly. Excluding the slight difference in sea temperature between the two sampling time points, it was speculated that the eDNA of *F. chinensis* in the water in this study was mainly from the physiological process of defecation. In this study, the time interval of sampling in Qingdao and Haiyang was only half a month, and the average body weight showed a significant increase ($p < 0.01$). Meanwhile, the eDNA concentration of *F. chinensis* in the two sampling areas also showed a significant difference ($p < 0.01$), indicating that with the increase of body weight of *F. chinensis*, the amount of eDNA released into the water also increased. From Qingdao to Haiyang offshore, which was called Qinghai fishing ground, a certain amount of *F. chinensis* migrate and enter Jiaozhou Bay and Dingzi Bay to spawn from March to May every year. Since the two sampling areas are adjacent and both belong to Qinghai fishing grounds, there will be no significant difference in population density between the two sampling times. If the average body weights combined with the distribution densities of the two samples (Section 3.2) were converted into the biomass of *F. chinensis* per 100 m^2, the effect of biomass on eDNA concentration can be described more objectively. The biomass of *F. chinensis* was 30.93 g per 100 m^2 in Qingdao and 33.17 g per 100 m^2 in Haiyang, which indicated that the increment of eDNA should be significantly correlated with the biomass. The increase in biomass is considered to be an important factor leading to the increase in eDNA release, which has been demonstrated in some freshwater and seawater fish with a linear correlation [18,19]. The positive correlation between biomass and eDNA concentration was not only confirmed by eDNA detection in some single species [7], but also in multi-species high-throughput sequencing of some marine bony fish: the reads sequence abundance of specific fish was also highly positively correlated with the corresponding catch abundance, and this positive correlation was reflected in multiple fishing seasons [5]. Water temperature, water layer, ocean current or

tide, physiological state and development stage of animals, etc., all have different degrees of influence on eDNA release and its concentration. With the gradual deepening of the research on these factors, the accuracy of eDNA estimation of biomass can be more accurately improved. However, the positive correlation between eDNA concentration and biomass was not changed.

In this study, the amount of eDNA released in 24 h per gram body weight was calculated by using the concentration of in culture ponds. Combined with the detected concentration of eDNA in Qingdao and Haiyang waters, and the degradation rate of eDNA under the current water temperature, the distribution densities of *F. chinensis* in Qingdao and Haiyang waters during two sampling points were estimated to be about one per 300 m^2 and one per 240 m^2, respectively. Historically, the amount of gravid female *F. chinensis* is quite abundant, and a significant spring shrimp-fishing season of *F. chinensis* is formed every year. Due to the disordered increase in fishing intensity, the capturing yield in spring declined sharply after the 1980s, and by 1990, the spring shrimp-fishing season disappeared completely [20]. Since then, there was no fishing boat to conduct the exclusive fishing of *F. chinensis* in spring, and it has become the concurrent fishing object in the fishing operation. This is also the reason why live and water samples in this study were not obtained in the same sampling area. In fact, due to its scarcity, there have been few reports on the biomass of gravid female *F. chinensis* in its traditional spawning grounds of the Yellow Sea and Bohai Sea in the past 30 years. The most recent correlational research showed that the density of gravid female *F. chinensis* in Yanwei (Yantai Weihai) fishery ground in late April ranged from 0.31 to 6.50 shrimp/net×hour Between 1989 and 1994 [21], and the average value was calculated as 2.665 shrimp/net×hour. Considering the general single trawl mouth is 10 m wide, and the trawl speed is 2 knots (about 3.70 km/h), the sweep area per hour is about 37,000 m^2 [22]. Besides, the escape index of *F. chinensis* during the trawling process is about 0.7 [22]. Based on the above information, the actual number of gravid female *F. chinensis* within a 37,000 m^2 area is about 8.85, which is equivalent to 0.07 shrimp per 300 m^2. It's important to note that this result is based on the survey from 1989–1994 [21], during which the resources of *F. chinensis* declined sharply. Although the autumn landings had a small peak in 1990 (13,000 tons), in the same year the spring landings plummeted from 764 t the previous year to zero. In the following years, the autumn landings of *F. chinensis* also sharply decreased, and it plummeted to 500 t in 1998 [23]). In the past 10 years, with the continuous propagation and release of *F. chinensis*, the autumn yield in the Yellow Sea and Bohai Sea has recovered to about 4000 t per year. Although it has not reached the historical peak, it has increased by about 8 times compared with the lowest year of 500 t. Assuming that the number of gravid female shrimp also increases in equal proportion, during the breeding migration every spring, the density of gravid female shrimp in Yanwei fishery ground should be restored to about 0.56 shrimp /300 m^2, which is very close to the results estimated in this study (1 shrimp /300 m^2 and 1 shrimp /240 m^2 in Qingdao and Haiyang, respectively). Prediction and assessment of fishery biomass using eDNA have become an interesting topic in the field of fishery resources research. In both relatively closed freshwater streams and open marine environments, eDNA has been used to reveal the biomass and its temporal and spatial changes to varying degrees [19,24,25]. In this study, due to the scarcity of gravid female *F. chinensis* in natural waters, simultaneous location and time sampling of live and water samples are not possible, which is one of the directions for improving the accuracy of eDNA assessment results in the future. As mentioned above, eDNA concentration is significantly affected by water temperature, ocean current, physiological state, etc. This is an important cause of uncertainty in the assessment of biomass using eDNA. Meanwhile, it should be noted that eDNA in water is always in a dynamic process of continuous accumulation and degradation, which needs to be taken into account. Compared with previous similar studies, the current study incorporated this dynamic process in analysis produced more credible results.

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