

# Influences of Three Salinities on the Growth Performance and Digestive Enzyme Activities at Different Stages of *Chrysaora quinquecirrha*

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**Abstract:** The Atlantic sea nettle (*Chrysaora quinquecirrha*) has been paid more and more attention due to its high ecological value and commercial cultivation. This study aimed to evaluate that culture salinity levels affected on the growth and digestive enzyme activities of *C. quinquecirrha*. Three salinity levels (10‰, 20‰ and 30‰) were used in cultured jellyfish at three stages of scyphula, juvenile and adult medusa. Results suggested that salinity had changed jellyfish physiological performance about growth and digestion. The scyphula was capable of growth and asexual reproduction in three salinity levels. All digestive enzyme activities (pepsin, cellulase, lipase and amylase) of scyphula were significantly higher than those of juvenile and adult ( $P < 0.01$ ). Appropriate salinity levels had improved growth, survival and digestive enzyme expression in artificial conditions. Salinity 10 was beneficial to the developed body and adult breeding and further desalting, and it could be used as the preservation salinity of scyphula for inland aquarium and home ornamental jellyfish. In salinity 20 jellyfish showed a good state of asexual reproduction, growth performance and vitality, and the activities of four digestive enzymes were the highest except for cellulose and amylase in juvenile and lipase and amylase in adult, which could be used as a suitable salinity for artificial breeding. The content of pepsin was the highest among the four digestive enzymes due to the high protein bait and the high protein demands of *C. quinquecirrha*. The results revealed the scyphula showed strong adaptability to external salinity changes, and its cell osmotic regulation ability was stronger than the developed body and adult medusa. Further desalination could be used in inland aquariums and home viewing jellyfish. Artificial domestication of freshwater was possible to improve the economic benefits in the future. Scyphula could asexual reproduce to permanently maintain its characteristic of feeding in fresh water and had strobilated in a certain condition to produce medusa which could be fed in fresh water. And this study provided a theoretical basis for the adjustment of bait, the activation and adaptation mechanism of *C. quinquecirrha* to environment and its commercial cultivation and development. This test detected a certain cellulase in the digestive cavity, however, further study was required to determine whether *C. quinquecirrha* has a complete cellulase system or a symbiotic relationship with a fungus, bacteria and alga, that could produce exogenous cellulose.

**Keywords:** *Chrysaora quinquecirrha*, Salinity, Digestive Enzyme, Scyphula, Medusa

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## 1. Introduction

Salinity is an important physical and chemical factor in the aquatic environment. The salinity changes can cause different levels of stress reaction for the animals and affect their internal stability, and trigger a series of physical changes of

the organism. Unless living in an isosmotic environment, the first effect of salinity changes on aquatic animals is osmotic stress. At present, studies on the influences of salinity on aquatic animals focus on fish, shrimp, shell, and other marine

animals [1-5], and no report has shown the influences of salinity on the growth and digestive enzyme activity of jellyfish. Fishes consume a large amount of energy and actively regulate the osmotic pressure with organs such as their liver and kidney, affecting their digestive physiological activity. Digestive enzymes are essential to the digestion and absorption of nutrients of fishes. The level of enzyme activity in fishes can reflect their digestive ability [6]. The salinity of the aquatic environment where fishes live can affect their digestive enzyme activity and ultimately affect their capability to digest and absorb food [7]. Pepsin is activated and produced by the pepsinogen secreted by the main cells in stomach. It breaks down protein in food into small peptide fragments or amino acid. Cellulase is a generic term of enzymes which can degrade celluloses to glucose. Some animals can secrete cellulase while some of them use cellulase secreted by symbionts in their digestive system. Lipase can be found in multiple parts of the digestive system. It breaks down fats to provides energy to organism for movement. The degree of amylase activity is greatly related to the feeding habits of animals [8, 9]. When feeding jellyfish, the salinity changes in water can trigger various physiological stress responses. The salinity can affect the physiological activities and functions, including metabolism by osmotic adjustment. The study suggests the existence of the ion rebalance in the *Aurelia coerulea* polyps, which the expression of the osmoregulation genes such as Vavulartype ATPase (VHA) and Aquaporin9 (AQP9) increases significantly during the strobilation interphase, and the expression of  $\text{Na}^+\text{-K}^+\text{-ATPase}$  (NKA), VHA, Aquaporin4 (AQP4), AQP9 gene and NKA enzyme activity increase significantly in strobilation stage [10]. Salinity has a positive effect on the supplementation of *Nemopilema nomurai* to the polypoid population via sexual reproduction, when salinity in sea environments is too low (15), the development process in the sexual reproduction is slowed, reducing the development rate and adversely affecting the supplementation of reproduction to the polyp population [11].

*C. quinquecirrha* is the genus of *Chrysaora* in the family Pelagiidae of the order of Semaestomeae in the class of Scyphozoa under the phylum of Cnidaria [12]. The Atlantic sea nettle has become one of the most suitable breeding species artificially and ornamentally due to its elegant appearance, fast growth, high aquatic production, etc. However, the study on the Atlantic sea nettle production and breeding technology has just started. Only a few researches in the basic phylogeny and toxic effects were published and none on the mechanism of environmental adaptation [13, 14]. This study focuses on the growth of the Atlantic sea nettle in different stages under different salinity by detecting and analyzing the influences of three salinities on the asexual reproduction rate of scyphistoma, the activity of pepsin, cellulase, lipase, and amylase, and the growth rate of larva and adult, and the activity of digestive enzymes. The study provides basic information about the responsive and adaptive mechanism of the Atlantic sea nettle to the environment

and the theoretical basis for artificial desalination breeding and baits adjustment.

## 2. Material and Methods

### 2.1. Test Materials

The experimental work was conducted in accordance with the laws and regulations controlling experiments and procedures on live animals in China. Testing animals were scyphistoma and medusa fed in Qingdao Aquarium in 2021. The low salinity scyphistoma was fed for more than three months in the stable salinity. The medusa was produced by the strobilation of scyphistoma with the same salinity. The water temperature was 20-23°C during the test. Scyphistoma and medusa were fed with *Artemia* nauplii incubated for 24h once a day and the mixture of *Artemia* nauplii, mysids, artificial feed and *Aurelia aurita* twice a day, respectively. The aquaculture water was adjusted with natural seawater (salinity of 32) and tap water and used after the tap water was aerated for more than 24h.

### 2.2. Test Methods

#### 2.2.1. Asexual Proliferative Capacity of Scyphistoma

One hundred scyphula were randomly cut from polyethylene corrugated plate and fed in a 1L beaker in an incubator at 22±1°C. The quantity of new scyphistoma and ephyra larva were counted, and new scyphula adhered to the plate were removed when changing the water every day. One hundred scyphula at the beginning of the test were bred for 30 days to calculate the capability of production and strobilation of each scyphistoma.

$$\text{Proliferative rate} = \frac{\text{Quantity of new scyphistoma}}{100} \times 100\%$$

$$\text{Strobilative capability} = \frac{\text{Quantity of ephyra larva}}{100}$$

#### 2.2.2. Cultivation of Juvenile and Adult Medusa

The scyphistoma was artificially fed in three salinities in the temperature-controlled incubator at 22±1°C. Then 100 ephyra larvae that were strobilated in two days were collected and moved to the bubble-driven water flows bath for breeding for 15 days and count the total quantity of the juvenile medusa. 30 juveniles are randomly selected and measured the umbrella diameter and weight while the tissue supernatant was prepared. The rest developed bodies were moved to the circular cylinder with a water pump driving flow to breed for 25 days before counting the number of the adult medusa. Then, adults of 40 days are randomly selected to measure their umbrella diameter and weight when tissue supernatant was prepared.

$$\text{Survival rate} = \frac{\text{Current quantity}}{\text{Quantity of moved } C. \text{quinquecirrha}} \times 100\%$$

#### 2.2.3. Preparation and Detection of Digestive Enzyme Samples

Whole scyphistoma was taken without feeding for 48

hours prior the experiment. During the test, 90 scyphula were randomly selected in any salinity and divided into 6 groups and were washed with sterilized seawater, removed from excessive moisture and ground in ice bath.

30 juveniles were randomly collected and divided into 6 groups. Then, the exumbrellas and oral arms were removed. The filaments in the gastric pouch and the gastrodermis on subumbrella were retained. The tissues of every five samples were put into one group and mixed for ice bath grinding in a grinding tube.

30 adults were randomly collected. Then, the exumbrellas and oral arms are removed. The contents in gastric pouch and the gastrodermis on subumbrella were kept. The tissues were put in the grinding tube for ice bath grinding. The grinding liquid of tissues was centrifuged in 5,000r/min for 5 minutes and then the supernatant was prepared for standby.

#### 2.2.4. Reagent and Apparatus

The insect pepsin ELISA kit, the insect cellulase ELISA kit, the insect lipase ELISA kit, and the insect amylase ELISA kit produced by Shanghai Jining Industrial Co., Ltd. were used for the testing. HBS-1096A Microplate Reader was used to detect 450nm absorbance to calculate digestive enzyme activity in every milliliter or liter of sample according to the standard curve.

#### 2.3. Statistical Analysis

Statistical analyses were performed following the followed methods described by Sokal and Rolf [15]. All data presented

were tested for normality and homogeneity of variance. Means and standard deviations (SD) were calculated for each parameter measured, which was presented in Mean  $\pm$  SD. Data were submitted to a one-way analysis of variance (ANOVA) and significant differences were considered when  $p < 0.05$ . When applicable, data were subjected to the best fit correlations, which were checked for significance at  $p < 0.05$ , and Pearson's coefficients were determined. Analyses were performed using the SPSS Statistical Software System v19.0 (SPSS, IBM, China).

### 3. Results

#### 3.1. Influences of Salinity on the Asexual Reproduction Capability of Scyphula

The scyphula of the Atlantic sea nettle was capable of growth and asexual reproduction in all three salinities (Table 1). The ephyra larvae produced was healthy and normal. In the salinity of 30, the reproduction rate of podocyst was the fastest and the most podocyst were grown up the new scyphula at 22°C. In the salinity of 20, scyphula had the strongest capability to strobilate with 0.45 ephyra larvae produced every day for each scyphula, which was significantly higher than 0.09 in the group of the salinity of 10 ( $P < 0.01$ ) and higher than 0.36 in salinity 30 ( $P < 0.05$ ). Compared with means, SD of reproduction rate and quantity of ephyra larvae by strobilation in salinity 10 was larger than others, which indicating that polys in salinity 10 were more susceptible and changeable.

Table 1. The capability of asexual reproduction of each scyphula in three salinities.

Reproduction capability	Salinity (‰)		
	10	20	30
Reproduction rate (each)	0.12 $\pm$ 0.06 <sup>B</sup>	0.27 $\pm$ 0.09 <sup>a</sup>	0.31 $\pm$ 0.03 <sup>a</sup>
Quantity of ephyra larvae by strobilation (each)	0.09 $\pm$ 0.04 <sup>B</sup>	0.45 $\pm$ 0.11 <sup>A</sup>	0.36 $\pm$ 0.10 <sup>a</sup>

Note: The same superscript means insignificant difference ( $P > 0.05$ ) while the different letters mean significant difference ( $P < 0.05$ ) and the capital letters mean the extremely significant difference ( $P < 0.01$ ) in the same row.

#### 3.2. The Influences of Salinity on the Growth of Juvenile and Adult

The developed body of *C. quinquecirrha* all grew healthily in the salinities of 10, 20, and 30 with the survival rate of 86.67%, 100% and 100%, respectively (Table 2). In the salinity of 20, the

diameter of umbrella grew the fastest to 18.25mm after 15 days, and they were significantly higher than those in salinity 30 ( $P < 0.05$ ) although there was no significant difference between salinity 20 and salinity 30 ( $P > 0.05$ ). Body quality in salinity 20 was the biggest with 3.01g, but there weren't significant difference in three groups ( $P > 0.05$ ).

Table 2. Influences of salinity on the growth of developed body.

Salinity (Unit: ‰)	Growth		
	Umbrella diameter (Unit: mm)	Body quality (unit: g)	Survival rate (unit: %)
10	13.61 $\pm$ 4.33 <sup>b</sup>	2.46 $\pm$ 0.32 <sup>a</sup>	86.67
20	18.25 $\pm$ 2.17 <sup>a</sup>	3.01 $\pm$ 0.25 <sup>a</sup>	100
30	15.79 $\pm$ 1.34 <sup>a</sup>	2.89 $\pm$ 0.26 <sup>a</sup>	100

Note: The same superscript means insignificant difference ( $P > 0.05$ ) while the different letters mean significant difference ( $P < 0.05$ ) and the capital letters mean the extremely significant difference ( $P < 0.01$ ) in the same column. The following tables show the same.

The survival rates of adult *C. quinquecirrha* in salinity 10, 20 and 30 are 80.77%, 96.67% and 93.33%, respectively

(Table 3). The umbrella diameter and body weight were the fastest to 86.43mm and 81.23g in salinity 20, after 40 days, and vitality were the best. The umbrella diameter ( $P<0.05$ ) and body weight ( $P<0.01$ ) were significantly higher than those in salinity 10, but there weren't significant difference

between salinity 20 and 30 ( $P>0.05$ ). The jellyfish in salinity 10 grew slowly with fewer cnidosac on the exumbrella. The survival rates were all high on the 40<sup>th</sup> day, with 26 jellyfish surviving 21 in salinity 10, 30 surviving 29 in salinity 20, and 30 surviving 28 in salinity 30 from juvenile to adult.

**Table 3.** Influences of salinity on the growth of adult *C. quinquecirrha*.

Salinity (Unit: ‰)	Growth		
	Umbrella diameter (Unit: mm)	Body quality (unit: g)	Survival rate (unit: %)
10	70.25±11.54 <sup>b</sup>	66.65±7.25 <sup>B</sup>	80.77
20	86.43±8.21 <sup>a</sup>	81.23±2.80 <sup>A</sup>	96.67
30	80.92±6.20 <sup>a</sup>	78.15±3.73 <sup>A</sup>	93.33

### 3.3. Influences of Salinity on Pepsin Activity in Three Stages

In this test, ELISA kit was used to obtain a curve of linear regression binomial expression for standards,  $y=220.28x^2+584.89x-42.341$  and correlation coefficient  $R^2=0.9999$  between standard linear regression and concentration. The pepsin activity of *C. quinquecirrha* scyphula was higher than the developed body and adult with the highest activity in salinity 20, 531.19 U/mL (Table 4). As growing, the pepsin activity in unit volume had gradually reduced in salinity 10 being 59.51U/mL. Pepsin activities of three stages in salinity 10 were lower than those in other salinity, which was consistent with their umbrella diameter and body weight, indicating that jellyfish in salinity 10 digested less protein and affected their growth.

**Table 4.** Influences of salinity on pepsin activity in three morphologies (Unit: U/mL).

Salinity (‰)	Scyphula	Developed body	Adult
10	336.10±41.21 <sup>b</sup>	80.09±16.18 <sup>a</sup>	59.51±5.39 <sup>B</sup>
20	531.59±28.37 <sup>a</sup>	88.17±10.42 <sup>a</sup>	92.93±7.28 <sup>A</sup>
30	435.19±35.21 <sup>a</sup>	81.43±16.57 <sup>a</sup>	64.12±7.57 <sup>B</sup>

### 3.4. Influences of Salinity on Cellulase Activity in Three Morphologies

With correlation coefficient  $R^2=0.9999$  between standard linear regression and concentration, the linear regression binomial expression was obtained as  $y=154.67x^2+432.36x-25.123$ . The cellulase activity of *C. quinquecirrha* scyphula was higher than that of developed body and adult (Table 5). With days increasing, the difference in cellulase activity among different salinity groups was getting smaller. The influence of salinity on the cellulase activity of scyphula was the biggest from 862.48 U/L in salinity 20 to 168.52 U/L in salinity 30. There was only 54.79U/L in salinity 10 and extremely lower than those in salinity 20 and salinity 30 ( $P<0.01$ ). The cellulase activity of scyphula and adult was the highest in salinity 20, while that of juvenile was the highest in salinity 30 group. They were similar to pepsin activity with the highest activity in salinity 20 for scyphula which was 862.48U/L.

**Table 5.** Influences of salinity on cellulase activity in three morphologies (Unit: U/L).

Salinity (‰)	Scyphula	Developed body	Adult
10	288.07±33.45 <sup>B</sup>	67.04±6.23 <sup>B</sup>	54.79±7.86 <sup>B</sup>
20	862.48±28.75 <sup>A</sup>	66.55±8.41 <sup>B</sup>	72.00±9.25 <sup>A</sup>
30	168.52±25.10 <sup>Ab</sup>	93.14±5.93 <sup>A</sup>	71.25±7.13 <sup>A</sup>

### 3.5. Influences of Salinity on Lipase Activity in Three Morphologies

**Table 6.** Influences of salinity on lipase activity in three morphologies (Unit: U/L).

Salinity (‰)	Scyphula	Developed body	Adult
10	154.90±35.97 <sup>B</sup>	91.72±5.68 <sup>B</sup>	84.37±8.53 <sup>a</sup>
20	349.85±23.31 <sup>A</sup>	150.45±3.93 <sup>A</sup>	83.64±6.82 <sup>a</sup>
30	148.91±20.96 <sup>B</sup>	97.61±7.79 <sup>B</sup>	86.58±6.45 <sup>a</sup>

With correlation coefficient  $R^2=0.9991$  between standard linear regression and concentration, the linear regression binomial expression obtained was  $y=89.852x^2+697.94x-58.809$ . The lipase activity of *C. quinquecirrha* in different salinities was always the highest in scyphula, followed by developed body with the lowest concentration in unit volume for adult (Table 6). The highest activity was in salinity 20 for scyphula, which was 349.85U/L while the lowest in the same salinity for adult which was 83.64U/L. The differences in three stages between salinity 10 and 30 were insignificant ( $P>0.05$ ), while there were extremely significant between salinity 20 and 30 for scyphula and juvenile ( $P<0.01$ ).

### 3.6. Influences of Salinity on Amylase Activity in Three Morphologies

With the linear regression and concentration related coefficient  $R^2=0.9999$ , the linear regression binomial expression of standards is obtained  $y=265.17x^2+393.67x-34.483$ . In all three forms, the amylase activity of *C. quinquecirrha* for scyphula is the highest in salinity 20 and is 260.20U/L (Table 7). The highest amylase activity of scyphula, juvenile and adult appeared in the salinity 20, 30 and 30, respectively. It indicated that scyphula had the strongest adaptability and it was easier to domesticate in freshwater if it was started at scyphula stage. The amylase activity of scyphula in salinity 10 was significantly higher

than in salinity 30 but the activities of juvenile and adult in salinity 30 were higher than those in salinity 10. Multiple individuals were mixed in scyphula and juvenile with small data differences, which conforms to normal distribution. The amylase activity of adult, which came from single individual, showed a big difference with the standard deviation large to the mean. It was possibly related to individual difference and low amylase activity. The amylase activity of adult among three morphologies showed insignificant difference ( $P>0.05$ ), though there were many differences in scyphula and juvenile.

**Table 7.** Influences of salinity on amylase activity in three morphologies (Unit: U/L).

Salinity (‰)	Scyphula	Developed body	Adult
10	198.26±40.40 <sup>a</sup>	21.18±5.04 <sup>B</sup>	13.84±4.09 <sup>a</sup>
20	260.20±35.62 <sup>A</sup>	30.06±4.25 <sup>B</sup>	13.39±4.15 <sup>a</sup>
30	86.33±41.27 <sup>B</sup>	55.86±4.81 <sup>A</sup>	15.66±4.40 <sup>a</sup>

## 4. Discussions

### 4.1. Influences of Salinity on the Growth of *C. quinquecirrha* in Three Morphologies

Salinity is an important environmental factor affecting various physiological activities, including the growth and metabolism of aquatic animals. The change of salinity can force an organism to adjust the dynamic balance between internal and external osmotic pressure following a series of physiological changes affecting biological indicators such as growth, survival, feeding, respiratory metabolism, enzyme and hormonal levels, and development. The direct effects of salinity on fish ecology and physiological actions are forcing the fishes with the capability of osmotic adjustment to counter the osmotic pressure. For fish without the capability of osmotic adjustment the salinity may cause discomfort, anesthesia, coma or death. The indirect effects are the influences on the material exchange and energy flow between the environment and fishes [16]. Studies show that maintaining normal metabolism plays an important role in salinity adjustment among aquatic animals. When the osmotic pressures in the body liquid of aquatic animals and external waters are equal, the organism will consume very little energy. In water with lower or higher salinity, shrimp need to maintain the ion concentration and osmotic pressure by consuming the energy in their body. Thus, the metabolism and energy supply of the caridean shrimp *Palaemon peringueyi* are inhibited in water with low salinity, which resulting a decrease of their growth rate and survival rate [17]. In salinity of 16-24, the certain growth rate of sea cucumber *Apostichopus japonicus* will increase with the rise of salinity [18].

Salinity directly affects the osmotic pressure regulation and plays an important role in the growth and development of jellyfish. Studies have shown that low salinity is good for larvae adhesion, larvae metamorphosis and polyps asexual reproduction of moon jellyfish *Aurelia coerulea* [19]. In salinity 18-33, the lower the salinity, the survival time of larvae is longer and more polyps are formed by larvae in moon jellyfish *A. aurita* [20, 21].

In this test, scyphula was domesticated in low salinity for a long time. The scyphula ingest, perform asexual reproduction and transverse fissure normally, in all three salinities. This shows that this test satisfy the condition for the growth and reproduction of the Atlantic sea nettle. The scyphula reproduction rate is the highest in salinity 30 while salinity 20 has the strongest capability of transverse fissure with 0.45 ephyra larvae by strobilation every day. As a result, salinity between 20 and 30 is the optimal environment for the normal growth of the Atlantic sea nettle scyphula with strong adaptation to the external salinity and strong capability of osmotic adjustment for further artificial domestication in fresh water. In salinity 10, scyphula can produce ephyra larva by transverse fissure. The ephyra larva grows normally in the same salinity with the umbrella diameter of the developed body after 15 days up to 13.61mm, though it is slower with a survival rate of 89.67. With the days of the testing increasing, the umbrella diameter and body weight increase the fastest with the best vitality and survival rate in salinity 20. The difference in the adaptation to and the growth rate in different salinities of the Atlantic sea nettle may be related to the capability of organism metabolism, ion transfer and energy synthesis.

### 4.2. Influences of Salinity on Digestive Enzyme Activity of *C. quinquecirrha* in Three Morphologies

Digestive enzyme is the enzyme for digestion secreted by the digestive system. Different digestive enzymes are distributed in different parts of the digestive system. All enzymes can supply energy for movement, growth, and reproduction by digesting and breaking down food. Talking animal species out of the factor, the activity of digestive enzyme is closely related to external factors, including food, temperature, salinity, and feeding method. The changes of salinity will vary the inorganic ion content in the water and then affect the activity of digestive enzyme. The study shows that the salinity has significant influences on the activity of digestive enzymes of fish, including of pepsin enzyme, amylase, and lipase [1]. The activities of three digestive enzymes in hepatopancreas and testine are the highest in salinity 26 on half-smooth tongue sole *Cynoglossus semilaevis* [22]. Shi et al. discussed the reactive response mechanism of yellowtail kingfish *Seriola aureovittata* to the gradual changes of salinity. The study shows that the activities of lipase in stomach, testine, liver and pyloric caecum is the highest at the gradient point of salinity 29. The pepsin activity in pyloric caecum is the highest at the gradient point of salinity 35. The amylase activity in stomach, testine and liver is the highest at the gradient point of salinity 29 while in pyloric caecum the highest is at the gradient point of salinity 35 [23]. In the salinity of 16 to 24, the activity changes of pepsin, amylase and lipase in digestive tract tend to be consistent for sea cucumber *Apostichopus japonicus*, which increase with the rise of salinity. When the salinity is higher than 24, the activity of three digestive enzymes reduces with the rise of salinity [18]. The activities of intestinal protease, amylase, cellulase and lipase are high in

the testine of *urechis unicinctus* in the salinity 25-35 while the vitality of digestive enzymes and immunocompetence significantly reduce in the low salinity of 15-20 [5].

Maintaining normal physiological metabolism plays an important role during jellyfish adapting to salinity changes. When the osmotic pressure between body fluid and the external water is equal, jellyfish consumes the least energy. This test takes the Atlantic sea nettle in stages of scyphula, developed body and adult in conditions of artificial feeding as the objects. All animals are fed at  $23\pm1^{\circ}\text{C}$  with *Artemia* nauplii incubated for 24 hours every day. With the same feeding conditions, the activities of pepsin, cellulase, lipase, and amylase were measured by insect Elisa kit at three salinity levels in three stages, because the metamorphosis, open circulation system and liquid flow cycle of jellyfish are similar to insects. In this study, the activities of four enzymes in scyphula are significantly higher than those in the developed body and adult. On one hand, it is because the water content in developed body and adult is higher than scyphula. On the other hand, it is because the feeding and digestive capability of scyphula is stronger for quick adaption to environmental changes. The Atlantic sea nettle scyphula is generally fed with *Artemia* nauplii, causing many challenges, including of individual shrinking and character degradation. This test shows that the activities of four digestive enzymes of scyphula are very high, which provides the theoretical evidence to the bait adjustment for scyphula. The content of pepsin is the highest among the four digestive enzymes due to the high protein in the bait and the high protein demands of the Atlantic sea nettle. However, the content of cellulase, lipase, and amylase is relatively low. The adult in salinity 20 only has 13.39U/L amylase activity which is significantly lower than the pepsin activity of scyphula 531.59U/ml in salinity 20. It means the Atlantic sea nettle has stronger capability to digest protein. It is suggested to appropriately increase the ratio of protein in daily bait to reduce the feeding cost. Cellulase can degrade the bait shell and other cellulose to absorbable glucose. This test detects a certain cellulase in the digestive cavity of the Atlantic sea nettle. However, It may be because the jellyfish has complete cellulase system which can produce endogenous cellulase, or there are symbiotic fungus, bacterial and zooxanthellae which can produce exogenous cellulase. Further study is required.

In this test, the survival rate, and the activities of four digestive enzymes are almost the highest in salinity 20 with the body weight of developed body and adult growing the fastest. It may be because the salinity is too high or too low to inhibit the activity of digestive enzyme and affect the digestion and absorption of nutrients. Thus, the scyphula lacks the energy for transverse fissure and the medusa is limited to survive and grow. Some inorganic ions in seawater have active or inhibitive effects on pepsin and the direct action of inorganic ions on pepsin is the main cause of salinity affecting the pepsin activity [22]. The results in this study show some inorganic ions will reach a certain concentration and inhibit the activity of pepsin when they enter the Atlantic sea nettle in high salinity environment.

However, very low salinity will decrease the ion concentration, which affected the activity of protease.

The activities of digestive enzymes are greatly related to the feeding habits of the animal. In general, the activity of amylase of omnivorous and herbivorous animals is higher while the activity of pepsin of Osteichthyes is higher [24, 25]. There are many factors affecting the activities of digestive enzymes, including of temperature, pH, and ion concentration [26].

This study analyzes the influences of three salinities on the growth and the activities of four digestive enzymes of the Atlantic sea. The strong adaptability to the salinity is observed in the external environment. Salinity 20 is the optimal for survival and asexual reproduction. The Atlantic sea can also quickly adapt to salinity 10. Different salinities also have different degrees of influences on the activities of digestive enzymes. The test results provide theoretical basis for the activation and adaptation mechanism of the Atlantic sea to environment and its commercial cultivation and development.

#### 4.3. Possible Mechanism of Jellyfish Adapting Salinity and Its Ecological Impact

Body wall is composed of ectoderm, mesoderm and endoderm in Cnidaria. And the tissue differentiation is simple. The internal cavity is a digestive circulation cavity, which has both digestive and circulation functions. When salinity stress occurs, jellyfish can produce a series of response mechanisms to reduce the damage of salinity stress on the body, such as shrinking tentacles, blocking the water-salt exchange, and adjusting the water in the mesoderm to adapt to the change of salinity. Some jellyfish with wide distribution, such as *Aurelia*, *Cyanea* and *Chrysaora*, show high tolerance to environmental conditions [27]. DONG found that salinity 10 and 40 are the lowest and highest limit for the survival of polyps of the giant jellyfish *Nemopilema nomurai* [28]. Rato evaluated the effects of 6 salinity regimes resembling estuarine and marine conditions (15, 20, 25, 30, 35 or 40) during 21 days on *Phyllorhiza punctata*, and believed that the survival rate of polyp was significantly correlated with salinity. The survival rate of polyp in salinity 40 was 50%, which was significantly lower than those in salinity 15, 20 and 25 after one week ( $P<0.001$ ). The settlement time of polyps isn't affected by salinity. The complete contraction of the polyp could be observed at  $21^{\circ}\text{C}$  and salinity 15 in 18 days, but the survival rate was 100% at the end of 21 days [29].

Phenomena such as global warming, rising sea temperatures and extreme weather and climate anomalies such as foods and heat waves have been shown to alter absolute salinity values. While affecting marine and estuarine population dynamics, these scenarios may also favour the invasion and proliferation of opportunistic and potentially harmful species in new geographical areas—such as blooming jellyfish.

This test starts to domesticate from polyps, and jellyfish with larger salinity adaptability is obtained. And individuals

adapted to the low salinity, also can quickly adapt even if they are suddenly back to the original environment. It indicates that the jellyfish has a strong tolerance to water environment, and climate change may be promoting jellyfish reproduction in new geographical areas, which will affect the ecological balance of the local area.

#### 4.4. Importance of Low Salinity Culture of Jellyfish

With the development of society, the public has increasing demands on ornamental species. Jellyfish as an emerging ornamental animal has made a figure in family feeding market due to its popularity. However, there are only few freshwater species and jellyfish in the class Hydrozoa with only a few months life, which cannot fully meet the increasing demands in the market. Thus, the technology of feeding seawater jellyfish in desalinated environment has become an urgent problem to be solved. The breakthrough of technical problem will be beneficial to developing more display forms of jellyfish and solve the problems of excessive processes for seawater preparation, high cost, easiness to crystallize, and easy growth of algae which may affect the appearance. Moreover, the technical breakthrough satisfies with the requirements of cultural and teaching product development, inland aquarium, and family feeding to greatly reduce the cost for production and maintenance.

At present, many marine economic animals have improved the economic benefits by desalination cultivation. However, jellyfish is featured with short generation, many filial generations, clear generic background, and easy achievement of filial generations. In artificial conditions, jellyfish can be fed, domesticated, and operated by experiment. Scyphula can asexual reproduce to permanently maintain its characteristic of feeding in fresh water and have transverse fissure in a certain condition to produce medusa which can be fed in low salinity. Jellyfish fed in low salinity avoid the reproduction of algae, bacterial, and parasites in breeding containers to ensure the feasibility of home feeding them as ornamental animals.

## 5. Conclusion

Salinity had changed jellyfish physiological performance about growth and digestion. All digestive enzyme activities (pepsin, cellulase, lipase and amylase) of scyphula were significantly higher than those of juvenile and adult due to the high protein bait and the high protein demands of *C. quinquecirrha*. Scyphula showed strong adaptability to external salinity changes because its cell osmotic regulation ability was stronger than the developed body and adult medusa. Salinity 10 was beneficial to the developed body and adult breeding and further desalting, and it could be used as the preservation salinity of scyphula for inland aquarium and home ornamental jellyfish. In salinity 20 jellyfish showed a good state of asexual reproduction, growth performance and vitality, which could be used as a suitable salinity for artificial breeding.

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