

Original Research

Effects of Hypersalinity on Serum Nonspecific Immune Indices in Three Marine Organisms

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Abstract

During the production of desalination plants, concentrated seawater is expelled to local marine environments, which may lead to hypersaline pollution. To assess environmental risk of hypersalinity, *Crassostrea angulate*, *Scylla paramamosain* and *Epinephelus akaara* were exposed to salinities of 30‰, 36‰ and 40‰, then changes of serum nonspecific immune indices were monitored for 96 hours, including superoxide dismutase (SOD) and acid phosphatase (ACP) activity, and contents of serum total protein and albumin. Two-way ANOVA revealed that salinity only affected contents of serum total protein and albumin in *C. angulate*, and content of serum albumin in *S. paramamosain* and content of serum total protein in *E. akaara*. Compared with the control, hypersaline treatments (36‰ and/or 40‰) first increased contents of serum total protein and albumin and then decreased them in *C. angulate*, suppressed content of serum albumin in *S. paramamosain*, and increased content of serum total protein in *E. akaara*. These results suggested that exposure to hypersalinity might enhance innate immunity of *C. angulate* and *E. akaara*, but suppressed it in *S. paramamosain*.

Keywords: saline stress, superoxide dismutase, acid phosphatase, serum total protein, albumin

Introduction

Desalination is an energy-intensive process. With the development of seawater desalination industries, a huge amount of hypersaline wastewater is produced. At present, the comprehensive utilization of hypersaline wastewater mainly focuses on extraction of potassium, magnesium and bromide. However, a considerable ratio

of concentrated seawater is still discharged to local seas directly, therefore raising ecological concerns to local marine environments [1].

Salinity can affect the physiological process of marine organisms such as enzymatic activity, reproduction, growth, breathing and behavior [2] by altering osmoregulation, protein synthesis, oxygen uptake, and extracellular acid-base balance [3]. Ecological investigations on areas close to desalination plants have revealed that discharge of concentrated seawater formed hypersaline areas that significantly destructed habitats; decreased densities of fish, echinoderms and planktons; inhibited shoot division of

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Posidonia oceanica; and negatively affected the growth of corals [4,5]. These results preliminarily demonstrated negative influences of desalination plants.

Innate immunity (nonspecific immunity) provides rapid and incomplete antimicrobial host defense before the development of slower, more definitive acquired specific immune responses, which needs several weeks to synthesize antibodies. Obviously, innate immunity contributes important roles to animal health. Up to data, the effects of hypersalinity on marine animals are still largely unknown. Compared with 28.5‰ salinity, *Dicentrarchus labrax* at 35‰ revealed higher levels of nephrosin in intestine, which represented the function of the immune system [6]. Matozzo et al. reported a decrease in the phagocytic index of the immune system cells in the clams *Chamelea gallina* exposed to hypersalinity (40‰) [7]. A similar decrease was also observed in *Macrocallista nimbosa* [8]. However, several reports also indicated that increased salinity did not significantly affect immune parameters in *Mytilus coruscus* and *Oreochromis niloticus* [9, 10]. These conflicted results suggested that the effects of hypersalinity on nonspecific immunity might differ among species. Investigations on more species are required.

Crassostrea angulata, *Scylla paramamosain* and *Epinephelus akaara* are three typical and ordinary species living in coastal marine habitats. They are possibly affected by hypersaline seawater released from desalination plants. SOD activity is closely associated with immune function in marine organisms [11, 12]. Acid phosphatase (ACP) is also a typical lysosome enzyme that plays a role in killing and digesting pathogens [13]. Serum total protein and serum albumin maintain the pH balance in the blood and the stability of the physiological environment, and function as an important endogenous antioxidant in plasma [14-16]. These indices are considered as indices of nonspecific immunity [17]. In the present study, to investigate the effects of hypersaline seawater on *C. angulata*, *S. paramamosain* and *E. akaara*, changes of SOD and ACP activities as well as contents of serum total protein and albumin were compared among treatments with 30‰, 36‰ and 40‰. These results might contribute to comprehensive assessment of environmental risk of desalination plants to local marine environments.

Materials and Methods

Hypersaline Treatment

C. angulata (weight: 98.6±21.7 g), *E. akaara* (weight: 135.9±20.6 g) and *S. paramamosain* (weight: 251.9±79.1 g, length: 10.26±0.85 cm) were purchased from a commercial aquafarm located as Haicang District, Xiamen City, Fujian Province, P. R. China.

Animals were maintained at 30‰ salinity for two days in order to observe their healthy status. Seawater was prepared by dissolving artificial sea salts in distilled water.

The general salinity at the sampling location was 30‰ and salinity of seawater near the local desalination plant was up to 40‰. In order to investigate the effects of hypersalinity on experimental animals, salinities of 36‰ and 40‰ were set as hypersaline treatments and 30‰ was included as control. Animals were randomly distributed into these three groups. Experiments were conducted in 50 L glass tanks. Each treatment included three tanks as three replicates.

After being treated for 0, 2, 6, 12, 24, 48 and 96 h, three individuals of each species were sampled from each treatment. Animals were sacrificed on ice. Blood was collected and stored at 4°C overnight. After centrifugation at 5,000 rpm and 4°C for 5 min, serum was collected for determining SOD and ACP activities as well as contents of serum total protein and albumin.

Determining SOD and ACP Activities

Activities of SOD and ACP were determined using commercial kits produced by Nanjing Jiancheng Biotechnology Company (Nanjing, China). Following the kits' protocols, SOD activity was assayed using the xanthine/xanthine oxidase method based on the production of O₂⁻, and ACP activity was measured using disodium phenyl phosphate as the substrate. One unit of SOD activity was defined as the amount of enzyme required for 1 mg tissue proteins in 1 ml of reaction mixture to achieve an SOD inhibition rate of 50%. One unit of ACP was expressed as the degradation of 1 mg of phenol at 37°C in 60 min.

Determining the Contents of Serum Total Protein and Albumin

A protein determination kit and albumin determination kit were purchased from Nanjing Jiancheng Biotechnology Company (Nanjing, China). The assays were carried out exactly following the kits' protocols. Determination of total protein and albumin employed the Coomassie brilliant blue G250 method and Bromocresol green colorimetric method, respectively.

Data Analyses

SPSS 18.0 software was used for statistical analyses. Two-way analysis of variance (ANOVA) was applied to check the effects of salinity, time and their iteration on each parameter. One-way ANOVA was applied to evaluate the effects of salinity on all parameters at the same time point, followed by multiple comparisons (SNK test). Differences were considered significant at $P < 0.05$. All data are expressed as mean±standard deviation (SD).

Results

Effects of Hypersalinity on *C. angulata*

Along with exposure time, at salinity of 36‰ SOD activity decreased from 2 h and then increased from 24 h to the maximum value at 48 h. A similar trend was observed in treatment with 40‰ (Fig. 1a). Compared with the control, SOD activity was significantly reduced in treatments with 36‰ and 40‰ within 12 h, but increased in treatment with 36‰ from 24 h to 96 h and in treatment with 40‰ at 48 h (Fig. 1a).

Compared with the control, ACP activity was significantly elevated in treatment with 36‰ at 2 h, 24 h and in treatment with 40‰ at 96 h, but significantly decreased in treatment with 40‰ at 48 h (Fig. 1b).

Compared with the control, the content of serum total protein increased in treatment with 40‰ at 2 h, but was significantly lower in treatments with 36‰ and 40‰ at 6 h and 48 h (Fig. 1c).

During the initial exposure period (from 2 h to 24 h), content of serum albumin was significantly higher in treatments with 36‰ and 40‰ than that in 30‰. Along with exposure time, the content of serum albumin

revealed decreasing trends from 2 h to 48 h at salinities of 36‰ and 40‰ (Fig. 1d).

Effects of Hypersalinity on *S. paramamosain*

Compared with the control, SOD activity at salinity of 36‰ showed no significant change during the experiment. However, at salinity of 40‰, it significantly increased at 2 h but decreased at 48 h and 96 h (Fig. 2a).

At 2 h and 12 h, ACP activity in the 36‰ group was significantly lower than those in control. In the 40‰ group it was significantly higher than that in control at 12 h (Fig. 2b).

Content of total serum protein in the 36‰ group significantly decreased at 2 h, but significantly increased at 96 h compared with control. Serum total protein content in the 40‰ group increased significantly (2 h-48 h) compared with control, with a maximum value at 6 h (Fig. 2c).

Compared with the control, serum albumin content in the 36‰ group significantly decreased at 2 h, 6 h and 12 h, with those in the 40‰ group significantly lower at 2 h and 24 h (Fig. 2d).

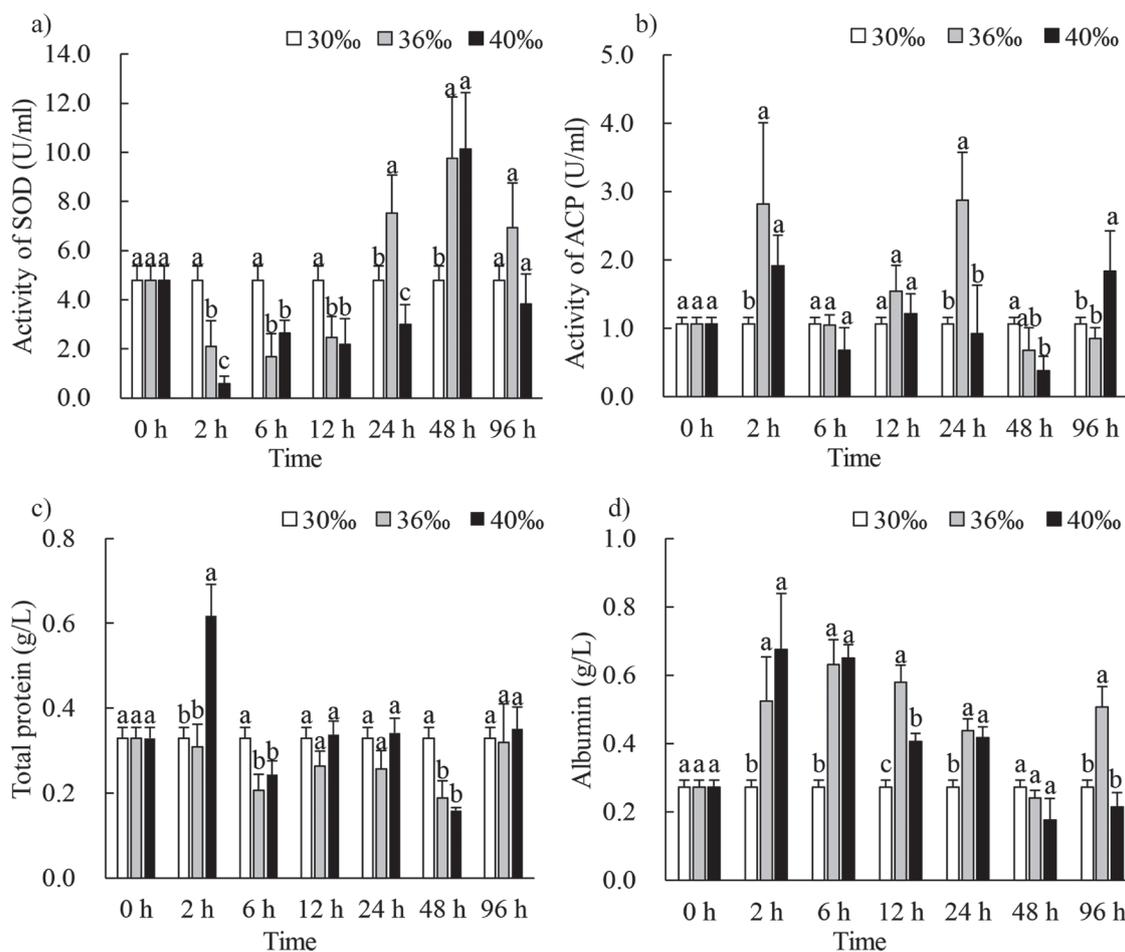


Fig. 1. Effects of hypersalinity on serum nonspecific immune indices in *Crassostrea angulata*; data represent mean±SD; different letters indicate significant differences among salinities in the same time slot (P<0.05).

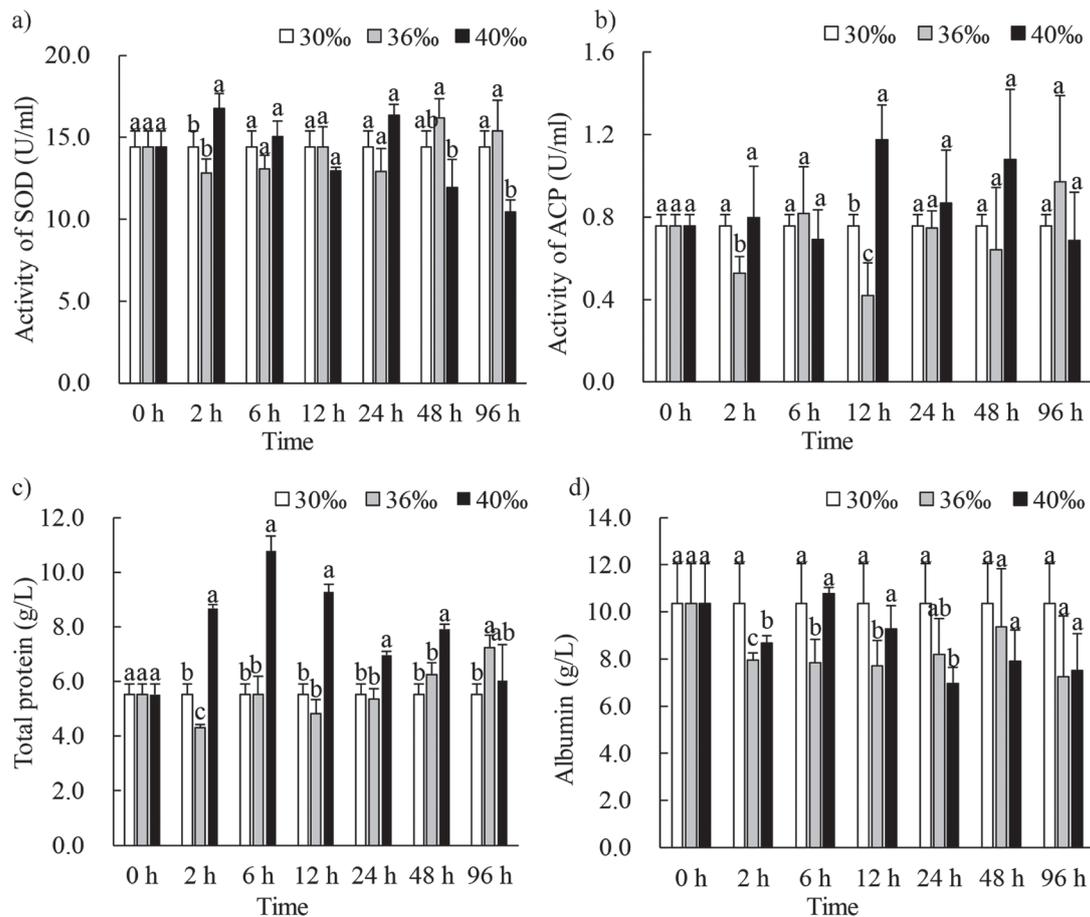


Fig. 2. Effects of hypersaline stress on serum nonspecific immune indices in *Scylla paramamosain*; Data represent mean±SD; different letters indicate significant differences among salinities in the same time slot ($P < 0.05$).

Effects of Hypersalinity on *E. akaara*

Compared with the control group, SOD activity significantly increased at 48 h and 96 h in treatments with 36‰ and 40‰. No significant difference was detected among three saline treatments from 0 h to 24 h (Fig. 3a).

ACP activity significantly decreased in treatment with 36‰ at 2 h, 12 h and 48 h, as well as in treatment with 40‰ at 2 h and 48 h, compared with the control. An increased level of ACP activity was detected in treatment with 40‰ at 6 h than that at 30‰ (Fig. 3b).

Content of total serum protein at salinities of 36‰ and 40‰ significantly increased from 2 h to 24 h, compared with control group (Fig. 3c).

Salinity and exposure time did not significantly affect the content of serum albumin in the present experiments (Fig. 3d).

Discussion

Salinity is a key environmental factor to determine the growth and health of marine organisms. Each marine species adapts to an optimal salinity and can tolerate

temporary or slight variation of salinity. However, exposure to large saline differences for a long time negatively affects growth of animals and even induces mortality. Previous studies have assessed impacts of brine discharges from desalination plants on marine ecosystems and particularly on marine fauna [18-20]. However, the tested indices in these studies were limited to survival, growth, and ecological changes. Toxicological impacts on innate immune responses were poorly investigated [21].

As we know, stress leads to a series of biochemical, physiological and behavioral changes in marine organisms, thus affecting normal body homeostasis. Activities of antioxidant enzymes and the levels of ROS have been found to correlate with various stress [22]. ROS generation is an essential mechanism during phagocytosis [23]. Besides, ROS level is a common feature representing oxidative stress in animals exposed to abiotic stress. Treatments with hypersalinity generally induce production of reactive oxygen species (ROS) in aquatic animals [23, 24], which are harmful to protein, lipid, DNA molecules, and finally damage cytomembrane, enzyme activity and cell function. To resist ROS, organisms may initiate a series of antioxidant reactions [25, 26]. Among them, SOD is

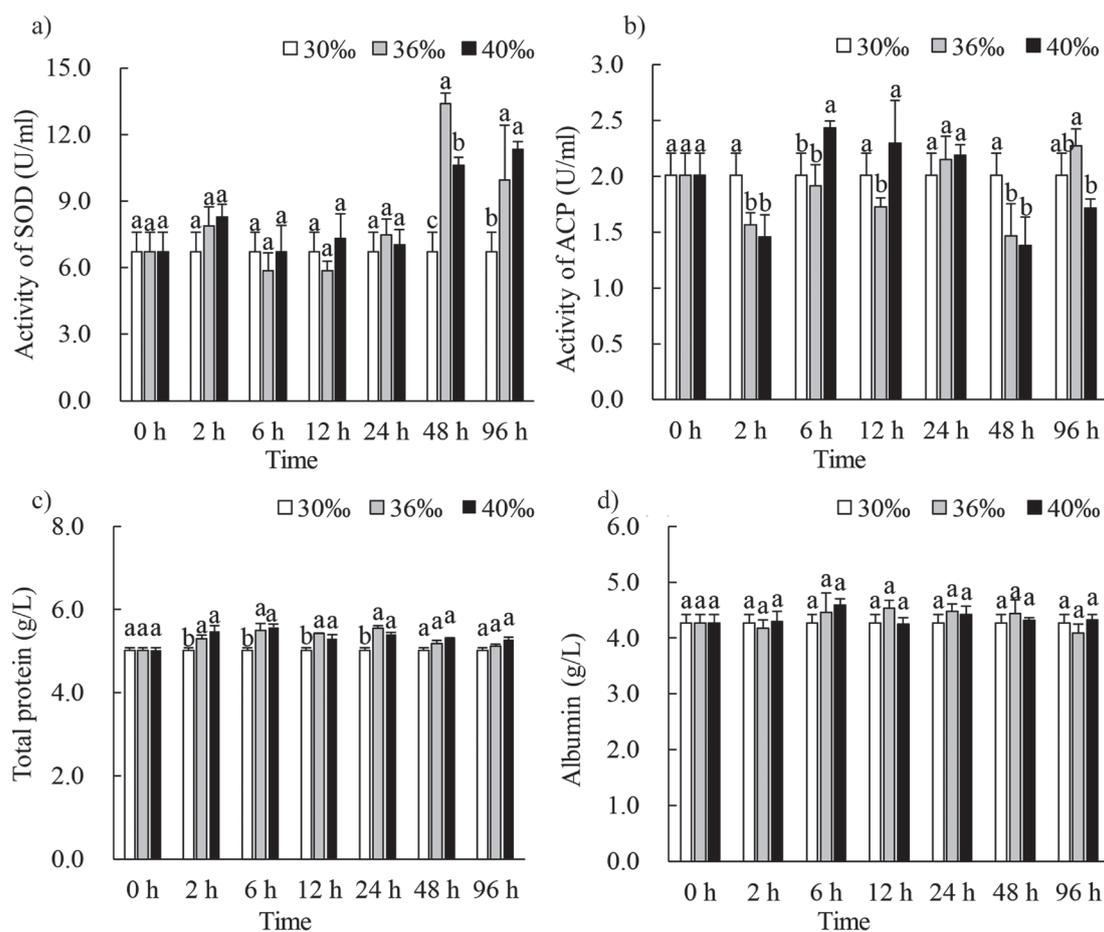


Fig. 3. Effects of hypersaline stress on serum nonspecific immune indices in *Epinephelus akaara*; data represent mean \pm SD; different letters indicate significant differences among salinities in the same time slot ($P < 0.05$).

a typical enzyme that catalyzes the dismutation (or partitioning) of the superoxide (O_2^-) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). In the present study, changes of SOD activity varied among species and exposure time. Hypersaline treatments elevated SOD activity in *C. angulate* and *E. akaara* but decreased in *S. paramamosain* at 48 h and 96 h, suggesting that phagocytes were more activated in *C. angulate* and *E. akaara*, but less activated in *S. paramamosain* in response to hypersalinity. Non-negligibly, changes of SOD might also be attributed to oxidative stress induced by saline variation [27, 28], or comprehensive effects of oxidative stress and innate response. Moreover, decreased SOD activities were observed in some treatments, which was similar to the results detected in *Echinometra lucunter* in response to hypersaline treatment [23]. The underlying mechanisms required further investigations.

Fang et al. found that ACP activity in the gill and kidney of the juvenile tongue sole (*Cynoglossus semilaevis*) showed no significant difference between low- and high-salinity treatments [29]. ACP activity was enhanced in *Penaeus vannamei* at salinity 30-40‰, but significantly reduced at higher salinity [17]. Decreased ACP activity represents an impaired ability

to degrade bacteria [30]. In the present study, changes in ACP activity were not consistent among the three tested species. In *C. angulate*, ACP activity was enhanced at initial exposure to hypersalinity but then decreased, suggesting that hypersalinity might first enhance but then suppress nonspecific immunity. Increased activity of ACP probably resulted from the proliferation of immune cells under hypersalinity [31]. In *E. akaara* and *S. paramamosain*, ACP activity decreased or did not change in hypersaline treatment compared with the control, displaying inhibitive effects on nonspecific immunity.

Increases in serum total protein and albumin are thought to be associated with a stronger innate immune response [32, 33]. In *C. angulate*, content of serum total protein increased at early contact and then decreased or did not change; content of serum albumin always increased or did not change compared with the control. These results at early contact were consistent with changes of ACP activity, suggesting that hypersaline treatment enhanced the immunity of *C. angulate* initially. In *S. paramamosain*, enhanced content of serum total protein and suppressed content of serum albumin were observed in hypersaline treatments. In *E. akaara*, content of serum total protein was always higher

Table 1. Two-way analyses of variance – SS: sum of squares; df: degree of freedom; MS: mean square; NA: not applicable .

| Sources | SS | df | MS | F | P |
|---|--------|----|--------|--------|-------|
| Serum albumin in <i>C. angulate</i> | | | | | |
| Time | 0.208 | 6 | 0.035 | 2.514 | 0.082 |
| Salinity | 0.125 | 2 | 0.062 | 4.519 | 0.034 |
| Time * Salinity | 0.165 | 12 | 0.014 | NA | NA |
| Serum total protein in <i>S. paramamosain</i> | | | | | |
| Time | 5.486 | 6 | 0.914 | 0.527 | 0.778 |
| Salinity | 25.432 | 2 | 12.716 | 7.33 | 0.008 |
| Time × Salinity | 20.816 | 12 | 1.735 | NA | NA |
| Serum albumin in <i>S. paramamosain</i> | | | | | |
| Time | 8.287 | 6 | 1.381 | 1.48 | 0.265 |
| Salinity | 15.274 | 2 | 7.637 | 8.185 | 0.006 |
| Time × Salinity | 11.196 | 12 | 0.933 | NA | NA |
| Serum total protein in <i>E. akaara</i> | | | | | |
| Time | 0.251 | 6 | 0.042 | 2.81 | 0.06 |
| Salinity | 0.414 | 2 | 0.207 | 13.902 | 0.001 |
| Time × Salinity | 0.179 | 12 | 0.015 | NA | NA |

in hypersaline treatment, but content of serum albumin never changed during the tested period. These results were inconsistent with ACP activity. Based on these data, it was hard to conclude the effects of hypersalinity on nonspecific immune function in *S. paramamosain* and *E. akaara*. Actually, the nonspecific immune system involves lot of complex molecular mechanisms.

To get more valuable information, two-way ANOVA was conducted on all tested parameters in the three species. The results showed that salinity only affected contents of serum total protein and albumin in *C. angulate*, content of serum albumin in *S. paramamosain* and content of serum total protein in *E. akaara*. No significant effects of time and time × salinity were detected on all tested indices (Table 1). Based on the changes of the indices significantly revealed by two-way ANOVA, we may conclude that hypersaline treatment enhanced innate immunity of *C. angulate* and *E. akaara*, but suppressed it in *S. paramamosain*. Different results among species might be due to the different sensitivities of marine organisms to environmental changes. Further studies are required to clarify the inconsistent changes in the present study.

Conclusions

Overall, in the present study SOD activity was elevated in *C. angulate* and *E. akaara* but decreased in *S. paramamosain* at 48 h and 96 h in hypersaline water. ACP activity was enhanced at initial exposure

to hypersalinity but then decreased in *C. angulate*, while it decreased or did not change in *E. akaara* and *S. paramamosain*. Content of serum total protein increased at early stages and then decreased or did not change in *C. angulate* but was always higher in *E. akaara* and *S. paramamosain* in hypersaline treatments. Content of serum albumin always increased or did not change in *C. angulate*, while it was suppressed in *S. paramamosain*, but it never changed in *E. akaara* during the tested period. Regardless, the widely accepted view is that the environmental impact of seawater desalination on marine environments will not be substantial if appropriate measures are implemented.

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Author Contributions

ZZ, ZWY and KBL designed the experiments. HG and DL collected the experiments and analyzed the

data. DL, YYZ and GZ drafted the manuscript and all authors revised it.

Conflict of Interest

There is no conflict of interest in this research.

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