High salinity effect on mortality of sandfish \textit{Holothuria scabra} (Jaeger, 1833)

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Abstract. The sandfish \textit{Holothuria scabra} is a sea cucumber with a very high economic value. Sandfish cultivated in brackish water ponds will face a high salinity problem in the dry season. This study aimed to analyse the effect of high salinity on sandfish mortality. The study was done in a controlled tank in which the sea water was allowed to evaporate naturally, without the addition fresh water, so that the salinity increased continually. High salinity can cause injury and death to sandfish; before death, there was an evisceration. The death salinity of sandfish was 47 ppt. Sandfish have a greater range of tolerance to salinity than other sea cucumber species.

1. Introduction

Sea cucumber has been consumed since more than 1000 years ago. Currently there are about twenty species of sea cucumber sea cucumbers that are consumed [1, 2]. One of the most consumed sea cucumber species is sandfish \textit{Holothuria scabra} [3]. Sea cucumber has high nutritional and economic value [4-6]. Sandfish is a sea cucumber which has very high economic value, whose prices can exceed US $ 100 per kilogram of dry weight [7]. The high price has caused Indonesian fishermen to look for sea cucumbers in Australian waters since hundreds of years ago [8, 9].

Sandfish have an elongated, cylindrical, and relatively fat body shape. The dorsal color varies from brownish gray to black, with dark wrinkles all over the body and small black papillae. The ventral part is relatively flat with a lighter color. Sandfish can grow to a length of 40 cm with a weight gain of up to 300 grams per year [10, 11]. It is not known exactly how long sandfish can live, but at least sandfish can live up to 10 years[10]. Like other sea cucumber species, sandfish can remove the contents of their internal organs (eviscerate) when under stress, and can regenerate their organs in about two months [10].

Sandfish live in shallow tropical waters with a depth of less than 20 m. Sandfish live in seagrass ecosystems and muddy substrates. Sandfish can tolerate low salinity [10, 12], so it can be found in brackish waters [10].

Apart from having economic value, sandfish also have a key role in the health of ecosystem or their habitat; like most sea cucumbers, sandfish are bioturbators that play a role in recycling sediment and ensuring that organic matter is distributed evenly on the sediment surface. Sandfish have a diet of detritus and other microorganisms [13-15].

Its high economic value causes the sandfish population to decline due to over exploitation [9]. To overcome this, hatchery and cultivation are the best options [7, 16]. Sandfish have the ability to tolerate the environment quite well so that they can be used as objects of co-cultures or multitrophic
cultures with other species. Sandfish are able to reuse waste material (metabolic waste) from other organisms and make nutrients usable [14]. Species that have been co-cultures with sandfish were black tiger prawn *Penaeus monodon* [17, 18], red seaweed *Kappaphycus striatum* [19], blue shrimp *Litopenaeus stylirostris* [20].

The market demand for sandfish still relies on catches in the sea. Several sandfish farming efforts have been carried out [16] in the ponds and coastal areas, but the results have not been as expected [9]. Sandfish that are cultured multitropically in brackish water ponds will face high salinity problems in the dry season. Therefore, it is necessary to study the effect of salinity on the mortality of sandfish. This study aims to analysis the high salinity effect on the sandfish mortality.

2. Materials and Methods

The study was conducted in a controlled tank which was allowed the sea water to evaporate naturally, and without the addition fresh water so that the salinity increase continually. The treatment used four tanks measuring 1 x 2 x 0.9 m (Figure 1a) consisting of three tanks for salinity enhancement treatment, and one tank for the wound healing process. Each tank was filled with water up to 45 cm high. The initial salinity of seawater used was 32 ppt. Each tank was aerated (Figure 1b). Before the sandfish were stocked, bottom microalgae were grown on the bottom of the tank to provide natural food for sandfish (Figure 1c). Microalgae were grown using organic sludge from brackish water ponds. Each tank contains 10 sandfish (Figure 1d). Each tank was given artificial feed in the form of flour as much as 20 g per tank every day as additional food. Salinity was recorded on every sandfish dead. Water quality parameters measured were temperature, pH and dissolved oxygen.

Correlation curves of mortality (%) and salinity (ppm) was drawn using trendline polynomials in the Microsoft Excel software. Death salinity of sandfish was estimated based on the mortality of 50%.

Figure 1. Tank conditions (a-b), benthic algae (c), and sandfish *Holothuria scabra* (d) used during the treatment
3. Results

During 87 days of treatment, the salinity increased from 32.3 ± 0.47 to 52.5 ± 0.50 (Figure 2). At salinity above 40 ppt, sea cucumbers have shown signs of unhealthiness, the tegument begins to shrink and the sandfish is less active (Figure 2). Some sandfish, the skin looks very wrinkled or rolled over (Figure 3), some other sandfish, the skin is slimy and injured (Figure 4). The injured sandfish then eviscerated (Figure 5), then died and was destroyed (Figure 6). The first deaths occurred at a salinity of 44 ppt, and the death salinity was 47 ppt (Figure 7). Wounded sandfish (Figure 7a) were transferred to tank with initial salinity (33 ppt), the wound stopped mucus on the fourth day (Figure 7b). The wound was completely healed by the 40th day after removal, almost no visible scar (Figure 7c). Of the twelve injured sandfish returned to initial salinity (32 pp), five sandfish completely healed from wounds.

![Figure 2. Salinity curve related to the treatment days](image)

![Figure 3. Sandfish Holothuria scabra condition was getting less healthy](image)

![Figure 4. Sandfish Holothuria scabra condition was getting worse](image)
Figure 5. Sandfish *Holothuria scabra* was severely injured

Figure 6. Condition of eviscerated sandfish *Holothuria scabra*

Figure 7. Dead sandfish *Holothuria scabra*

Figure 8. Mortality rate of sandfish *Holothuria scabra* related to salinity
Figure 9. Sandfish Holothuria scabra condition when wound (a), started to heal (b), completely healed (c).

The water temperature during treatment ranges from 26.4-30.2 with an average of 28.18 ± 0.9, the pH of the water ranges from 7.35-7.72 with an average of 7.53 ± 0.1, and dissolved oxygen ranges from 3.35-4.27 with an average of 3.82 ± 0.33.

4. Discussion
This study showed that the sandfish died in total at a salinity of 55 ppt. With a death salinity of 47 ppt, the sandfish were only able to tolerate an increase in salinity during treatment of 15 ppt from the initial salinity of treatment (32 pp). This salinity range was slightly smaller than the salinity drop range that sandfish can tolerate, which was around 20 ppt [12].

Salinity variations affect the activity of catalase, myeloperoxidase, and lysozyme. Catalase activity decreased significantly after only one hour of exposure to 20 ppt salinity. Myeloperoxidase and lysozyme activities indicate that A. japonicus tolerates limited salinity stress [21]. As an animal of marine origin, the sea cucumber H. spinifera lives in optimum conditions at a salinity of 35 ppt [22].

Salinity is a major limiting factor for sea cucumber [23]. This study indicated that sandfish have greater salinity tolerance (up to 47 pp), compared to other sea cucumber species Apostichopus japonicus (40 ppt) [24, 25]. The optimum salinity of Apostichopus japonicus was lower in the juvenile phase (30 ppt) [26]. Salinity that is not in the maximum range has an impact on energy allocation [27].

At high salinity, severe injury will be followed by evisceration. Evisceration is a biological process that can occur when sandfish suffer from stress [28]. The phenomenon of evisceration is common in sea cucumbers [16, 29]. Naturally, evisceration at sea cucumber does not cause death usually because the internal organs removed during evisceration can be regenerated [30].

This research shows that sandfish can recover from wounds due to poor environmental conditions (salinity). The previous studies showed that sandfish can heal completely from wounds caused by predators [31]. Another species of sea cucumber H. glaberrima also has extraordinary wound healing abilities [32]. The ability of wound healing or organ regeneration due to evisceration in H. glaberrima was related to analogous cellular mechanisms during wound healing and organ regeneration [32]. Rapid process of integument wound healing also occurs in other sea cucumber species such as Thyone briareus [33] and Stichopus badionotus [34]. Morula cells appear to be involved in the healing of epidermal and dermal wounds, although their exact role is unknown [33]. Healing of skin tissue in sea cucumbers is also related to the formation of new collagen [34].

5. Conclusion
High salinity can cause the sandfish to seriously injure and die. Before dying, there was an evisceration. The death salinity for sandfish was 47 ppt. Sandfish have a greater range of tolerance to salinity than other sea cucumber species.

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