Low salinity reduces survival rate of a commercially important sea cucumber (Sandfish: *Holothuria scabra*)

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Abstract. Sandfish culture had been implemented for more than 20 years but has frequently failed to yield the expected results due to low survival rates. A decrease in salinity during the rainy season was suspected as a cause of sandfish mortality. This study aimed to assess the influence of salinity on sandfish survival rates. Treatments included sudden shock and gradual acclimation trial methods. Even though the results of this study indicate a mean survival rate of 50% at 14.6 ppt, the survival rate fell drastically as water salinity was reduced below the salinity of saltwater bony fish body fluids (around 18 ppt). Sandfish cannot live in water with salinity lower than the osmotic body fluid of freshwater bony fish (around 14 ppt). Therefore, for the cultivation of sandfish in coastal ponds, salinity should not be allowed to fall below a minimum of 18 ppt.

1. Introduction
Southeast Asia is the principal source of sea cucumbers worldwide, with around 80,000 tons harvested each year from tropical regions [1], while Indonesia has been (and remains) the top producer of dried sea cucumber (*teripang*) [2-8]. Many species of sea cucumbers from this region have a high economic value [9-11]. Sea cucumber fisheries provide an income to many poor coastal fishers [12]. In many islands and coastal villages, income from sea cucumber fishery is used to constitute a significant portion of the total income of many families [13].

Sandfish is one of the largest catches of sea cucumbers in the world [14]. This commodity, as well as other commercial sea cucumber species [15] is an important source of income for marginal fishermen in poor coastal villages of Southeast Asian countries, especially in Indonesia [9]. Sea cucumbers, as bycatch, can contribute as much as 41% to the daily take-home income of fishers in The Philippines [16]. In these countries, sandfish is highly exploited to the point where overfishing has been causing declines in the wild populations and in sandfish production [17, 18]. Current populations of commercially sea cucumber in many shallow coastal areas have been overfished [14], and the income derived from sea cucumber gleaning has, therefore, become less important [19]. This situation requires an alternative strategy to preserve the sea cucumber populations, increase sea cucumber production, and improve coastal community welfare. Aquaculture is one alternative for maintaining
the sustainability of sandfish production [20]. In Southeast Asian countries, sandfish is the only sea cucumber species that currently cultivate [9, 21].

Sandfish have been cultivated in coastal ponds for more than 20 years [22], but this effort has often not yielded the expected results [20, 23]. The lack of success in culture is due to low survival rates, especially for the culture of sandfish in brackish-water ponds [24]. It has been suggested that low sandfish survival rates in these coastal ponds might be due to excessive fluctuations of salinity. This hypothesis was based on previous research on the effects of sub-lethal stress on the salinity responses of marine organisms [25, 26]. Salinity stress could affect marine invertebrates in a variety of mechanisms [27, 28]. Although there have been many results of previous studies that support directly or indirectly for the success of cucumber cultivation [29-33], advance research is still needed to be able to make sea cucumber ecologically and economically feasible.

The effects of salinity on the physiology of marine invertebrates have been documented for several species of echinoderms [34-36]. Echinoderms are generally considered as one of the stenohaline phyla in the animal kingdom, but several species show remarkable abilities to acclimate and survive in euryhaline habitats [37]. Yuan (2006) had shown that sea cucumbers could tolerate a range of salinity from 22 to 36 ppt. However, salinities of 28–34 ppt are preferred for the growth of sandfish [26]. In addition to their economic value, sandfish are better suited for cultivation compared to other sea cucumber species [38], owing to their capacity to tolerate salinity fluctuation [9].

Sandfish cultivation has been attempted for many years; however, until the present time, it has not been economically viable due to high mortality rates, especially during the rainy season. It is suspected that sandfish deaths in coastal ponds culture may be due to decreases in salinity. Even though previous research has reported that sea cucumbers, including sandfish, can be found in estuarine areas [26], from a biological perspective, sea cucumbers are primitive animals that do not have good regulatory systems [28, 39, 40]. There is a lack of studies in assessing the capacity of sandfish to adapt to salinity changes or other environmental stress factors. The present study aims to assess the effect of salinity changes to sandfish survival rates, both in a sudden event or in a situation where the salinity decrease gradually.

2. Materials and methods

Sandfish were collected during low tide at nighttime in the waters of Liukang Tupperbiring Village, Pangkep Regency, South Sulawesi, Indonesia (4°42′13" S, 119°37′04" E). There were a total of 120 sandfish used in two separate experiments. The sandfish were acclimatized for 30 days in a holding tank with a volume of two tons of seawater with salinity 36 ppt. During the acclimatization and experimentation process, dried spirulina and artificial feed are given every 6 AM and 6 PM, respectively, in an amount equivalent to 2 ppm in the experimental tank. The experimental tanks are cylindrical in shape, with a 55 cm diameter and a height of 45 cm, filled with seawater to a depth of 49 cm which made up to 95 l volume. During the experiments, all of the tanks were kept outdoors.

To experiment on the sudden decrease in salinity, 21 tanks were filled with seawater with seven different treatments, each with three replicates. The seven salinity treatments are 35, 30, 25, 20, 15, 10, and 5 ppt, chosen based on the fact that the salinity in coastal ponds during the rainy season ranges from 35 to 5 ppt (personal observations). Apart from the salinity difference, all other physical aspects such as temperature and pH were kept the same. The experimental animal used in a range of 5.0–13.5 cm (8.65±2.34 cm in average), while the gutted body weight ranges from 13.92–106.33 g (49.40±29.20 g in average). The experiment began when four sandfish were put into each tank and ended when 100% mortality was reached. Observations were carried out everyday at 6 AM and 6 PM just before feeding.

The second experiment was aimed to observe the survival rate during a gradual decrease in salinity. Using nine experimental tanks, filled with seawater in 36ppt salinity. A total of 36 sandfish, with an average length of 3.5–10.0 cm (6.11±1.50 cm in average) and a gutted body weight of 5.62–56.54 g (19.47±12.66 g in average), were placed evenly into the tanks, resulting in four sandfish each tank. The treatment was obtained by replacing the experimental tank water by 7.5%, 5%, and 2.5% with
fresh water for treatment a, b and c, respectively. This water replacement is carried out every 12 hours during the study. The study was ceased when 100% mortality was reached. The survival rate (Sr) was calculated using Sparre, Ursin, and Venema [41] equation: 

\[ Sr = \left( \frac{N_t}{N_0} \right) \times 100, \]

where \(N_0\) is the number of sandfish at the beginning of the treatment, and \(N_t\) is the number of sandfish at a given time. The significance level of the difference between means was tested using a One-sample t-test [42]. The relationship between length and gutted body weight as well as the theoretical curve of decreasing salinity were both determined using Scherrer [42] quadratic equation:

\[ Y = ae^{bX} \]

The salinity value at 50% survival rate was estimated using Scherrer [42] linear regression equation:

\[ Y = a + bX \] (3).

3. Results

3.1. Sandfish size

The length-gutted bodyweight relations for the samples used in the sudden decrease of salinity experiment was \(Y = 4.322e^{0.258X}\), with \(R^2 = 0.847\) (Figure 1A), while the length-gutted body weight relation of sandfish used in gradual decrease salinity experiment was \(Y = 1.767e^{0.362X}\) with \(R^2 = 0.804\) (Figure 1B).

![Figure 1. The curves of length-gutted body weight relation of sandfish Holothuria scabra used in sudden salinity decrease experiment (A) and gradual salinity decrease experiment (B).](image)

3.2. Survival rate

3.2.1. Sudden salinity decrease experiment. In the sudden salinity decrease treatment, at salinities of 5, 10, and 15 ppt the sandfish had not moved from their original place by the third day of the treatment and did not produce feces. On the first day, some sandfish had their ventral face-up, indicating an attempt to turn around, while the mouth and anus were tightly closed. On the second day, the condition of the sandfish remained the same as on the first day, showing no signs of life. On the third day, the skin appeared crushed, indicating that the sandfish had been dead for two days; based on our observations over many years, sea cucumber skin begins to disintegrate two days after death. At salinities of 20, 25, 30, and 35, sea cucumbers appeared to be actively moving and passing feces. From the start of the treatment until the end of observation, the survival rate was still 100% (Table 1). Sandfish were still alive in these tanks after 30 days of treatment.

![Table 1. Survival rate of sandfish Holothuria scabra exposed to sudden salinity decrease treatments.](image)
3.2.2. **Gradual salinity decrease experiment.** Under gradual salinity decrease treatment, the first mortality occurred on the sixth day of treatment for 7.5% freshwater substitution per 12 hours, which coincides with a decrease in water salinity below 18 ppt (Table 2).

**Table 2.** Survival rate of sandfish *Holothuria scabra* exposed to gradual salinity decrease treatments.

| Day | Hour | 2.5% Salinity (ppt) | Survival rate (%) | 5.0% Salinity (ppt) | Survival rate (%) | 7.5% Salinity (ppt) | Survival rate (%) |
|-----|------|---------------------|-------------------|---------------------|-------------------|---------------------|-------------------|
| 1   | 0    | 36.0±0.0            | 100.0±0.0         | 36.0±0.0            | 100.0±0.0         | 36.0±0.0            | 100.0±0.0         |
|     | 12   | 36.0±0.0            | 100.0±0.0         | 36.0±0.0            | 100.0±0.0         | 36.0±0.0            | 100.0±0.0         |
| 2   | 24   | 34.7±0.3            | 100.0±0.0         | 34.3±0.6            | 100.0±0.0         | 33.5±0.5            | 100.0±0.0         |
|     | 36   | 32.7±0.6            | 100.0±0.0         | 32.7±0.6            | 100.0±0.0         | 29.3±0.3            | 100.0±0.0         |
| 3   | 48   | 31.7±0.6            | 100.0±0.0         | 30.7±0.6            | 100.0±0.0         | 29.0±0.0            | 100.0±0.0         |
|     | 60   | 31.3±0.3            | 100.0±0.0         | 29.5±1.7            | 100.0±0.0         | 27.7±0.3            | 100.0±0.0         |
| 4   | 72   | 30.7±0.6            | 100.0±0.0         | 27.7±0.6            | 100.0±0.0         | 25.0±0.0            | 100.0±0.0         |
|     | 84   | 30.3±0.6            | 100.0±0.0         | 26.7±0.6            | 100.0±0.0         | 24.3±0.6            | 100.0±0.0         |
| 5   | 96   | 30.0±0.0            | 100.0±0.0         | 26.0±0.0            | 100.0±0.0         | 23.2±0.3            | 100.0±0.0         |
|     | 108  | 30.0±0.0            | 100.0±0.0         | 25.0±0.0            | 100.0±0.0         | 21.0±0.0            | 100.0±0.0         |
| 6   | 120  | 28.3±0.6            | 100.0±0.0         | 23.3±0.6            | 100.0±0.0         | 17.7±0.6            | 100.0±0.0         |
|     | 132  | 26.7±0.6            | 100.0±0.0         | 21.7±0.6            | 91.7±14.4         | 16.2±0.3            | 91.7±14.4         |
| 7   | 144  | 26.7±0.6            | 100.0±0.0         | 20.0±0.0            | 91.7±14.4         | 15.0±0.0            | 91.7±14.4         |
|     | 156  | 25.0±1.0            | 100.0±0.0         | 19.7±0.6            | 83.3±28.9         | 15.0±0.0            | 91.7±14.4         |
| 8   | 168  | 25.0±1.0            | 91.7±14.4         | 18.0±0.0            | 83.3±28.9         | 14.7±0.6            | 75.0±25.0         |
|     | 180  | 24.5±0.5            | 91.7±14.4         | 17.3±0.3            | 83.3±28.9         | 14.7±0.6            | 0.0±0.0           |

Mortality increased and reached 100% on eight days after treatment started (Figure 2a), with salinity below 15 ppt. Under the 2.5% and 5.0% freshwater substitution treatments, mortality was not significantly different (P < 0.05) between treatments, with survival rates still above 80% at the time treatment was stopped. This indicates that up to 80% of sandfish can remain alive at salinities of 15 ppt and above for at least a week. The salinity level at which survival rate would be 50% was estimated at 14.561 ppt (Figure 2b).
Figure 2. Salinity decrease curves for 2.5%, 5.0% and 7.5% freshwater substitution at 12 hourly intervals (A), and survival rate of sandfish *Holothuria scabra* (B) under the gradual salinity decrease treatment.

Water quality parameters other than salinity, such as temperature, pH, and dissolved oxygen, were maintained within or very close to the optimum ranges for sandfish (Table 3). Temperature, dissolved oxygen and pH of the treatment medium were measured in the morning and evening during the study and varied significantly (p < 0.05) between times of day but not between treatments (p > 0.05).

4. Discussion

Poor water quality can affect normal sandfish development, and therefore good water quality is a key factor for success in sandfish culture [43]. During the study, water quality parameters (temperature, dissolved oxygen, and pH) were in the recommended range for sandfish culture and rearing (Table 3); it is therefore unlikely that these parameters influenced the salinity treatments. The optimum water temperature range for sandfish culture 26-30°C; while the optimal temperature range for sea cucumber hatcheries is 27-29°C [44, 45]. In sandfish hatcheries, dissolved oxygen should be maintained above 5-6 ppt [45]. Although dissolved oxygen was below 5 ppt on one occasion, this is not considered likely to have been a problem because the tanks were aerated continuously throughout the study. The pH range was in the optimal pH range for sandfish throughout the study (Table 3).
Table 3: Water quality parameters during the experiments.

|                | Temperature (°C) | Dissolved Oxygen (ppm) | pH    |
|----------------|------------------|------------------------|-------|
|                | 06:00am          | 06:00pm                | 06:00am | 06:00pm | 06:00am | 06:00pm |
| **Sudden salinity decrease treatment** | | | | | | |
| N              | 9  | 9  | 9 | 9 | 9 | 9 |
| Range          | 28.3-28.5  | 31.1-32.5  | 5.00-5.60  | 4.80-5.00  | 7.7-7.9  | 7.7-7.9 |
| Average        | 28.4±0.1   | 31.5±0.5   | 5.20±0.21  | 4.92±0.07  | 7.8±0.1  | 7.8±0.1 |
|                |                |                        |       |    |    |     |
| **Gradual salinity decrease treatment** | | | | | | |
| N              | 9  | 9  | 9 | 9 | 9 | 9 |
| Range          | 27.0-28.5  | 29.0-30.0  | 5.60-6.70  | 4.80-5.60  | 7.5-7.7  | 7.6-7.8 |
| Average        | 27.7±0.6   | 29.3±0.4   | 6.1±0.4   | 5.2±0.3    | 7.6±0.1  | 7.7±0.1 |
|                |                |                        |       |    |    |     |
| **Optimum**    |                |                        |       |    |    |     |
| Range          | 26-29 (Pitt et al. 2004; Giraspy and Ivy 2005) | 5-6 (Pitt et al. 2004) | 6-9 (Pitt et al. 2004) |

Salinity is one of the factors that can limit the distribution and survival of marine invertebrates through a variety of mechanisms [46]. Salinity can affect marine invertebrate immune systems, especially in sea cucumbers [47]. The immune system in echinoderm is in the form of coelomocytes, which are found in coelomic, water, and circulatory systems. These cells are known to be activated when sea cucumbers are exposed to environmental changes or other stressors that cause stress [48-54]. The concentration of coelomocytes increased when the animals were exposed to lower (25 ppt) or higher (45 ppt) salinity levels [48].

Salinity has a positive effect on immune responses ROS (reactive oxygen species) of Echinoderms. *Asterias rubens*, amoebocyte ROS production increased at low salinity [36]. Salinity can also affect sea cucumbers through its influence on the activity levels of superoxide dismutase (SOD), catalase (CAT), myeloperoxidase (MPO), and lysozyme (LSZ). Limited tolerance of salinity stress has been reported in *A. japonicus*; where SOD, MPO, and LSZ activity were significantly affected by salinity change, while CAT activity decreased rapidly after exposure to the salinity of 20 ppt [55]. Decreasing salinity can also interfere with breathing and excretion systems in *Apostichopus japonicus*. *A. japonicus* respiration and excretion rates were lowered at salinities of 22 and 27 ppt, and within one hour, the immune capacity was significantly showed a difference between salinity levels of 25 ppt and 35 ppt [56].

In nature, salinity fluctuations are relatively small and, in general, did not last very long, so that sandfish can overcome the situation by burying themselves into the sediment. These conditions are different from the situation in coastal ponds, where a decrease in salinity can be much greater and tends to last for a long time. Sandfish cannot overcome the decrease in salinity with their natural burying behavior.
Changes in salinity will affect all organisms in the sea, especially invertebrates, but some types of animals are equipped with mechanisms that help to minimize these effects. Observations by Chen and Chen [25] found that mollusks abundance and population size in low tide zone were not much different compared to the one in 10 m depth. This was due to their ability to prevent or limit osmotic stress by closing their shell or by burying themselves into the sediment. In contrast to what happens to mollusks, the diversity and abundance of echinoderms are very dependent on fluctuations in the salinity of the environment. This might be due to the nature of the outer layer of the sea cucumber, which has high permeability [34]. Like other echinoderms, although sensitive to salinity change, sandfish is more tolerant compared to some other sea cucumbers. The metabolism of *Lytechinus variegatus* is disrupted by salinity levels below 25 ppt [57]. Although the calculated salinity, where the survival rate reached 50%, is 14.6 ppt (Figure 2B), the studies indicate strong evidence that *H. scabra* appeared to be able to tolerate salinity fluctuation as lower as 18 ppt, for at least several days with relatively low levels of mortality.

The body fluid salinity of freshwater bony fish is around 14 ppt [27], while that of saltwater bony fish is around 18 ppt. The results of this study indicate that sandfish have limited resistance to fluctuations or decreases in salinity. The lower limit of salinity tolerance of sandfish seems to be located within the range of salinity of bony fish body fluids. At salinity levels below that level, sandfish seem to begin facing osmotic problems; while at salinity levels below the body fluid salinity of freshwater bony fish, sandfish mortality quickly reaches 100%.

5. Conclusion
The current study indicates that the mortality of *H. scabra* exposed to low salinities is due to environmental salinity has exceeded its lowest tolerance limit, regardless of whether the decrease is due to a sudden or gradual process. Although the calculated salinity, where the survival rate reached 50%, is 14.6 ppt, the studies indicate strong evidence that the survival rates will decrease when salinity reaches below the body salinity of saltwater bony fish, which are 18 ppt. The survival rate of sandfish reached 0% when approaching the body salinity of freshwater bony fish, which is around 14 ppt. Therefore, to ensure the sandfish cultivation in coastal ponds is succeeding, the salinity should not be allowed to fall below 18 ppt. The first paragraph after a heading is not indented.

Acknowledgment
We would like to thank Universitas Hasanuddin for providing research funding (contract number 123/SP2H/LT/DPRM/III/2016; 005/SP2H/LT/DPRM/IV/2017; and 123/SP2H/PTNBH/DPRM/2018); and to Australia Awards Fellowship under Grant R170570. We wish to thank also to the James Cook University Team of Dr Naomi Gardiner and Dr Laurence McCook for their valuable advice. We would also like to thank Abigail Moore for proof reading the manuscript.

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