Evisceration rate of sandfish *Holothuria scabra* during transportation

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Abstract. The sandfish *Holothuria scabra* is a benthic animal of high ecological and economic value. The high economic value of sandfish has led to declining populations due to overfishing. Initiatives to improve sandfish production have included restocking, sea-ranching and aquaculture ( captive breeding and grow-out). These initiatives require a supply of animals (seed and/or broodstock) that often originate far from the location where they are needed. This calls for a good transportation system so that the sandfish arrive in good condition. This study aimed to assess the evisceration rate of sandfish transported using open and closed transportation modes. During the study, different modes of packing, density, transportation time, and holding time were evaluated. This study indicated that delivery using open systems gave poor results; besides being less practical, post-transport evisceration was very high. The ratio of oxygen and water and the packing density did not appear to affect the sandfish evisceration rate. Evisceration seemed to be triggered by the presence of dead and decaying sandfish in the bag. Sandfish that survived transportation generally recovered swiftly after being placed in a pre-prepared holding tank. The transported sandfish were used for multitrophic aquaculture and the post-transport survival ratio was very high.

1. Introduction
The sandfish *Holothuria scabra* is an animal belonging to the phylum Echinodermata, subphylum Echinozoa, class Holothuroidea, order Aspidochirotida, family Holothuriidea, and genus Holothuria. The sandfish is a reef-associated organism with a wide Indo-Pacific distribution stretching from east Africa to the Pacific Islands but does not reach Hawaii (Figure 1). Sandfish are generally found in silty sand, often near low saline areas and frequently in *Cymodocea* beds, from the intertidal area to a depth of 10 m. They tend to remain buried in the sand for part of the day, with juveniles generally living in shallower waters, and tending to move deeper as they grow, though rarely below 10 m [1]. Golden sandfish are mostly found on the inner reef flats of fringing and lagoonal reefs, coastal areas under terrigenous influence, and near mangroves. Sandfish burrow in mud and sandy-mud substrates where the population density can reach one per m² [2]. Sandfish are also found in intertidal seagrass beds [3].

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Sandfish are benthic detritus feeders; they can assimilate organic matter from muddy and sandy sediments, as well as several other food sources [4]. *H. scabra* is a species with high potential fecundity and early sexual maturity [2]; in New Caledonia, Sandfish spawn from August to September and December to January [5]. Sexual maturity can be reached at a length of 16 cm [6], with a common length of 22.0 cm [2] and a maximum published weight of 1.5 kg [6].

The sandfish *Holothuria scabra* is important from both ecological and economic viewpoints. The high economic value of sandfish has led to declines in many populations due to overfishing [7]. Various initiatives to improve sandfish production have included restocking, sea ranching and aquaculture (breeding and grow-out) [8-12]. These initiatives all require a supply of sandfish juveniles (seed) or broodstock that generally originate from a location far from the site where they are needed. This situation means that good transportation systems are required to ensure that the sandfish arrive in good condition. This study aimed to assess the evisceration rate of sandfish transported using open and closes transport systems.

**Figure 1.** Sandfish *Holothuria scabra* (A) and a map of sandfish distribution (B) [13]

2. Materials and Methods

2.1. Materials and Methods

*Holothuria scabra* specimens were collected from the coastal waters of Liukang Tupabbiring Sub-District, Pangkep District in South Sulawesi Province, Indonesia (Figure 2). The sandfish were picked up by hand at low spring tide during the dark moon phase.

**Figure 2.** Sampling station of Sandfish *Holothuria scabra* in Liukang Tupabbiring Sub-District
During the study, different transport models were trialled, including packing methods and density, transportation time, and holding time (Table 1). Five hours holding time was added to the transport time to simulate the time taken to complete package processing procedures at the airport. A one-hour stop-over on the way was used to simulate the airport transit process.

**Table 1.** The treatment detail of Sandfish *Holothuria scabra* during transportation

| Transport Model | Treatment applied (density and time)                        | Density (ind/bag or box) | No of Replicates |
|-----------------|-------------------------------------------------------------|--------------------------|------------------|
| Open model      | Low density                                                 | 5                        | 2                |
| Closed model    | Low density                                                 | 1                        | 4                |
|                 |                                                              | 2                        | 3                |
|                 |                                                              | 5                        | 2                |
|                 | Low density plus five hours holding time                     | 2                        | 3                |
|                 |                                                              | 4                        | 3                |
| Closed model    | Medium density                                              | 15                       | 6                |
|                 | Medium density plus five hours holding time                  | 8                        | 3                |
|                 |                                                              | 10                       | 3                |
|                 |                                                              | 15                       | 3                |
|                 | Medium density plus one hour rest on the way, and five hours holding time | 10                       | 5                |
| Closed model    | High density                                                | 50                       | 6                |
|                 | High density plus five hours holding time                    | 30                       | 3                |
|                 | High density plus one-hour rest on the way, and five hours holding time | 25                       | 5                |

The first packing model was an open model using styrofoam boxes. The second packing model was a closed model using plastic bags containing water and oxygen. The ratio of oxygen to water was around 30% to 70%. Closed transportation involved two stages, namely packing and transportation (Figure 3).

**Figure 3.** Sampling station of Sandfish *Holothuria scabra* in Sub-District Liukang Tupabbiring (top row); Closed model packing (middle and bottom rows)
During closed system transportation, the plastic bags were placed in Styrofoam boxes. The densities trialled were low density (1, 2, 4 and 5 sandfishes per bag); medium density (8, 10 and 15 sandfishes per bag); and high density (25, 30 and 50 sandfishes per bag). Each bag was numbered for easy recognition (Figure 3).

The density range used in this study was based on a previous study in which 100 to 200 juvenile sandfish weighing 1-5 g were packed in each bag [14]. In the preliminary study, it was found that the average weight of the sandfish to be transported was 42.68g ±20.16 (n = 25). Therefore, biomass for a packing density of 25 sandfish/bag was equivalent to the biomass of 213 juveniles weighing 5 g size, while 50 sandfish per bag would be equivalent to more than 400 juvenile sandfish weighing 5 g.

The plastic bags used for low density packing were 25 cm wide (the diameter after being filled with water and oxygen was about 18 cm). For medium density packing, the bags used were 35 cm wide (diameter after filling ≈ 25 cm); and for high density the bags were 50 cm wide (diameter after filling ≈ 33 cm). The length of the plastic bags after being filled with water and oxygen was ≈25 cm for low density, ≈35 cm for medium density, and ≈65 cm for high density.

The holding time was five hours after transportation. During the holding time, the plastic bags were left in the Styrofoam box. The parameters observed were the number of sandfishes which had eviscerated after transport and after transport + holding time.

3. Results

3.1. Open Model
Transportation was carried out on March 9, 2016. The journey from the sampling location to the laboratory was around 3.5 hours (13.30-16.30) drive by car. During transportation only one sandfish eviscerated (8.3%). Around 4 hours after completion of transportation, the number of eviscerations had reached 50% (Table 2 and Figure 4). The next day, all of the specimens were dissected and none of the sandfish had intestines. This means that the five remaining sandfish had eviscerated before being transported (Table 2).

Table 2. Eviscerated condition of Sandfish Holothuria scabra after transportation in a styrofoam box

| Box | Density (ind) | 16.30 (just after transportation) | 20.48 | 20.59 | 08.30 (dissection the day after transportation) |
|-----|---------------|-----------------------------------|-------|-------|-----------------------------------------------|
| 1   | 5             | 1 Evisceration                    |       |       | 3 without internal organs                      |
| 2   | 5             | 1 Evisceration                    |       |       | 2 Eviscerations                                |
|     |               |                                   |       |       | 2 without internal organs                      |

Figure 4. Evisceration of sandfish Holothuria scabra in styrofoam boxes and after transportation by styrofoam box. A and B: sandfish in styrofoam boxes; C to F: sandfish evisceration in styrofoam boxes
3.2. Closed transport model

3.2.1. Low density
The low density closed model transportation was carried out on April 11th, 2016. A total of 20 adult sandfish were transported at different densities in nine plastic bags. During the transport process, no sandfish eviscerated in the bags (Table 3).

| No. | Bag number | Density (sandfish/bag) | Evisceration (After 3.5 hours transportation) |
|-----|------------|------------------------|-----------------------------------------------|
| 1   | 1          | 1                      | 0                                             |
| 2   | 2          | 1                      | 0                                             |
| 3   | 3          | 1                      | 0                                             |
| 4   | 4          | 1                      | 0                                             |
| 5   | 5          | 2                      | 0                                             |
| 6   | 6          | 2                      | 0                                             |
| 7   | 7          | 2                      | 0                                             |
| 8   | 8          | 5                      | 0                                             |
| 9   | 9          | 5                      | 0                                             |

Transportation at low density plus five hours holding time was carried out on May 11th, 2016. During the transportation process and holding time, there were 3 eviscerations in the bag, and a further 2 after the sandfish were removed from the bag (Table 4). These eviscerations were caused by three dead and decaying sandfishes in the bag. In bags number 10 and 17, sandfish which had already eviscerated before being transported (two and one, respectively) died in transport and quickly decayed, becoming foul-smelling.

| No. | Bag number | Density (ind/bag) | Evisceration inside the bag | Evisceration outside the bag during length and weight measurement | Total eviscerations |
|-----|------------|-------------------|-----------------------------|------------------------------------------------------------------|--------------------|
|     |            |                   | ∑   | %      | ∑   | %      | ∑   | %      |
| 1   | 16         | 2                 | 0   | 0.00   | 0   | 0.00   | 0   | 0.00   |
| 2   | 17         | 2                 | 1   | 50.00  | 0   | 0.00   | 1   | 50.00  |
| 3   | 18         | 2                 | 0   | 0.00   | 0   | 0.00   | 0   | 0.00   |
|     |            |                   | Average                      | 0.33                | 16.67         | 0.00 | 0.00 | 0.33 | 16.67 |
|     |            |                   | STD                          | 0.58                | 28.87         | 0.00 | 0.00 | 0.58 | 28.87 |
| 4   | 10         | 4                 | 2   | 50.00  | 0   | 0.00   | 2   | 50.00  |
| 5   | 11         | 4                 | 0   | 0.00   | 1   | 25.00  | 1   | 25.00  |
| 6   | 12         | 4                 | 0   | 0.00   | 1   | 25.00  | 1   | 25.00  |
|     |            |                   | Average                      | 0.67                | 16.67         | 0.67 | 16.67 | 1.33 | 33.33 |
|     |            |                   | STD                          | 1.15                | 28.87         | 0.58 | 14.43 | 0.58 | 14.43 |

Table 4. Evisceration of Sandfish *Holothuria scabra* transported in bags at low density with five hours holding time

3.2.2. Medium density
Medium density transport of *H. scabra* in the closed system (bags) was carried out on July 20, 2017. The transport with this system at a medium density gave very good results, as no sandfish eviscerated during transport or during the post-transport measuring process (Table 5).
Table 5. Evisceration rate of *H. scabra* transported in bags at medium density

| No. | Bag number | Density (ind/bag) | Evisceration inside the bag | Evisceration outside the bag during length and weight measurement |
|-----|------------|-------------------|----------------------------|---------------------------------------------------------------|
|     |            |                   | ∑   %                      | ∑   %                                                       |
| 1   | 1          | 15                | 0  0                       | 0  0                                                         |
| 2   | 2          | 15                | 0  0                       | 0  0                                                         |
| 3   | 3          | 15                | 0  0                       | 0  0                                                         |
| 4   | 4          | 15                | 0  0                       | 0  0                                                         |
| 5   | 5          | 15                | 0  0                       | 0  0                                                         |
| 6   | 6          | 15                | 0  0                       | 0  0                                                         |
|     | **Average** |                  | 0.00 0.00 | 0.00 0.00 |
|     | **STD**    |                  | 0.00 0.00 | 0.00 0.00 |

The transport of medium density plus five hours holding time was carried out on May 11th, 2016. During the transportation process a total of nine sandfish eviscerated (Table 6). In bag 14, four sandfishes had eviscerated and there were three dead and decaying sandfishes in the bag (one survived). In bag 4 and five, one and two sandfishes respectively had eviscerated and died. The water in both bags was heavily contaminated with the products of the dead and decaying sandfish.

Table 6. Evisceration of *H. scabra* transported in bags at medium density with five hours holding time

| No. | Bag number | Density (ind/bag) | Evisceration inside the bag | Evisceration outside the bag during length and weight measurement | Total evisceration |
|-----|------------|-------------------|----------------------------|---------------------------------------------------------------|-------------------|
|     |            |                   | ∑   %                      | ∑   %                                                       | ∑   % |
| 1   | 13         | 8                 | 0  0.00 | 0  0.00 | 0  0.00 | 0  0.00 |
| 2   | 14         | 8                 | 4  50.00 | 0  0.00 | 4  50.00 |
| 3   | 15         | 8                 | 0  0.00 | 0  0.00 | 0  0.00 |
|     | **Average** |                  | 1.33 16.67 | 0.00 0.00 | 1.33 16.67 |
|     | **STD**    |                  | 2.31 28.87 | 0.00 0.00 | 2.31 28.87 |
| 4   | 1          | 10                | 0  0.00 | 0  0.00 | 0  0.00 |
| 5   | 2          | 10                | 0  0.00 | 0  0.00 | 0  0.00 |
| 6   | 3          | 10                | 0  0.00 | 0  0.00 | 0  0.00 |
|     | **Average** |                  | 0.00 0.00 | 0.00 0.00 | 0.00 0.00 |
|     | **STD**    |                  | 0.00 0.00 | 0.00 0.00 | 0.00 0.00 |
| 7   | 4          | 15                | 1  6.67 | 0  0.00 | 1  6.67 |
| 8   | 5          | 15                | 2  13.33 | 0  0.00 | 2  13.33 |
| 9   | 6          | 15                | 0  0.00 | 0  0.00 | 0  0.00 |
|     | **Average** |                  | 1.00 6.67 | 0.00 0.00 | 1.00 6.67 |
|     | **STD**    |                  | 1.00 6.67 | 0.00 0.00 | 1.00 6.67 |

Medium density transportation plus a one-hour stop-over on the way, with air temperatures reaching 35 °C, and five hours post-transport holding time was carried out on June 20 2019. Evisceration rates were quite high in the bag (14.11 ± 11.40) and outside the bag during length and weight measurement (22.00 + 19.24) (Table 7).
Table 7. Evisceration of *H. scabra* transported in bags at medium density with one hour stop-over on the way and five hours holding time

| No. | Bag number | Density (ind/bag) | Evisceration inside the bag | Evisceration outside the bag during length and weight measurement | Healthy and actively moving |
|-----|------------|-------------------|-----------------------------|---------------------------------------------------------------|-----------------------------|
|     |            |                   | %                           | %                                                            | %                           |
| 1   | 6          | 10                | 3                           | 30.00                                                        | 1                           | 10.00                                      | 0                       | 0.00                              |
| 2   | 7          | 10                | 2                           | 20.00                                                        | 0                           | 0.00                                      | 0                       | 0.00                              |
| 3   | 8          | 10                | 0                           | 0.00                                                         | 3                           | 30.00                                     | 0                       | 0.00                              |
| 4   | 9          | 10                | 1                           | 10.00                                                        | 2                           | 20.00                                     | 0                       | 0.00                              |
| 5   | 10         | 10                | 1                           | 10.00                                                        | 5                           | 50.00                                     | 0                       | 0.00                              |
|     |            |                   |                             |                                                               |                             |                                           |                         |                                   |
|     | Average    |                   | 1.40                        | 14.00                                                        | 2.20                        | 22.00                                     | 0                       | 0.00                              |
|     | STD        |                   | 1.14                        | 11.40                                                        | 1.92                        | 19.24                                     | 0                       | 0.00                              |

3.2.3. High density

High density transportation was carried out on July 20, 2017. During transport, two sandfish eviscerated, representing 2% of the respective bag contents. After the transportation, no sandfish eviscerated during the measurement process (Table 8).

Table 8. Evisceration of *H. scabra* transported in bags at high density

| No. | Bag number | Density (ind/bag) | Evisceration inside the bag | Evisceration outside the bag during length and weight measurement | Total evisceration |
|-----|------------|-------------------|-----------------------------|------------------------------------------------------------------|-------------------|
|     |            |                   | %                           | %                                                               | %                 |
| 1   | 7          | 50                | 1                           | 2.00                                                             | 0                 |
| 2   | 8          | 50                | 0                           | 0                                                                | 0                 |
| 3   | 9          | 50                | 1                           | 2.00                                                             | 0                 |
| 4   | 10         | 50                | 0                           | 0                                                                | 0                 |
| 5   | 11         | 50                | 0                           | 0                                                                | 0                 |
| 6   | 12         | 50                | 0                           | 0                                                                | 0                 |
|     |            |                   |                             |                                                                   |                   |
|     | Average    |                   | 0.33                        | 0.67                                                             | 0.00              | 0.00                                      |
|     | STD        |                   | 0.45                        | 0.89                                                             | 0.00              | 0.00                                      |

High density transportation of *H. scabra* in bags with five hours holding time was carried out on May 11, 2019. The mean overall evisceration rate was quite low in the bags (2.2 + 1.92%) and no evisceration occurred outside the bag during length and weight measurements (Table 9).

Table 9. Evisceration of *H. scabra* transported in bags at high density with five hours holding time

| No. | Bag number | Density (individual) | Evisceration inside the bag | Evisceration outside the bag during length and weight measurement | Total evisceration |
|-----|------------|----------------------|-----------------------------|------------------------------------------------------------------|-------------------|
|     |            |                      | %                           | %                                                               | %                 |
| 1   | 7          | 30                   | 1                           | 3.33                                                             | 0                 | 0.00                                      | 1                       | 3.33                              |
| 2   | 8          | 30                   | 1                           | 3.33                                                             | 0                 | 0.00                                      | 1                       | 3.33                              |
| 3   | 9          | 30                   | 0                           | 0.00                                                             | 0                 | 0.00                                      | 0                       | 0.00                              |
|     |            |                      |                             |                                                                   |                   |                                           |                         |                                   |
|     | Average    |                      | 0.67                        | 2.22                                                             | 0.00              | 0.00                                      | 0.67                    | 2.22                              |
|     | STD        |                      | 0.58                        | 1.92                                                             | 0.00              | 0.00                                      | 0.58                    | 1.92                              |
High density transportation with a one-hour stop-over in air temperatures reaching 35 °C and five hours post transport holding time was carried out on June 20, 2019. Evisceration occurred mostly in the bags (9.60 ± 6.69 %) and some further eviscerations occurred (2.40 ± 2.19 %) outside the bag during the length and weight measurements. Because the water in the bag had become fouled with the products of decomposition from the ejected viscera and the dead and dying sandfish, morbidity and mortality were high. All the dead or injured sea cucumbers (foul-smelling, wrinkled, and passively moving) were dissected. These sandfishes (69.60 + 13.15%) had no intestines, meaning that those which did not eviscerated during transport had already eviscerated before being transported (Table 10).

Table 10. Evisceration of *H. scabra* transported in bags at high density with one-hour stop-over on the way and five hours holding time

| No. | Bag number | Density (individual) | Evisceration inside the bag | Evisceration outside the bag during length and weight measurement | Died, wound skin, foul-smelling, without internal organs |
|-----|------------|----------------------|----------------------------|---------------------------------------------------------------|----------------------------------------------------------|
|     |            |                      | ∑  | %   | ∑  | %   | ∑  | %   | %   |
| 1   | 1          | 25                   | 4  | 16.00 | 0  | 0.00 | 16 | 64.00 |
| 2   | 2          | 25                   | 4  | 16.00 | 0  | 0.00 | 13 | 52.00 |
| 3   | 3          | 25                   | 2  | 8.00  | 1  | 4.00 | 18 | 72.00 |
| 4   | 4          | 25                   | 2  | 8.00  | 1  | 4.00 | 18 | 72.00 |
| 5   | 5          | 25                   | 0  | 0.00  | 1  | 4.00 | 22 | 88.00 |
|     | Average    |                      | 2.40 | 9.60 | 0.60 | 2.40 | 17.40 | 69.60 |
|     | STD        |                      | 1.67 | 6.69 | 0.55 | 2.19 | 3.29 | 13.15 |

4. Discussion

Evisceration is an autotomy process that causes expulsion of the sea cucumber viscera [15]. Evisceration is a body response that involves a series of events, including muscle contractions and failure of three autotomy structures [15]. Evisceration is associated with spontaneous damage from three autotomy tissues [16, 17]: (i) introverted, anterior extensible parts of the body wall; (ii) tendons that connect pharyngeal retractor muscle with longitudinal body wall muscles; and (iii) the intestinal-cloaca junction [15]. The evisceration process begins with a softening and rupture of the introverted wall followed by the release of viscera [18, 19]. Evisceration can occur naturally under natural conditions and is sometimes seasonal [20]. At certain locations and times, evisceration can occur in up to 76% of the holothurian population [21]. Evisceration is a phenomenon commonly found in sea cucumber [22], and needs to be considered in the selection of sandfish broodstock [8].

This study indicated that the delivery with an open system did not work well; besides being less practical, post-transport evisceration was also very high. It was difficult to know the exact cause, but the greater shock and friction with Styrofoam box walls compared to plastic bags was thought to place greater stress on the sandfish.

This study also indicated that, with the ratio of oxygen and water used, density did not cause evisceration in sandfish. Evisceration appeared to be triggered by the presence of dead and decaying sandfish in the bag. Sandfish that survived transportation generally recovered almost immediately after being placed in a pre-prepared holding tank. The sandfish which survived transportation were used for multitrophic aquaculture, and the post-transport survival ratio was very high. Previous research reports the successful transportation of juveniles for sandfish restocking [14].

During transport for 3.5 hours, without an additional five hours of holding time, even though there were some sandfish which had eviscerated before being transported, and two sandfish eviscerated in high density bags, these events did not cause further eviscerations or mortality. Apparently, evisceration started to have an impact if the eviscerated sandfish died and began to rot during the treatments with additional holding time. This finding shows that the transport time will affect the evisceration rate, especially if there are sandfish that die in the bag. It seems that the high air temperature during transportation, especially during the stop-over and holding time, also affected
evisceration. The evisceration rate was worse (and the mortality higher) when there were sandfish in the bags that had eviscerated before being transported.

Up to a density of 50 sandfish, for relatively short journeys density did not noticeably increase sandfish evisceration, even though when referring to previous studies [14], the density of 50 sandfish, was equivalent to 400 sandfish juveniles weighing 5 g. Overall, the results can be interpreted as indicating that with a good selection of sandfish to be transported (avoiding eviscerated individuals) and good handling before, during and after transportation, even at such high densities the closed model can provide successful H. scabra transportation.

5. Conclusion
This study indicated that the delivery model using an open system was neither practical nor effective, with high post-transport evisceration. This study also indicated that with the ratio of oxygen and water used (30:70), density did not cause evisceration in sandfish. Evisceration appeared to be triggered by the presence of dead and decaying sandfish in the bag, mostly sandfish that had eviscerated before transport. Sandfish that survive transportation generally recover almost immediately after being placed in a pre-prepared holding tank. Sandfish from this transportation were used for multitrophic aquaculture, and the post-transport survival ratio was very high.

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