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Experimental test of optimal holding conditions for live transport of temperate sea cucumbers

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ABSTRACT

Sea cucumber is one of the top five luxury seafoods in Asia and its commercialization primarily revolves around the processed body wall. Hence, live transport and storage of sea cucumbers prior to processing must preserve the condition of the body wall and underlying muscles. Unlike most commercial shellfish on which industry standards are chiefly based, sea cucumbers lack a protective exoskeleton and have the ability to autolyze. Here, we tested the efficacy of different live storage methods on *Cucumaria frondosa*, a commercial species that is widely distributed in the North Atlantic and the Arctic. Current technologies and low-cost variants were experimentally tested under conditions prescribed for the transport of seafood in Canada. Markers of post-storage health, body wall condition and muscle integrity were compared among treatments. The most common method currently in use (icing and salting) yielded the highest rates of mortality and skin necrosis, whereas iced seawater emerged as the best storage condition. These findings should help stakeholders adapt their methodologies to optimize the exploitation of temperate and cold-water sea cucumber resources.

1. Introduction

Sea cucumbers have been consumed and used in traditional medicine for centuries in Asia; over the past 50 years they have become one of the most prized seafoods in the world (Fabinyi, 2012; Purcell, 2014; Yang et al., 2015). The most commonly traded product, known as beche-de-mer or trepang, consists of the sea cucumber body wall (skin), generally including the muscle bands, which is dried and sold as a luxury seafood (Purcell, 2014). In North America, muscle bands are sometimes fresh frozen and marketed separately (Hamel and Mercier, 2008a). Dried aquapharyngeal bulbs (labelled flowers), liquid or gel extracts and various supplements can also be found on the market. It is believed that the consumption of sea cucumber has significant health benefits (Bechtel et al., 2013; Cheung and Wu, 2012). Studies have shown that the body wall and muscles of sea cucumbers are important sources of high-value compounds exhibiting anticoagulant, anticancer, antioxidant and anti-inflammatory properties (Bordbar et al., 2011; Wen et al., 2010; Xia and Wang, 2015). Because body wall is the chief commercial product, special attention should be given to the storage and transport of live sea cucumbers following harvest in order to maintain their organoleptic and nutritional properties.

Compared to other shellfish, live sea cucumbers have proven difficult to store and transport, because they lack a protective exoskeleton and have the ability to autolyze when they are stressed or taken out of seawater (Duan et al., 2010; Zang et al., 2012). Autolysis is a physiological response which leads to dermis (body wall) degradation through protein breakdown (Wu et al., 2013). Endogenous proteases have been reported to be responsible for the autolysis process, which is often associated with changes in the organoleptic properties of the meat in various marine species (Sun et al., 2013). The formation of new compounds, following lipid oxidation and protein breakdown, alters the color, odour, flavour and texture of the meat (Ghaly et al., 2010; Rodríguez et al., 2009). During storage, transport, handling and processing, sea cucumbers are exposed to air and UV and undergo abrupt changes in temperature and salinity (Ji et al., 2008; Zhu et al., 2009). These factors may lead to damage of the body wall, promote the development of strong odour and even cause the animal's death (Zhu et al., 2009). Furthermore, the digestive tract secretes enzymes (e.g., trypsin, chymotrypsin and cathepsin) which act in the hydrolysis of collagen (Yan et al., 2014), the main component of the body wall (~70%). Dead or unhealthy sea cucumbers exhibit deteriorated body walls

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that decrease the final products' quality and result in economic losses (Saito et al., 2002; Sun et al., 2013; Wu et al., 2013).

Market price for sea cucumber is based on many criteria that include general appearance (e.g., color, shape, texture) and smell (Kinch et al., 2008; Purcell, 2014; Tuwo, 2005), all of which can be affected by handling. Storage and transport methods of live marine organism have been investigated in fishes (Berka, 1986; Froese, 1998; Harmon, 2009), crustaceans (Barrento et al., 2012; Fotedar and Evans, 2011) and molluscs (Buzin et al., 2011; Wyatt et al., 2013). The few studies that have been conducted on sea cucumbers are restricted to the transport of hatchery-produced juveniles for restocking programs (Purcell et al., 2006; Zamora and Jeffs, 2014), and more generally apply to tropical species.

The shortage of data on storage and transport of live sea cucumbers for food processing, and on temperate or cold-water species in particular, may be explained by the chiefly artisanal nature of harvesting and processing techniques used in the Indo-Pacific countries, where harvests outside China have traditionally concentrated. Tropical sea cucumbers are generally handpicked, and immediately eviscerated, boiled and dried on the shore or a nearby site; processing is essentially manual (Hair et al., 2012; Purcell et al., 2013). In China, hatchery-produced juveniles of the temperate species *Apostichopus japonicus* are transported to the restocking sites either in damp sealed plastic bags without seawater or in buckets inside fiberglass tanks full of aerated seawater (Guo et al., 2014; Tan et al., 2014). Depending on the distance from the hatchery to the restocking site and the accessibility, transport can take more than 10 h (Tan et al., 2014).

In recent years, the overexploitation of high-valued sea cucumbers from Asia and the Indo-Pacific has led to the development of new fisheries for under-utilized species around the world (Anderson et al., 2011; So et al., 2010; Therkildsen and Petersen, 2006). The sea cucumber *Cucumaria frondosa* is the focus of an emerging fishery in the North Atlantic. This species is widely distributed in temperate and cold waters, occurring from the Arctic Ocean to Cape Cod as well as along the coasts of northern Europe and Russia (Hamel and Mercier, 2008a). The state of Maine (USA) was the first region to start a commercial fishery for *C. frondosa* in 1980, followed by several eastern Canadian provinces (Hamel and Mercier, 2008a; Rowe et al., 2009), as well as Iceland and Russia (Garcia et al., 2006; Gudimova et al., 2005; Hamel and Mercier, 2008b; Therkildsen and Petersen, 2006). Commercial harvest of *C. frondosa* was initially carried out with scallop gears; eventually, specific drag nets were designed to minimize bycatch and suit local conditions (Barrett et al., 2007). Storage of *C. frondosa* between the fishing wharfs and the processing plants can range from a few hours in Iceland to almost 2 days on the east coast of Canada (B. L. Gianasi, unpublished data).

With the expansion of sea cucumber fisheries in North and South America and northern Europe, and developing aquaculture ventures, the need to optimize storage and transport of cold-water and temperate species of sea cucumber from wharfs or farms to processing plants is increasing. Optimum storage conditions that minimize stress and mortalities are of significant value not only for the emerging industry around *C. frondosa* in the North Atlantic, but also for other commercially important temperate species around the world such as *A. japonicus*, *Parastichopus californicus*, *C. japonica*, and *Australostichopus mollis*.

The present study investigated the use of different media for refrigeration during live storage of the sea cucumber *C. frondosa*. Current methods used by the industry and low-cost variants were investigated, following the general guidelines of the fish inspection regulations of Canada (FIR, 2014). Individuals were classified according to health and body wall condition immediately after storage. Measurements of pH of the meat (body wall and muscle bands) were conducted and water quality in the storage tanks assessed. Finally, individuals were monitored post storage for survival and development of skin damage to identify the optimal storing method in order to obtain high-quality body wall and meat products.

2. Material and methods

2.1. Sea cucumber collection

Sea cucumbers weighing 8.1 ± 1.3 g (immersed weight) and measuring 12.2 ± 2.4 cm (contracted body length) were collected by divers in Bay Bulls, Newfoundland (47°17′44.6″N, 52°46′8.9″W), eastern Canada, at depths between 5 and 10 m. Dive collections were performed by the Field Services of the Department of Ocean Sciences with the required permits from the Department of Fisheries and Oceans Canada (DFO). Sea cucumbers were kept in holding tanks with running seawater at ambient temperature (1.2–2.1 °C) for over a month before using them in any trial. Only healthy and undamaged individuals displaying normal pigmentation and feeding activity, firm attachment to the substrate and no skin lesion were selected.

2.2. Experimental conditions and data collection

2.2.1. Testing storage of sea cucumbers

The Canadian fish inspection regulations (FIR, 2014) do not explicitly regulate the transport and storage of sea cucumbers. However it mentions that fish and shrimp that are being transported or held in a preparatory room prior to entering the processing line must be iced or chilled to maintain their temperature not higher than 3–4 °C. The same regulation stipulates that trucks designed to transport seafood should be equipped with appropriate insulated/sealed fish containers in order to avoid the discharge of any fluid and effluent during transport (FIR, 2014).

Sea cucumbers were distributed in plastic tanks 0.40 m long × 0.28 m wide × 0.22 m height, total volume of 24 l without a drainage system in order to retain any water resulting either from the storage media or from the sea cucumbers, as per regulations (FIR, 2014). The tanks were stored for 48 h, representing the maximum time that sea cucumbers are held in fish vats during transport on the east coast of Canada, in a dark and cold room between 1 and 3 °C (FIR, 2014). Sea cucumbers were distributed in one layer of 12 individuals on the bottom of each tank. This design ensured that every sea cucumber had comparable exposure to any storage medium. Triplicate groups of sea cucumbers were submitted to one of six storage treatments: Seawater ice (T1), freshwater ice (T2), freshwater ice with fish salt (T3), iced seawater (T4) and bagged freshwater ice (T5). The no-medium group (T6) consisted of sea cucumbers stored humid without any ice or water in the experimental tanks. The various transport media tested (Table 1) were based on the technology already available, and on potential improvements that would demand only minimal investments from the fishing industry.

Seawater ice used in treatment 1 was made by freezing seawater with salinity of 35 at −20 °C for 48 h prior to the start of the experiment. The seawater ice was then crushed manually until it reached approximately the same texture (granule size) as commercial freshwater ice. The freshwater ice used in treatments 2–5 was prepared by an ice machine (ITV IQ300Ca). According to the manufacturer, the ice is granular with pieces ranging from 1 to 2 mm in diameter. For treatments 2 and 3, freshwater ice was removed straight from the ice machine and spread on top of the sea cucumbers. Fish salt (Avalon®) was added over the freshwater ice in treatment 3 in order to replicate the method currently used by many companies to transport shrimp, fish and sea cucumber on the east coast of Canada. The amount of salt added followed the proportion used

Table 1
Description of the various media and conditions used in treatments 1 through 6 (T1 to T6) over 48 h.

Code	Storage medium	Description
T1	Seawater ice	Crushed seawater ice prepared by freezing seawater with a salinity of 35
T2	Freshwater ice	Freshwater ice (granule type, ~1 mm) prepared by an ice machine (ITV IQ300Ca) at −2 °C
T3	Freshwater ice + fish salt	Freshwater ice spread uniformly over the sea cucumbers with fish salt added on top
T4	Iced seawater	A slush solution made of freshwater ice and seawater, with a temperature of 0 °C and salinity of 29
T5	Bagged freshwater ice	Freshwater ice kept in plastic bags and placed on the bottom of the tanks with sea cucumbers positioned above the bags
T6	No medium	No storage medium was added to the storage tanks

Table 2
Classification of external health conditions exhibited by the sea cucumbers immediately after the storage treatments.

Health Condition	Description
Very Good (VG)	No visible alterations on the body wall such as scratches, blisters, frostbites or skin lesions. Individuals respond immediately to handling by contraction, have faint/fresh fish smell and thin layer of mucus over the body wall
Good (G)	Less than 20% of the total body surface showing presence of blisters, scratches, frostbites or any other skin lesion. Individuals respond immediately to handling by contraction and have faint/fresh fish smell
Slightly Deteriorated (SD)	Clear signs of blisters, scratches, depigmentation, swelling, frostbites or any other skin lesion that represent 20–50% of the total body surface. Individuals covered with sticky mucus, displaying relaxed and flaccid body, slow contraction response and acrid, slightly fishy odour. Sea cucumbers with partially or completely extruded tentacles are included in this category if they do not show signs of skin alterations
Deteriorated (D)	Multiple skin/body alterations covering more than 50% of the total body surface. Individuals do not response to handling, present strong fish odour and are abundantly covered with sticky mucus. In addition, eviscerated (dead/dying) sea cucumbers are included in this category

by the industry of 6 g l^{-1} (based on a volume of 12 l occupied by the sea cucumbers and the ice in the tank). Iced seawater used in treatment 4 was prepared by mixing equal amounts of freshwater ice and seawater with salinity of 35 at ambient temperature (2.1 °C). The result was a slushy solution with a temperature of 0 °C and salinity of 29 (measured immediately after preparation with a YSI® 556 MPS probe). In treatment 5, freshwater ice was kept in plastic bags and placed on the bottom of the tanks with sea cucumbers packed on top of the bags to avoid applying pressure with the weight of the ice. In preliminary experiments with bagged ice, individuals were crushed when bagged ice was placed over them. The bags were used to avoid straight contact of the sea cucumbers with the ice and meltwater while maintaining a low temperature. The amount of ice used in each treatment followed the proportion used by fishing industries in eastern Canada, which is 2 volumes of sea cucumbers for 1 volume of ice.

2.2.2. Evaluation of post storage condition

Following the 48-h storage, sea cucumbers were evaluated and classified according to morphological alterations: signs of evisceration (expulsion of internal organs) and abnormally extruded tentacles (i.e., limply hanging outside the body), frozen body parts (i.e., difference in skin texture along the body wall that may indicate frostbites), excessive odour and mucus production, blisters, skin necrosis and evidence of swollen body. Alterations to the skin were scored relative to the total surface area they covered on the body of the sea cucumbers. Conditions recorded after storage were classified according to the rubric in Table 2. Pictures from the alterations caused by the storage were taken from all treatments for comparison purposes.

Following the initial evaluation, 3 sea cucumbers were sampled haphazardly from each treatment tank. Their muscle bands and body wall were removed and first inspected for color and texture. Another group of 9 individuals were collected haphazardly from holding tanks and were labelled live non-exposed controls (NE). Following visual inspection, the muscle bands and the body wall from each sample (3 individuals per storage tank and 9 non-exposed controls) were blended using a dilution of 1 g of tissue in 10 ml of deionized water until the solution was smooth and uniform (Benjakul et al., 1997). The final solution was sieved (1 mm mesh size) to remove any remaining particles and triplicate pH measurements performed at 18 °C (air temperature in laboratory) using a YSI® 556 MPS probe.

The remaining sea cucumbers from each treatment were transferred into 24 l flow-through tanks (9 sea cucumbers per tank, 3 tanks per treatment) for a recovery/monitoring period of 30 days. Conditions included a flow of ~10 l h^{-1}, natural photoperiod of 14L/10D, water temperature between 1 and 3 °C and ambient salinity of 35. Each tank was monitored daily for survival rates, number of sea cucumbers with tentacles deployed and signs of skin necrosis. Tentacle deployment was used in this study as an indicator of health, because healthy sea cucumbers tend to fully extend their oral tentacles in order to capture food in the water more often than stressed individuals (Hamel and Mercier, 1998). Skin necrosis was used as an indicator of damage caused by the storage condition; it included sloughing of the outer layer of the dermis resulting in the exposure of the white connective tissue. Evisceration of internal organs such as intestine, gonads and/or respiratory tree led to death of the sea cucumbers and eviscerated individuals were thus removed promptly.

2.2.3. Water quality in the storage tanks

Water quality was assessed in each storage tank as soon as sea cucumbers had been sampled and transferred to recovery tanks. The water in the storage tank, which resulted either from the storage media and/or from the sea cucumbers, was mixed with a glass rod. Dissolved oxygen, salinity and pH were measured with a YSI® 556 MPS probe. In addition, total ammonia nitrogen was quantified with La Motte® Smart 3 Colorimeter. A measurement was taken in each tank from all 6 treatments, at a water temperature of 3 °C.

2.3. Data analysis

The proportion of sea cucumbers under each health category (Table 2) immediately after storage (48 h) violated the assumptions for parametric statistics even after transformations. For this reason, Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks ($\alpha = 0.05$) followed by Tukey test was used to compared condition of sea cucumbers among treatments. One-way ANOVA was used to compare pH in the muscle and body wall of sea cucumbers among all treatments, followed by Holm–Sidak test for pairwise comparisons.

One-way ANOVA was used to compare the proportion of sea cucumbers with tentacles fully extended among treatments (inte-

grated over the period of 30 days). Pairwise comparisons were made using the Holm–Sidak test. Data on the proportion of sea cucumbers that had developed skin necrosis at the end of the recovery period (30 days) violated the assumptions for parametric statistics. Hence, one-way ANOVA on ranks was carried out to compare the proportion of sea cucumbers with skin lesion among treatments, followed by Tukey test as appropriate. Ammonia nitrogen, dissolved oxygen, water pH and salinity in the tanks after storage (48 h) were compared among treatments using one-way ANOVA followed by Holm–Sidak test for pairwise comparisons.

Logrank survival analysis ($\alpha = 0.05$) was used to compare survival rates among treatments. Survival rates were estimated with the Kalpan–Meier estimator (followed by multiple comparison Holm–Sidak test) at the end of storage, as well as on days 10, 20 and 30 of the subsequent recovery period.

Tentacle deployment, skin lesions and survival rates were not assessed in treatment 3 (T3), because all sea cucumbers had died after the storage period of 48 h. Data in the text are expressed as mean ± standard error. Statistical analyses were conducted with Statistica®.

3. Results

3.1. Immediate post storage condition

3.1.1. Health condition of sea cucumbers in the storage tanks

Treatment 4 (iced seawater) resulted in the highest proportion of sea cucumbers (92 ± 4%) scored as exhibiting very good health (VG) after storage in the cold room for 48 h (Fig. 1A). No visible skin lesions could be observed in these individuals (Fig. 2A and B). They looked like freshly collected individuals, responding immediately to handling by contraction of the body and presenting a very faint fish smell. A single sea cucumber (8 ± 4%) in treatment 4 was scored lower (G), because of a small blister on the dorsal anterior side of its body wall (Fig. 1B).

Treatments 1 (seawater ice) and 6 (no storage medium) also yielded relatively high proportions (75 ± 4%) of sea cucumbers scored as VG (Figs. 1 A and 2 A). These individuals had no visible damage on the body wall and a slight fish smell. Few sea cucumbers (25 ± 4%) were classified as G (Fig. 1B) as small blisters could be detected on their dorsal tegument, although it covered <20% of the total body wall surface.

Treatment 5 (bagged ice) resulted in high proportion (75 ± 4%) of sea cucumbers in VG condition (Figs. 1 A and 2 A). Few individuals were classified under G condition (17 ± 5%), because of small blisters were detected over the dorsal tegument covering less than 20% of the total body surface (Fig. 1B). However, treatment 5 also yielded sea cucumbers under slightly deteriorated (SD) condition (8 ± 5%; Fig. 1C). Individual categorized under SD showed blisters covering more than 20% of their body (Fig. 2C) and a more pronounced odour, as detailed in Table 2. In addition, abundant mucus on the body wall made the sea cucumber very slippery. Normally, healthy sea cucumbers contract their body immediately when handled; however, these individuals contracted their body ~5 times slower than other sea cucumbers under VG condition.

Treatment 2 (freshwater ice) resulted in 58 ± 5% of VG condition (Figs 1 A and 2 A). One quarter of sea cucumbers exposed to this treatment were classified under G after storage (Fig. 1B), because of small blisters on the dorsal tegument. Sea cucumbers scored as VG and G responded well to handling and did not exude any strong fish smell. Treatment 2 showed the highest proportion of sea cucumbers scored as SD (25 ± 4%; Fig. 1C) due to blisters covering more than 30% of their body (Fig. 2C), abundant mucus on the body wall, fishy odour and reaction to handling ~5 times slower than individuals under G condition.

In treatment 3 (freshwater ice with sea salt), all sea cucumbers (100%) were classified as deteriorated (D) after 48 h in the cold room (Fig. 1D). They were all eviscerated (Fig. 2D), did not respond to handling and exhibited multiple skin lesions covering more than 50% of their body wall (Fig. 2E). In addition, abundant sticky mucus was noticed on the surface of the water in the storage tanks.

One-way ANOVA on ranks revealed that the proportion of sea cucumbers in VG condition was significantly higher in treatment 4 when compared to the other treatments (Fig. 1). Also, treatments 1, 5 and 6 had similar proportion of sea cucumbers under VG condition and did not differ statistically ($H = 13.87$, df = 5 $p = 0.016$). Treatment 3 had the lowest proportion of sea cucumbers under VG condition and the highest proportion under D condition and both were significantly different than in all the other treatments (Table S1).

3.1.2. Visual assessment and pH of muscle bands and body wall

Live non-exposed control sea cucumbers (NE) collected from holding tanks displayed firm and pink colored muscle bands and their body wall was firm and elastic. The pH for non-exposed individuals was of 7.5 ± 0.1 in the muscle bands and 7.2 ± 0.1 in the body wall (Fig. 3).

The muscle bands (longitudinal and circular) and body wall of sea cucumbers exposed to treatment 4 exhibited a color and texture comparable to those of non-exposed sea cucumbers. Muscle bands and body wall were firm and easily separated from each other (Fig. 2B). The pH of muscle bands and body wall of sea cucumbers exposed to treatment 4 was of 7.6 ± 0.1 and 7.3 ± 0.0, respectively (Fig. 3).

Individuals exposed to treatments 1, 2, 5 and 6 also exhibited standard organ color and texture, similar to freshly collected live sea cucumbers. The pH of muscle bands for these treatments ranged from 7.4 ± 0.1 in treatment 1 to 7.5 ± 0.1 in treatment 5. The pH of body wall ranged from 7.1 ± 0.1 in treatment 1 to 7.2 ± 0.0 in treatment 6 (Fig. 3).

Sea cucumbers exposed to treatment 3 also exhibited pinkish muscle bands, comparable to non-exposed sea cucumbers; however, red spots could be observed all over the longitudinal muscles (Fig. 2F). In addition, tissues were extremely flaccid and were very easily torn during the dissection procedure so that separation of the muscle bands and body wall was hard to achieve. The pH measurements in the muscle bands and body wall of individuals exposed to treatment 3 were the lowest among all treatments, at 6.8 ± 0.1 for the muscle bands and 6.6 ± 0.1 for the body wall (Fig. 3).

Results of one-way ANOVAs showed that pH of the muscle bands did not differ among treatments or between treatments and non-exposed live controls, except for treatment 3 where values were significantly lower ($F_{5,48} = 39.87$, $p < 0.001$). Measurements of pH in emulsions of the body wall varied from 6.6 ± 0.1 in treatment 3 to 7.4 ± 0.1 in treatment 4. The statistical analyses showed that the pH of body wall was significant higher in treatment 4 than in the other treatments and in non-exposed sea cucumbers ($F_{5,48} = 36.53$, $p < 0.001$). In contrast, measurements of body wall pH in individuals from treatments 1, 2, 5 and 6 and non-exposed controls did not differ statistically. Finally, the pH of body wall from sea cucumbers exposed to treatment 3 was significant lower than in all other treatments (Fig. 3; Table S2).

3.2. Water quality in the storage tanks

At the end of the trials, the water in the tanks from treatment 4 was clear, did not present any marked odour and the freshwater ice had melted completely. Also, there was no evidence of abundant froth/mucus on the surface of the water. Tanks from treatments 4 had ammonia nitrogen levels of 2.7 ± 0.2 ppm, dissolved oxygen levels of 6.5 ± 0.3 mg l^{-1}, pH values of 7.2 ± 0.0 and salinity levels of 30.7 ± 0.3 (Fig. 4).

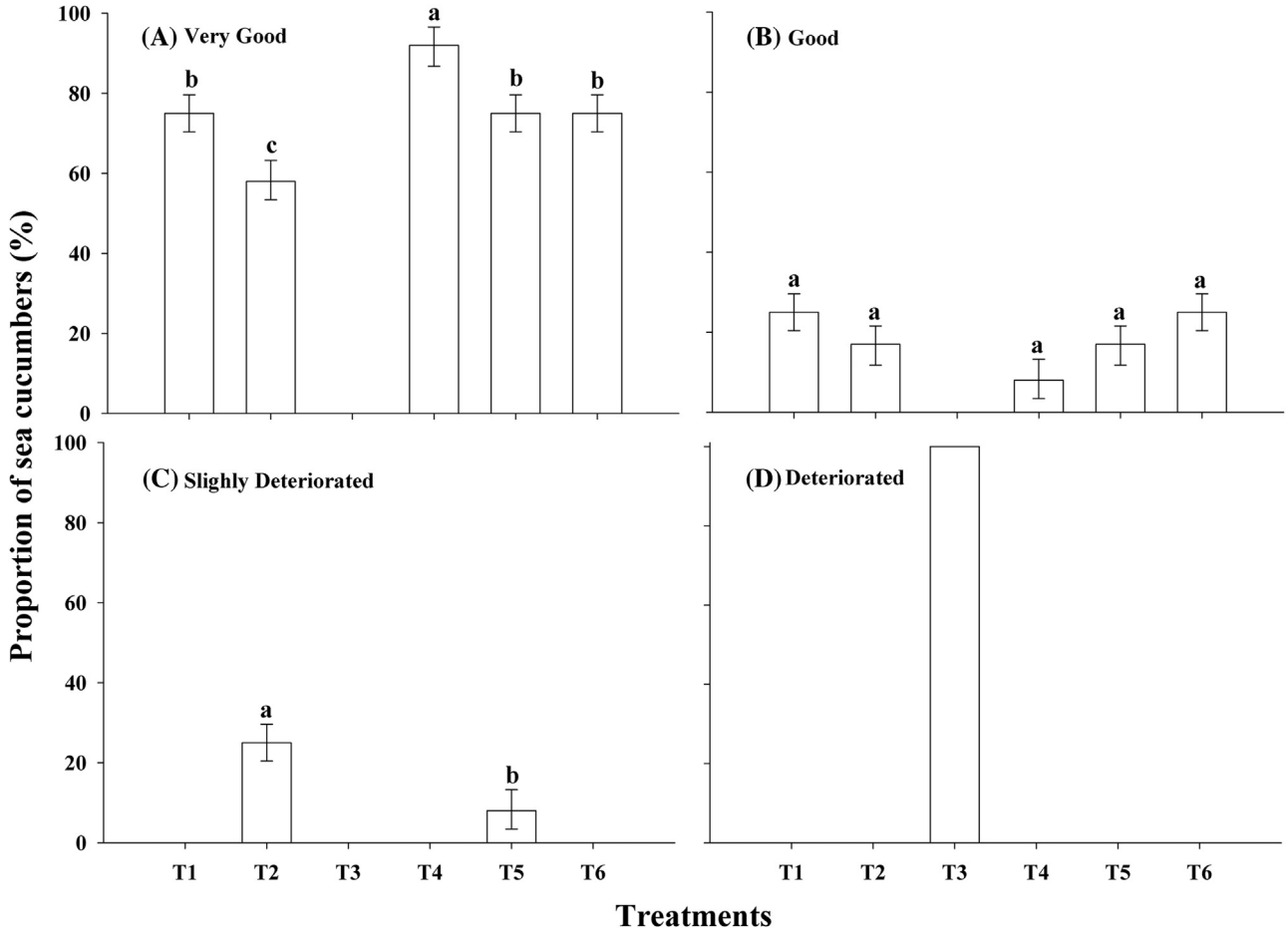

Fig. 1. Status of sea cucumbers after exposure to various storage treatments for 48 h.
Treatments consisted of seawater ice (T1), freshwater ice (T2), freshwater ice with fish salt (T3), iced seawater (T4), bagged freshwater ice (T5) and no medium (T6) as per Table 1. Sea cucumbers were scored as very good (VG), good (G), slightly deteriorated (SD) and deteriorated (D) as per Table 2. Data shown as mean ± se ($n = 3$). Means with different letters are significantly different (ANOVA on ranks, $\alpha = 0.05$). See Table S1 for full statistical results.

Treatments 5 and 6 also showed clear water at the end of the trial and no fish smell could be noticed. Big chunks of ice could still be found in the bags (60% melted) from treatment 5. The ammonia nitrogen level in theses tanks was the highest among all treatments at 4.0 ± 0.7 ppm in treatment 5 and 3.6 ± 0.8 ppm in treatment 6. Levels of dissolved oxygen were the second lowest among treatments at 2.2 ± 0.2 mg l^{-1} in treatment 5 and 2.5 ± 0.6 mg l^{-1} in treatment 6. Water pH values of 7.3 ± 0.1 in both treatments were the highest among all treatments. Salinity in these tanks ranged from 34.0 ± 2.8 in treatment 5 to 35.0 ± 0.7 in treatment 6 (Fig. 4).

Tanks from treatment 1 exhibited clear water with no strong fishy odour at the end of the storage period. Approximately 90% of the ice had melted. The concentration of ammonia nitrogen in tanks from treatment 1 was the second lowest (1.7 ± 0.1 ppm) among treatments. Dissolved oxygen was 4.4 ± 0.2 mg l^{-1}, pH was 7.1 ± 0.0 and salinity was 26.2 ± 1.0 (Fig. 4).

The water in the storage tanks from treatment 2 was also clear with no strong fishy odour. Approximately 90% of the ice had melted during storage. The concentration of ammonia nitrogen was 3.0 ± 0.4 ppm. Dissolved oxygen reached the highest concentration among all treatments at 7.0 ± 0.3 mg l^{-1} after storage. Water pH was 7.1 ± 0.0 and salinity 17.1 ± 1.8, which were the lowest values among the treatments (Fig. 4).

Tanks from treatment 3 exhibited a strong fishy odour and the color of the water was reddish (Fig. 2D). Mucus was also noticed on the surface of the water in such high concentration that it could stick to the fingers. The freshwater ice had melted completely during storage. The concentration of ammonia nitrogen (2.6 ± 0.4 ppm), dissolved oxygen (2.6 ± 0.4 mg l^{-1}) and the pH (6.8 ± 0.0) in tanks from treatment 3 were the lowest among all treatments. However, salinity (66.3 ± 2.2) measured in the water from the storage tanks was the highest among all treatments (Fig. 4).

Results of one-way ANOVAs showed that similar concentration of ammonia nitrogen was observed in treatments 1, 2 and 4 (Fig. 4). The concentration of ammonia nitrogen was significantly higher in treatments 5 and 6 than in the other treatments. Also, treatment 3 had lower ammonia nitrogen concentration when compared to the other treatments ($F_{5,12} = 12.06$, $p = <0.001$). Dissolved oxygen was significantly higher in treatments 2 and 4 than in the other treatments (Fig. 4). The lowest concentration of dissolved oxygen could be observed in treatment 3 ($F_{5,12} = 40.80$, $p < 0.001$). Water pH was similar in all treatments, except treatment 3 which was significantly lower than in the other ones ($F_{5,12} = 19.84$, $p < 0.001$). Salinity was statistically higher in treatment 3 then in the other treatments. Also, treatment 2 had significantly lower salinity when compared to the other treatments ($F_{5,12} = 22.19$, $p > 0.001$; Table S3).

3.3. Post storage monitoring

Survival rates immediately after the 48-h storage in the cold room was recorded as 100% for all treatments except for treatment 3, which resulted in massive mortality of sea cucumbers (Fig. 2D) and was, therefore, excluded from post-storage analysis.

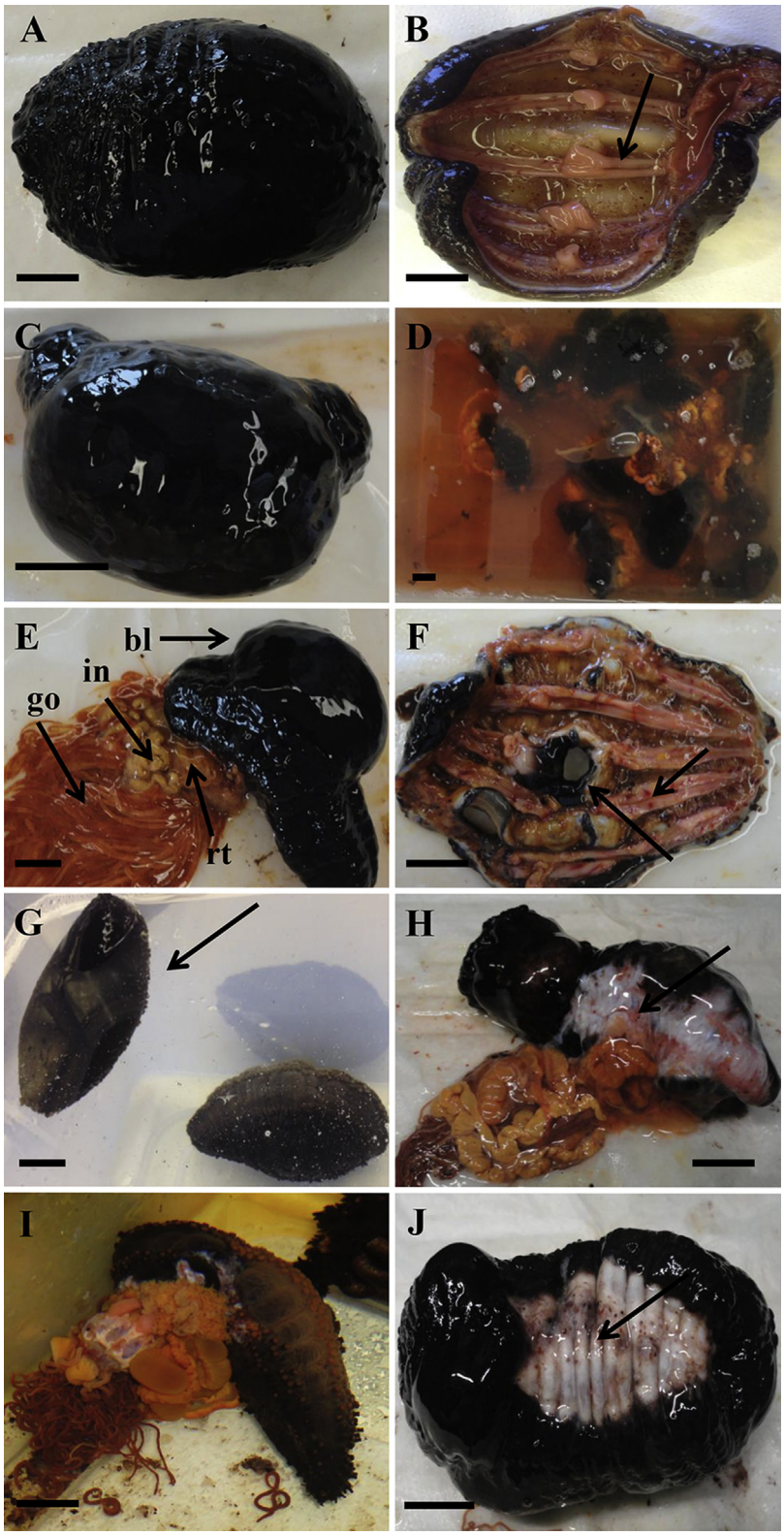

Fig. 2. Post storage condition of sea cucumbers stored in various media. (For interpretation of the references to color in the text, the reader is referred to the web version of this article.)
(A) External aspect of a sea cucumber in very good condition, showing firm body wall and no visible skin damage. (B) Internal view of sea cucumber in very good condition, showing healthy pinkish longitudinal muscle bands (arrow). (C) Slightly deteriorated sea cucumber showing blisters covering 20–50% of the total body surface. (D) Worst example of sea cucumbers scored as deteriorated (treatment 3). All individuals were eviscerated and dead/moribund (not responding to handling). The water was reddish, had a strong fish smell and was covered with mucus. (E) Example of a deteriorated sea cucumber, with a blister on the body wall (bl) and eviscerated gonad (go), intestine (in) and respiratory tree (rt). (F) Internal view of a deteriorated sea cucumber, showing tears in body wall and red spots on longitudinal muscle bands (arrows). (G) Some sea cucumbers stored with freshwater ice were floating (arrow) when transferred to the recovery tanks. (H) Skin necrosis and hole (arrow) in the sea cucumber's body wall resulting in evisceration of internal organs. (I) Dead and eviscerated sea cucumbers were recorded in treatments 1, 2, 5, and 6 during the recovery period. (J). Sea cucumber showing signs of skin necrosis (arrow). The outer dermis layer (epidermis) is shedding off, exposing the connective white tissues (arrow). Scale bars represent 2 cm. Treatments are defined in Table 1 and sea cucumber health conditions are defined in Table 2.

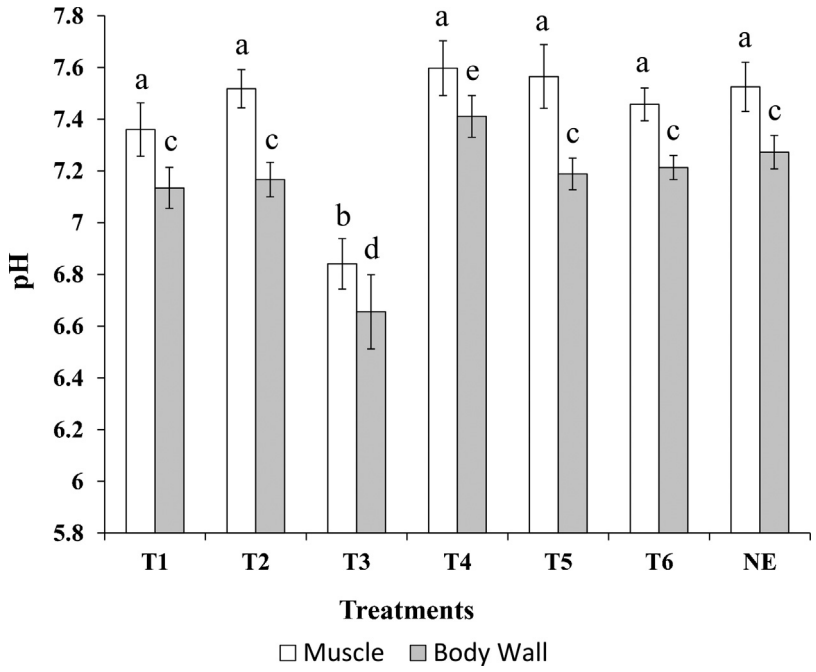

Fig. 3. Post-storage pH in muscle and body wall of sea cucumbers.
Treatments consisted of seawater ice (T1), freshwater ice (T2), freshwater ice with fish salt (T3), iced seawater (T4), bagged freshwater ice (T5) and no medium (T6) as per Table 1. NE refers to non-exposed control sea cucumbers sampled from holding tanks. Measurements were conducted immediately after 48-h storage. Data shown as mean ± se ($n = 3$). Means with different letters are significantly different (ANOVA, $\alpha = 0.05$). See Table S2 for full statistical results.

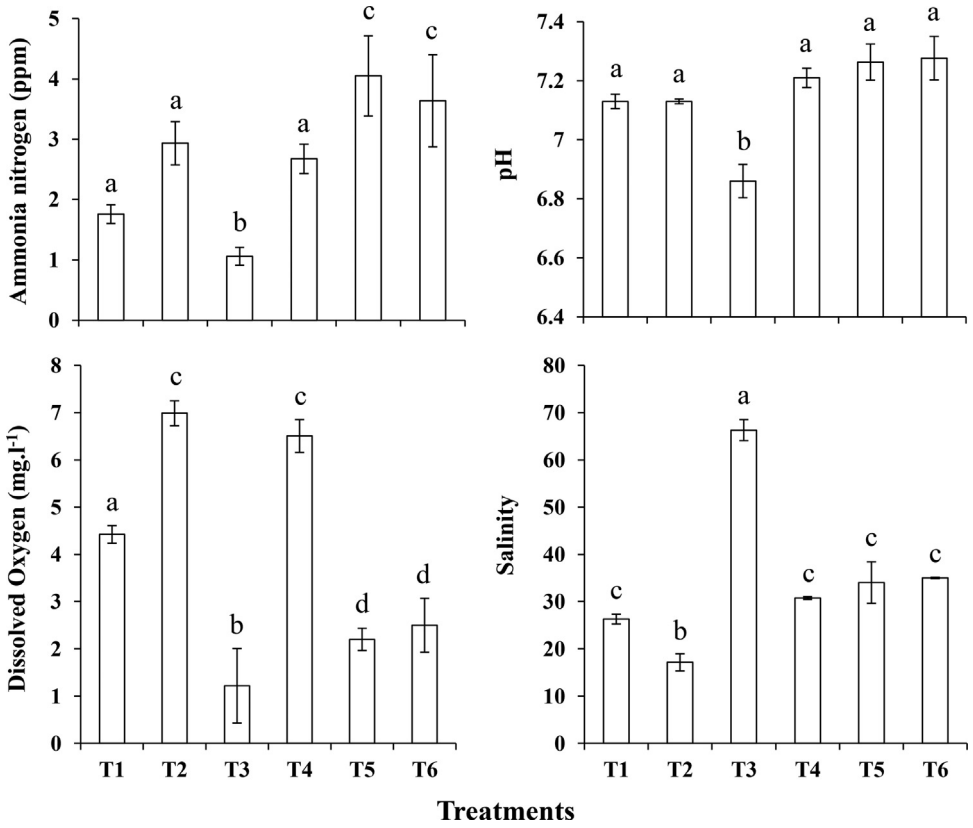

Fig. 4. Water quality in the tanks immediately after storage.
Treatments consisted of seawater ice (T1), freshwater ice (T2), freshwater ice with fish salt (T3), iced seawater (T4), bagged freshwater ice (T5) and no medium (T6) as per Table 1. Ammonia nitrogen, dissolved oxygen, pH and salinity were measured in water resulting either from the melted ice and/or water expelled from the respiratory tree of sea cucumbers in the storage tanks after 48 h. Data shown as mean ± se ($n = 3$). Means with different letters are significantly different (ANOVA, $\alpha = 0.05$). See table S3 for full statistical results.

Table 3
Survival rates (mean percent ± se) of sea cucumbers exposed to different storage treatments for 48 h. Treatments consisted of seawater ice (T1), freshwater ice (T2), iced seawater (T4), bagged freshwater ice (T5) and no medium (T6), as per Table 1. Survival rate was not assessed in T3 (freshwater iced with fish salt), because all sea cucumbers died after the initial storage period of 48 h. Means with different letters are significantly different (Logrank survival analysis, $\alpha = 0.05$). See Table S4 for full statistical results.

Days	Treatments				
	T1	T2	T4	T5	T6
0	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
10	78 ± 7^{b}	67 ± 13^{b}	100^{a}	89 ± 7^{b}	89 ± 7^{b}
20	67 ± 13^{b}	55 ± 18^{b}	100^{a}	67 ± 6^{b}	78 ± 5^{b}
30	44 ± 18^{b}	55 ± 7^{b}	100^{a}	55 ± 13^{b}	67 ± 18^{b}

When sea cucumbers were transferred to flow-through recovery tanks, the majority sank to the bottom of the tanks and became firmly attached to the bottom within 1 h. However, few individuals (17 ± 10%) from treatment 2 remained floating on the water surface and could not get attached to the tank walls or bottom (Fig. 2G). Attachment of these individuals was finally observed 48 h after they had been transferred to the tanks.

Treatment 4 exhibited the highest survival rate (100%) after the full 30 days. For all other treatments, survival rates decreased over time (Table 3). Treatment 4 also resulted in the lowest proportion of sea cucumbers (11 ± 6%) developing skin necrosis over 30 days (Fig. 5A). Moreover, this treatment yielded the highest overall proportion of sea cucumbers with tentacles fully deployed (91 ± 3%) during the recovery period of 30 days (Fig. 5B).

Treatment 6 showed the second highest survival rate during recovery. Survival decreased to 89% after 10 days and to 67% after 30 days (Table 3). This treatment also resulted in the second lowest proportion (22 ± 7%) of sea cucumbers with skin lesions (Fig. 5A) and the second highest proportion (71 ± 7%) of individuals with tentacles fully deployed during the recovery period (Fig. 5B).

Treatment 1 showed the lowest survival rate among treatments during the recovery period, at only 44 ± 18% (Table 3). The proportion of skin necrosis in individuals exposed to this treatment was high (47 ± 7%; Fig. 5A); the body wall and muscle bands were both affected and internal organs eviscerated (Fig. 2H). Treatment 1 also resulted in low proportion (44 ± 6%) of sea cucumbers with tentacles fully deployed during recovery (Fig. 5B).

Treatments 2 and 5 showed similar results for survival rates, development of skin lesions and tentacle deployment over the recovery phase. Treatment 2 had a survival rate of 67 ± 13% in the first 10 days, dropping to 55 ± 7% after 30 days. Survival for treatment 5 was 89 ± 7% in the first 10 days decreasing to 55 ± 13% after 30 days (Table 3). The proportion of sea cucumbers developing skin lesion during recovery was the highest for treatment 2, with 51 ± 6%, followed by 48 ± 8% for treatment 5 (Fig. 5A). The body wall (comprising epidermis and connective tissues) and the muscle bands were both affected. In those instances, the body wall and muscle bands were torn away, leaving a hole in through which internal organs (e.g., intestinal tract, gonads and respiratory tree) were eviscerated, resulting in the death of the sea cucumbers (Fig. 2H). Additionally, both treatments displayed the lowest proportion of sea cucumbers with tentacles deployed during recovery, at 33 ± 6% for treatment 2 and 32 ± 7% for treatment 5 (Fig. 5B).

Mortality of sea cucumbers (Fig. 2I) was always accompanied by evisceration of internal organs (e.g. gonad, intestine and respiratory tree) and was observed in all treatments except in treatment 4. Also, necrosis usually affected the outer layer of the dermis (epidermis) exposing the white subepidermal connective tissue (Fig. 2J).

Statistical analyses showed that survival rates were significantly higher in treatment 4 than in all other treatments over the entire recovery period of 30 days ($\chi^2 = 11.48$, df = 4, $p < 0.001$; Table S4).

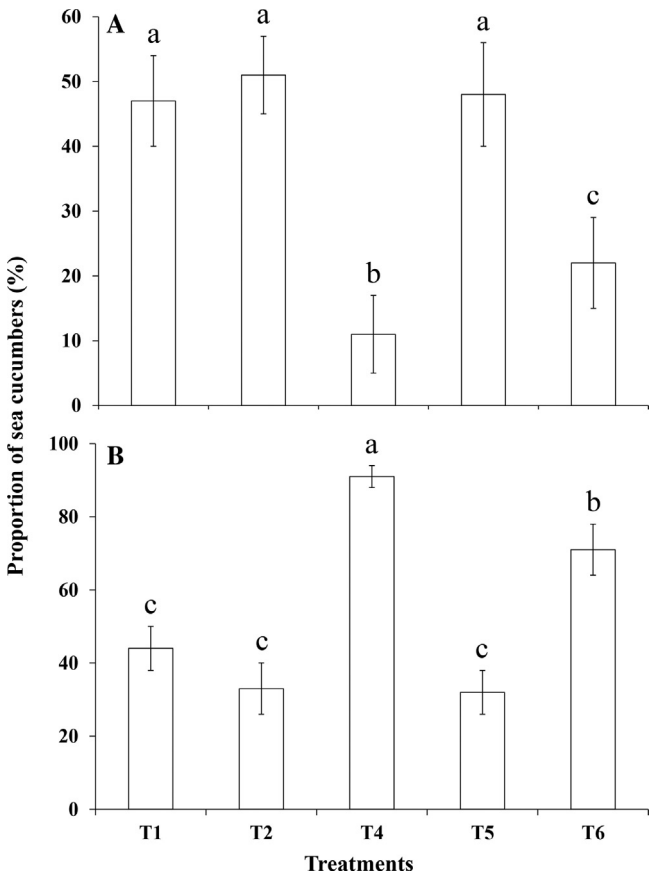

Fig. 5. Proportion of sea cucumbers with skin necrosis and tentacle deployment during recovery period of 30 days.
(A) Development of skin necrosis and (B) tentacle deployment. Treatments consisted of seawater ice (T1), freshwater ice (T2), freshwater ice with fish salt (T3), iced seawater (T4), bagged freshwater ice (T5) and no medium (T6) as per Table 1. Data shown as mean ± se ($n = 3$). Means with different letters are significantly different. Skin necrosis data was analyzed by ANOVA on ranks ($\alpha = 0.05$) and tentacle deployment by ANOVA ($\alpha = 0.05$). See Tables S5 and S6 for full statistical results.

Treatment 4 also had significantly lower proportions of sea cucumbers with skin necrosis ($H = 18.32$, df = 4, $p < 0.001$; Table S5). In addition, the proportion of sea cucumbers with skin lesions in treatments 1, 2 and 5 was significantly higher than in the other treatments (Fig. 5A). The results of one-way ANOVA revealed that treatment 4 had the highest proportion of sea cucumbers with tentacles fully deployed when compared to the other treatments ($F_{4,86} = 133.04$, $p < 0.11$; Fig. 5B). The proportion of sea cucumbers with tentacles deployed was significantly higher in treatment 6 than in treatments 1, 2 and 5, with no significant differences among the latter (Table S6).

4. Discussion

Live storage of sea cucumbers is a challenge due to their soft unprotected body wall and ability to autolyze when they are stressed or taken out of seawater (Duan et al., 2010; Zheng et al., 2012). Unlike fish and crustaceans, on which industry standards are largely based, sea cucumbers are not protected by any scales or hard exoskeleton that would prevent contact with ice, salt or any other media during storage. Deterioration of the body wall and underlying muscles, which together constitute the chief marketable sea cucumber products, will likely translate into commercial products of a lower grade. As fisheries of *C. frondosa* expand in the North Atlantic in order to supply Asian countries, optimal methods for

their live storage and transport on the way to the processing line will become crucial to the production of high-quality end products.

Among the media tested here, iced seawater was the most effective for live storage of *C. frondosa*, yielding 100% survival and largely intact body wall and muscles. This mix of cold seawater and freshwater ice maintained conditions of salinity, pH, ammonia and dissolved oxygen in the storage tanks within acceptable ranges, consistent with the seawater characteristically found in the natural habitat of *C. frondosa*. The storage temperature was also within the range experienced by this species in nature (Hamel and Mercier, 1996). The use of iced seawater minimized direct contact between ice and the body wall, avoiding the burns observed in the treatments where ice was applied directly over the sea cucumbers. Also, iced seawater surrounded the sea cucumbers completely, buffering them against changes in environmental conditions and preventing exposure to air, which is known to cause stress in sea cucumbers (Duan et al., 2010; Zang et al., 2012). This medium offered no jagged or sharp edges, which can inflict injuries to the body wall. Overall, iced seawater offered a clean, cold and oxygenated storage medium, which decreased stress on the sea cucumbers compared to conditions that favoured contact with air, ice and/or salt.

Salinity is known to be one of the principal factors driving physiological responses in marine organisms. As sea cucumbers lack an osmoregulatory mechanism, their coelomic fluid remains isosmotic with the environment (Yu et al., 2013). Nevertheless, *C. frondosa* can be found in a variety of habitats with a wide range of salinities, from maritime estuaries to oceanic waters (Hamel and Mercier, 1996). The salinity of 29 measured in the storage tanks with iced seawater falls inside the range where extensive populations of *C. frondosa* occur, such as the St. Lawrence Estuary, in eastern Canada (Hamel and Mercier, 1996).

Another key driver of marine animal physiology is dissolved oxygen (DO). Values of DO measured were relatively high in the water surrounding the sea cucumbers after 48 h of storage in iced seawater. These high values evoke low sea cucumber metabolism and minimal release of ammonia during storage. Ammonia is known to be highly toxic for marine organisms (Burgess, 2000; Wang et al., 2014), especially at high pH, where skin ulcers, necrosis and deaths can occur, as observed in fish (El-Shafai et al., 2004). It was also shown that an increase in water temperature resulted in greater oxygen consumption and ammonia excretion rates in the temperate sea cucumber *A. japonicus* (Yang et al., 2006).

Although treatments with freshwater ice, bagged ice and no medium also yielded high proportions of sea cucumbers exhibiting very good condition immediately after storage, they all resulted in post-storage skin lesions, eviscerations and mortalities, indicating that these storage methods were less ideal (or suboptimum) for the transport of live sea cucumbers when compared to iced seawater. Frequent development of skin necrosis and low survival during the recovery period might be explained by direct skin contact with the frozen media, which induced the development of blisters and frostbites. The dermis of sea cucumbers appears to be much more sensitive to ice than that of fish or shellfish, which is protected by scales and an exoskeleton, respectively. It is more reminiscent of mammal skin, in which response to contact with a low-temperature surface varies from skin necrosis, which can also lead to inflammatory wounds, to tissue destruction as a result of progressive failure of the microcirculation in the affected area (Gage and Baust, 1998). Moreover, the fact that sea cucumbers stored in freshwater ice had more difficulty initially attaching to the substrate during the recovery period might be due to freshwater from the melted ice somehow impeding attachment. This effect was not permanent and eventually dissipated.

Sea cucumbers stored with either bagged ice or without any medium suffered mainly from exposure to low DO levels and high concentrations of ammonia nitrogen measured in the product water found in the tanks after storage. This water emanated from their respiratory trees and intestines; it therefore contained metabolic wastes. Because there was no medium in contact with the sea cucumbers in these treatments, the wastes were not diluted, resulting in low DO levels and high levels of ammonia. As previously discussed, high concentrations of ammonia can elicit skin ulcers and necrosis (El-Shafai et al., 2004) and might be the reason for the rapid development of skin lesion and mortalities during the recovery period in individuals stored without any medium or with bagged ice.

Of all the treatments, freshwater ice with added fish salt proved to be the most inefficient to store live sea cucumbers. All sea cucumbers were found dead and eviscerated after the storage period of 48 h. The added fish salt increased the salinity in these tanks to very high values that may have triggered evisceration. Unlike certain tropical sea cucumbers, *C. frondosa* does not eviscerate to deter predators and is not known to readily regenerate viscera, making this process a lethal outcome of stress (J.-F. Hamel, unpublished data).

Apart from effects on the health and skin condition of sea cucumbers immediately after storage, the type of storage medium also influenced meat quality. Irrespective of external appearance and health, individuals stored in seawater ice, freshwater ice, iced seawater, bagged ice and without any medium all exhibited firm and pinkish muscle bands and an overall firm body wall, comparable to those of non-exposed sea cucumbers. In contrast, sea cucumbers stored in freshwater ice with fish salt exhibited very soft tissues, which were easily torn during sampling, likely a result of autolysis, which is associated with degradation of abdominal tissue and muscle softening in many marine organisms (Ando et al., 1999; Sun et al., 2013; Zheng et al., 2012). The firmness of fresh muscle is directly correlated with the amount of collagen in the tissue; intestinal proteases can degrade collagen leading to muscle softening (Ando et al., 1999). Sea cucumbers stored with freshwater ice and fish salt presented an unpleasant appearance and a pronounced smell. In addition, the red spots observed in the longitudinal muscle bands of these sea cucumbers can be associated with lipid oxidation and protein breakdown (Fu et al., 2005; Wu et al., 2013).

The lower pH measured in tissue emulsions following exposure to freshwater ice with fish salt can be the result of *post mortem* bacterial and chemical degradation when compared to values measured in individuals stored in iced seawater and in non-exposed sea cucumbers. Although there is no documentation of pH in the muscle bands or body wall of sea cucumbers, it was expected that sea cucumbers classified under deteriorated condition (D) would exhibit lower tissue pH, as acidity is typically associated with softening of tissues in fish (Dunajski, 1980; Martinez and Gildberg, 1988). It was also initially presumed that pH associated with the body wall of *C. frondosa* would be higher than the pH associated with the muscle bands, due to the presence of calcareous ossicles in the body wall. However, the emulsions of muscle had a higher pH than those of body wall. Measurement of pH in fresh sea cucumber tissues (meat) should be investigated further, as it might serve as an indicator of integrity and quality after live storage.

5. Conclusion

Taken together, the results of the present study indicate that storage in iced seawater generates the best overall health conditions, survival rates, and meat quality. This medium should therefore be favoured for transporting and storing the sea cucumbers before processing. While the method differs from the current industry standard used with *C. frondosa* along the eastern coast of Canada, the modification proposed should not involve any major

financial cost. In the end, as the organoleptic properties of the body wall account for most of the commercial value of sea cucumber, this adjustment could help producers obtain higher prices. In addition, it would be greatly beneficial for preserving the quality of the meat, especially since fresh frozen muscle bands are the second most valuable product derived from *C. frondosa*. Overall, the findings presented here provide important information about the live storage of sea cucumbers that will hopefully help stakeholders optimize the quality of end products in countries that commercialize *C. frondosa* and other temperate or cold-water sea cucumbers.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fishres.2015.11.004.

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