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Response of cultured *Holothuria atra* to thermal and salinity stressors: growth, survival rates, and physiochemical studies

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ABSTRACT

This study investigated the response of cultured *Holothuria atra*, a sea cucumber species inhabiting the Red Sea's Egyptian coast off Hurghada City, to thermal and salinity stressors. It aimed to identify the growth, survival rates, and physiochemical adaptations of *H. atra* to understand their potential for aquaculture in the region. The study used a range of experimental manipulations to expose the species to different temperature and salinity conditions, measuring key physiological responses such as respiration rate, execration rate, and growth rate. The results suggested that thermal and salinity stressors significantly impacted *H. atra*, with growth rates decreasing and physiological function disrupted under extreme conditions. As revealed, temperature and salinity variations harm the *H. atra* growth as it showed a noticeable decline under any deviation from their typical temperature and salinity conditions. The species also exhibited some adaptive responses, i.e., evisceration, regeneration (autotomy), and survivorship. Overall, the study offers important insights into the potential for sea cucumber aquaculture in the Red Sea, highlighting the need for careful management of environmental conditions to maintain optimal health and growth.

INTRODUCTION

Holothuroids play an important role in conservation as they are considered vital members of the benthic communities and important in nutrient cycling and sediment turnover. They have a high economic value and are used as food. They are also useful in treating weakness, impotence, elderly debility, constipation caused by intestinal dryness, and excessive urination (**Abdel-Razek *et al*., 2005**). The dried sea cucumber (beche-de-mer) has a high commercial value and is in great demand from China since it is believed to have aphrodisiac and therapeutic powers (**Chen, 2004**). The sea cucumber sandfish is among the most common species along the Egyptian Red Sea coast (**Ahmed and Lawrence, 2007**). *Holothuria atra* is a third-class commercial species exploited in Egypt as part of the Bech-de-mer trade since 2002. *H. atra* had the relatively highest density of echinoderms in the littoral zone of the Red Sea Coast of Egypt (**Mahdy *et al*., 2018**). Most commercial sea cucumber species live in shallow clear areas of sandy reef flats or seagrass beds; harvesting these sessile animals is relatively simple (**Ahmed, 2015**). The commercial holothurian species were generally found in the depth range of 5-10 m off the Egyptian shore of the Red Sea (**Lawrence *et al*., 2009**). Sea cucumber harvesting in the Red Sea involves two processes; trawlers that use benthic trawl nets to catch sea cucumbers in the Gulf of Suez and the Mediterranean. It was initially caught as a bycatch; they were specifically targeted and subjected to heavy trawling activity. The second process is SCUBA diving, which collects animals in the central Red Sea and the Gulf of Aqaba. Furthermore, several species are picked by hand on the reef flats at low tide. (**Lawrence *et al*., 2004**). By 2000, the fishery had grown significantly, raising concerns about overfishing. Several years later, many areas of the Red Sea experienced severe exhaustion of sea cucumber populations (**Lawrence *et al*., 2004**). Fishing pressure is increasing primarily due to increased export demand to China. This ongoing overfishing will substantially impact the overall ecosystem and marine environment (**Conand, 2004**). Overfishing and unsustainable harvesting practices have led to a decline in the population of sea cucumbers in the Red Sea, including *Holothuridae atra*. However, this depletion occurred in all sea cucumber species, regardless of their economic value (**Hasan, 2019**). Local authorities and conservation organizations have implemented measures to regulate fishing and promote sustainable harvesting practices to address this issue. Hence, restoration projects, in addition to standard policies and firm punishment for illegal fishermen, may be the only way to restore these species to their original habitat. As a result, sustainable sea cucumber production is critical for the conservation of species in the Red Sea and the industry's long-term viability. It is noteworthy that sea cucumber was not previously regarded as a cultural species. (**Ahmed & Lawrence, 2007**), Nevertheless, the current state of sea cucumber fishing makes this activity more important than ever. They can tolerate their surroundings well and are suitable for co-culture or multi-trophic culture with other species. Moreover, like most invertebrates, they can reuse metabolic waste from other organisms and convert it into usable nutrients (**Purcell, 2004**). However, several sandfish farming attempts have been made in ponds and coastal areas, but the results were disappointing (**Tuwo, 2004**). Aquaculture success is fundamentally dependent on the ecology of the aquatic environment, which is a major concern given the far-reaching effects of climate change **(IPCC, 2007**). According to Bell *et al*. (**2013**), climate change will result in a 30% decrease in tropical Pacific coastal aquaculture production by the end of the century. For the current case, sea cucumbers in the Red Sea are adapted to high salinity and temperature. They have mechanisms to regulate their internal osmotic pressure and maintain their body fluids in a hypertonic state to the surrounding seawater (**Al-Yaqout *et al*., 2021**). Sea cucumbers also have a high tolerance to temperature fluctuations, which allows them to survive in the warm waters of the Red Sea. However, The Red Sea is particularly vulnerable to the effects of climate change, including rising sea temperatures and changes in salinity levels. These changes can significantly impact the marine ecosystem, including sea cucumbers. Changes in salinity and temperature can still impact sea cucumber populations. As sea temperatures rise, sea cucumbers may become stressed, leading to decreased growth rates and increased susceptibility to disease. Additionally, changes in salinity levels can affect the ability of sea cucumbers to maintain proper osmotic balance, further impacting their health and survival (**González-Durán *et al*., 2021**). Moreover, extreme events like El Niño can cause significant changes in the Red Sea environment, leading to mass mortality of sea cucumbers (**Kühnhold, 2017**). In addition, previous research has recognized temperature and salinity as two of the dominant extrinsic factors influencing the physiology of marine invertebrates (**Taylor *et al*., 2016; Kang *et al*., 2015**). Hence, controlling water quality in aquaculture, i.e., temperature, salinity, and ammonia, is crucial for the growth and survival of sea cucumbers, as it is a key abiotic factor that affects their well-being. Regular monitoring is necessary to ensure that water quality meets the needs of these organisms, as thermal and osmotic stress occurs when water temperature or salinity exceed their ideal ranges, altering normal physiological functioning and initiating energy-intensive stress responses. (**Aknaf *et al*., 2017; Romin *et al*., 2021**).

For instance, nitrogen, the main component of protein, is taken up by the individual during their metabolic processes and excreted as ammonium (NH_4^+), the primary nitrogenous waste in the water. This nitrogen is typically discharged within 24 hours (**Ip and Chew, 2018**). Ammonia also exists in the unionized NH_3 form (non-dissociated) and the NH_4^+ ion (ionic form). The proportion of these forms in water depends on pH and temperature (**Svobodova *et al*., 2017**). In addition to ammonium, there are two other nitrogen compounds: nitrite (NO_2^-) and nitrate (NO_3^-). Nitrite is produced through the oxidation metabolism of ammonia and accumulates when there is an imbalance in nitrifying bacterial activity (**Yun *et al*., 2019**). High nitrate levels can lead to nitrite accumulation in the blood plasma, affecting physiological functions such as oxygen capacity, immune system health, physiological metabolism, and endocrine regulation (**Ha *et al*., 2019**).

Hence, this study aimed to investigate the impact of temperature and salinity on sea cucumbers to determine the optimal thermal range, delimiting temperature levels for best aquaculture practices, and predict the susceptibility of *H. atra* to future global warming scenarios. The metabolic rate was also measured to assess physiological stress by examining growth rates, survival, oxygen consumption, and ammonia excretion. The study aimed to establish a basic framework for physiological research and evaluate sea cucumbers' growth and survival under different treatment conditions.

MATERIALS AND METHODS

Sampling collection and acclimation

The research was conducted from August 2022 to October 2022. A total of 65 sub-adult *Holothuria atra* samples of average weight 110.2 gm and length 13.5 cm were collected manually from the intertidal zone at a depth of 1-5 m by scuba diving. Off the northern Red Sea coast, in front of the National Institute of Oceanography and Fisheries (NIOF) branch. The sampling site is approximately 5 kilometers north of Hurghada City, at 27° 17' 07'' N latitude and 33° 46' 30'' E longitude (Figure 1).

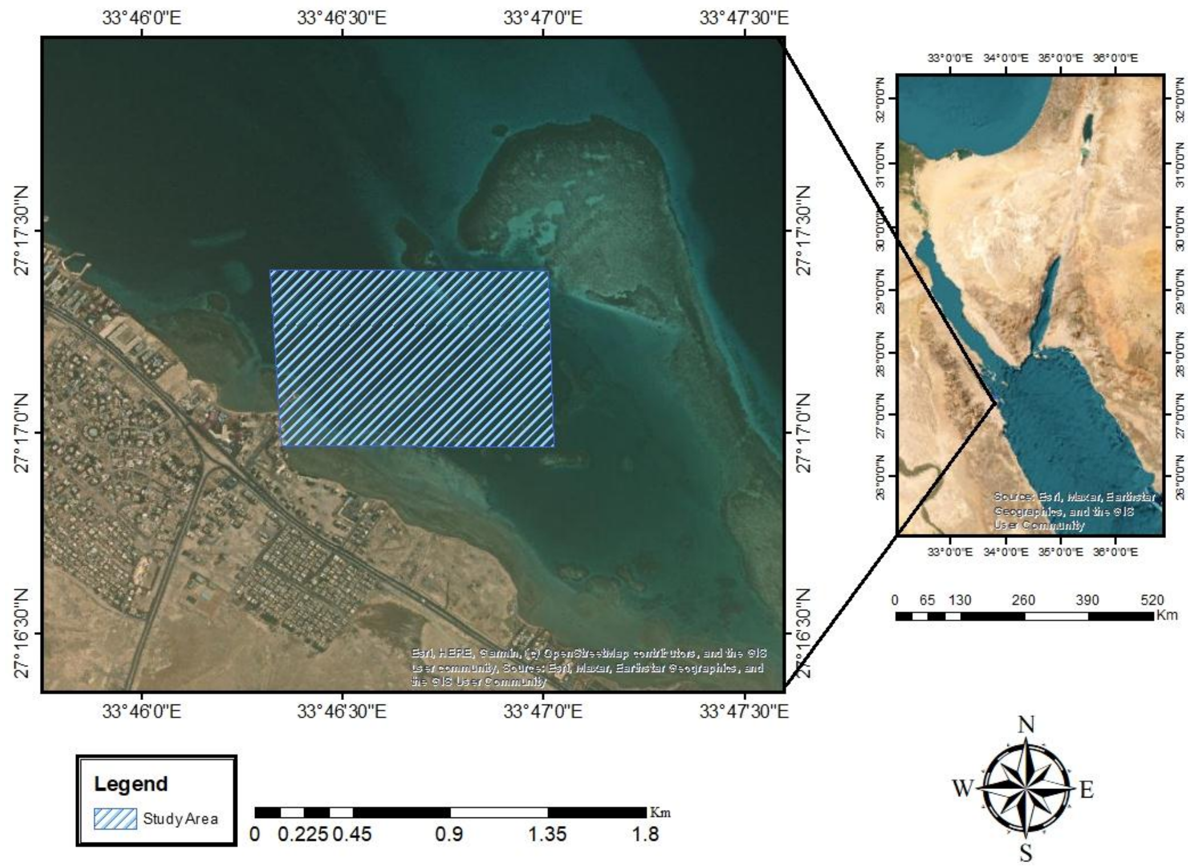

Fig. (1): Sampling location off the NIOF in Hurgahda, Red Sea.

Experimental design

Handling of the *Holothuria atra* samples was done under observation and after agreement of the NIOF Committee for Ethical Care of Marine Organisms and Experimental Animals (NIOF- IACUC) (2023/No: AQ2I23R019) upon arrival of samples to the lab. It has transferred to tanks. Furthermore, following a 24-hour fast, the external water was removed from the specimens by drying them with a sponge. Specimens were weighed within one minute of being removed from the seawater (Battaglene *et al*., 1999; Dong *et al*., 2006). Individual weights were recorded (to the nearest 0.01 gram) after gentle pressure was applied to the anterior region of each individual to remove the water content from the respiratory trees (Sewell, 1990). The experiment used sea cucumbers with similar wet weights of 110 gm to ignore the weight difference factor. Samples were divided into 13 groups, each of 5 individuals in 100 L glass tanks; one group was used as control, three groups were exposed to high salinity stress (H.S.), three groups to low salinity stress (L.S.), three groups to high-temperature stress (H.T.), and three to low-temperature stress (L.T.). All samples were left for one week for acclimation before starting the experiment.

During the acclimation period, normal water parameters were applied to all the tanks, the average temperature was 26.00 ± 0.82 °C, the average salinity was 40.00 ± 0.78 PSU, the average pH was 7.50 ± 0.54, and the average dissolved oxygen was 5.00 ± 0.35 mg/L in all tanks of the experiment.

For high salinity tanks, the normal water evaporation raised the salinity level gradually every day, and the water did not change till reaching the maximum salinity of 46.00 ± 1.26 PSU. To reach the desired low salinity level, a daily 10 % of the water tanks were replaced by a mixture of fresh and saltwater till reaching 33.00 ± 0.45 PSU at 26.00 ± 0.82 °C. 150-watt water heaters were used in high-temperature tanks to reach 30±0.16°C, and freshwater was added daily to prevent rising salinity by the act of the water temperature that might happen by heaters application in these tanks. Ice packs were replaced twice daily in low-temperature tanks to keep the tanks in a low-temperature state at 20.00 ± 0.71°C. Every two days, the water in each tank was replenished. Any excess food and excretory pellets were removed by siphoning at this time. Care was taken during sampling to avoid stress in these animals. In control tanks, the average temperature was 26.00 ± 0.82 °C, and the average salinity was 40.00 ±0.78 PSU were used.

Feeding plan

Both control and treatment tanks will be fed with *Horomphys sp.* algae collected from the same sampling site of *H. atra*; the algae were dried in the lab. Furthermore, using spice and herb grinder Model HR-16B, the feeding amount was calculated as 2% of body weight (Broom *et al*., 2021). They will be fed during the day at 10:00 a.m. The diet was soaked for 12 hours in water before feeding.

Survival rate

The survival rate was calculated with the following formula (Effendie, 1997):

$$SR = \frac{\mathrm{Nt}}{\mathrm{N0}}\, x\ 100$$

SR = Survival rate (%), N_o = Initial number of animals, and N_t = Final number of animals.

Water quality measurements

To estimate the number of factors, the physicochemical parameters of water samples using the Multi-function environmental sensor Model (Hana, HI98194) were measured daily; temperature (ºC), pH, salinity (‰), total dissolved solids (TDS) mg/l and dissolved oxygen (DO) mg/l after calibration during the duration of the experiment. The dissolved oxygen (DO) levels were determined using the modified Winkler method, while the spectrophotometer Jenway UV-VIS model 6800 was used to measure nutrient concentrations using standard methods (APHA, 2012). During analysis, Synthetic samples and reference materials of different nutrient salts were used to obtain the calibration curve and precision and accuracy as quality control tools.

Specific growth rate (SGR)

The specific growth rate was the percentage increase in individual weight that could be calculated using the following equation (Huisman, 1987):

$$SGR = \frac{\ln Wt - \ln W0}{\mathrm{t}}\ x\ 100$$

SGR = Specific growth rate (%/day), W_o = sea cucumber weight on initial rearing (g), W_t = Sea cucumber on t-rearing period (g), t = Rearing period.

Ingestion Rate

Following acclimation, food and water were completely changed, and the sea cucumbers were fed as previously stated. Fecal samples were carefully siphoned from the bottom of each tank every 6 hours for 24 hours. To eliminate excess salt, the fecal samples were rinsed with deionized water and then dried at 60°C until they reached a consistent weight. To analyze inorganic matter content (ash), the samples from each tank were combined into a single sample and milled into a fine powder. The dry samples were burned in a muffle furnace at 550°C for 6 hours to estimate the percentage of inorganic matter content by dry weight, and the remaining sample weight after combustion was utilized to calculate the percentage of inorganic matter content. The ingestion rate (IR, mg $g^{-1}h^{-1}$) of the sea cucumbers can be calculated from its defecation rate (DR, mg $g^{-1}h^{-1}$) using equations (1) and (2) (Yuan *et al*., 2010).

$$\text{DR (mg g}^{-1}\text{h}^{-1}\text{) = F/W/t} \qquad (1)$$

$$\text{IR (mg g}^{-1}\text{h}^{-1}\text{)} = \frac{DRXIMf}{IMs} \qquad (2)$$

Where F is the feces' dry weight (g), W is the wet weight of the sea cucumber (g), t is the duration of experiment (d), DR is the defecation rate (mg $g^{-1}d^{-1}$), IM_f is the inorganic matter content of the feces (%), IMs is the content of inorganic matter in the sediment from the natural habitat (%).

Oxygen Consumption and Ammonium Excretion Rates

To evaluate sea cucumbers' oxygen consumption and ammonium excretion rates, a fasting period of 24 hours was implemented to ensure the emptying of their digestive tracts. Each experimental sea cucumber was placed in a 3-liter brown jar and then sealed underwater to eliminate air bubbles. Concurrently, three control jars without animals were prepared. After incubating for 3-4 hours and ensuring that the oxygen levels in the experimental jars reached at least 80% of the control jars, the sea cucumbers were removed, and the water inside the jars was gently stirred. The oxygen concentration was promptly measured using an MPS meter (Hana, HI981946) within 10 seconds, and water samples were collected for ammonium concentration analysis following the method described in Grasshoff *et al*. (1983). All experiments were conducted between 8:00 a.m. and 12:00 p.m. At the end of the experiment, the sea cucumbers were drained, weighed, and their body volumes were determined using the water displacement method. The oxygen consumption rate (OCR, mgO_2/g/h), ammonium excretion rate (AER, μg/(g·h), and O/N rate were computed in atomic equivalents by dividing the quantity of oxygen ingested by the amount of nitrogen excreted using Equations (3), (4) and (5) (Sabourin & Stickle, 1981; Yang et al., 2006).

$$\text{OCR} = \frac{(\mathrm{DO0}-\mathrm{DOt})V}{WT} \qquad (3)$$

$$\text{AER} = \frac{(Nt-N0)V}{WT} \qquad (4)$$

$$O/N = (OCR/16)/\ (AER/17) \qquad (5)$$

Where the initial and final concentrations of dissolved oxygen (DO) ($mgO_2\ g^{-1}$) and ammonia ($\mu g\ g^{-1}$) are represented by subscripts 0 and t, respectively, the volume of the respiration jar is denoted as V (L), the dry weight of the sea cucumber as W (g), and the time between the initial and final measurements as T (h).

RESULTS

Abnormal symptoms

The evisceration of internal organs (autotomy) (Fig. 2) was noticed during the period of the experiment at 13.3% of all samples in each of the high-salinity, low-salinity, and high-temperature tanks; the eviscerated samples survived and regenerated during the period of the experiment with no mortalities noticed in these samples. Tanks exposed to low temperature and control tanks showed no evisceration.

Fig. (2): Evisceration of internal organs (autotomy) in *H. atra*.

Survival rate

All samples used in the experiment survived till the last day (100%) except in the high-salinity tanks, which had a survival rate of 93.30%.

Physico-chemical parameters measurements

Water temperature, pH, TDS, salinity, conductivity, dissolved oxygen (DO), saturated dissolved oxygen (% DO), and nitrogen compound were recorded at different values depending on the treatments used in the experiment. All the Physicochemical parameters measured throughout the experiments were summarized in Table (1). The water temperature ranged from (20.00 ± 0.71 to 30.00 ± 0.16) °C while salinity ranged from (33.00 ± 0.45 to 46.00 ± 1.26) PSU. DO and % DO varied between (4.96 ± 0.57 - 5.37 ± 0.20) mgO_2/L and (59.04 ± 2.37 - 66.20 ± 0.75) respectively. It was noticed that pH and

DO values showed slight variations within the temperature and salinity change. However, TDS and conductivity showed a considerable change within each treatment. The pH ranged from (7.15 ± 0.22 to 7.52± 0.54) in high salinity and control, respectively. However, DO recorded the highest values (5.37 ± 0.20 and 5.37 ± 0.24) mgO_2/L in both low salinity and low temperature, respectively. It recorded the lowest value under high-temperature treatments (4.96 ± 0.57). However, % DO recorded the highest value in low salinity treatments and lowest in the low temp treatment.

Table (1): Physical parameters in each treatment tank.

	Control	H.S.	L.S.	H.T.	L.T.	Quality standard values
Water Temperature (ºC)	26.00±0.82	26.12±0.45	26.42±0.33	30.00±0.16	20.00±0.71	24.00 – 30.0 0 27.00 – 29.00 Environmental condition
pH	7.52± 0.54	7.15± 0.22	7.41± 0.12	7.49± 0.08	7.48± 0.06	6.90 - 8.50 7.50 – 8.50
TDS (mg/l)	24.46 ± 0.11	28.67 ± 0.43	21.33 ± 0.07	30.21 ± 3.94	25.28 ± 1.40	
Salinity (PSU)	40.00 ± 0.78	46.00 ± 1.26	33.00 ± 0.45	40.80 ± 0.50	40.78 ± 0.42	32.00 – 35.00
Conductivity (µS/cm)	48.92 ± 0.21	57.34 ± 0.88	42.66 ± 0.14	56.81 ± 0.90	51.15 ± 1.97	
DO (mg/l)	5.15 ± 0.35	5.21 ± 0.37	5.37 ± 0.20	4.96 ± 0.57	5.37 ± 0.24	> 5.00
Saturated DO (%)	63.49 ± 0.76	64.23 ± 3.08	66.20 ± 0.75	65.65 ± 4.53	59.04 ± 2.37	

Specific growth rate

Specific growth rate (SGR) showed variations for *H. atra* groups inhabiting different treatments (i.e., Low temp, Low salinity, High temperature, High salinity) (Fig. 3). It was noticed that high salinity and temperature treatments accompanied by a sharp decline in the SGR. However, the decrease in the SGR in specimens under low temp and salinity was somewhat moderate compared to other treatments. The highest SGR was recorded for specimens under control conditions (0.30±0.1 %). All *H. atra* under other treatments showed negative SGR, which ranged from -1.69 ± 0.74 to -0.82 ± 0.57 %. The lowest was recorded for specimens under high salinity treatment (-1.69 ± 0.74 %), followed by specimens under high temperature, which revealed a low SGR (-1.44 ± 0.79 %). The specimens under low salinity conditions showed a high SGR (-0.82±0.57 %). The low-temperature Treatment exhibited a relatively high SGR compared to other treatments.

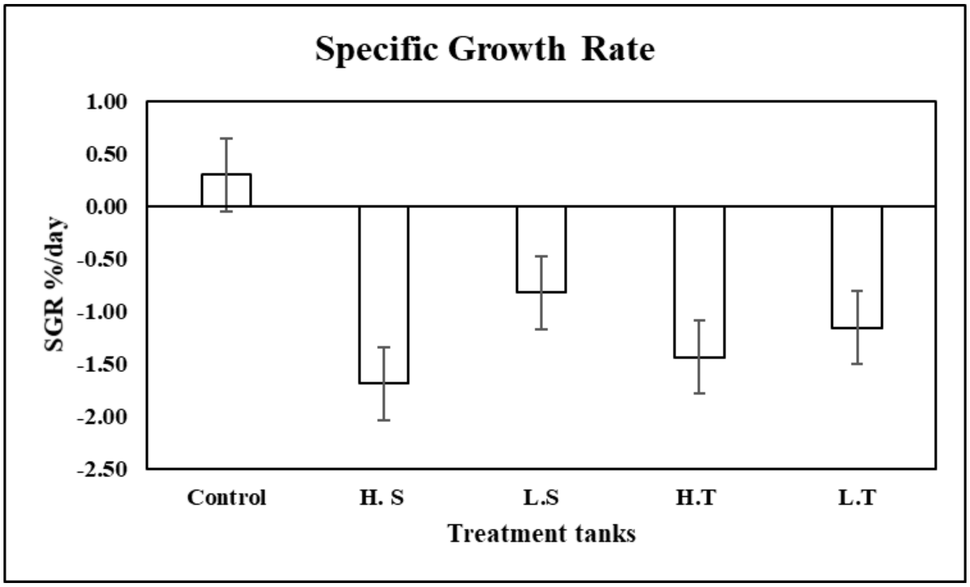

Fig. (3): Specific growth rate of *H. atra* groups inhabiting different conditions.

Ingestion rate

Throughout the 24-hour ingestion rate (IR) experiment, fecal production was observed in all treatments. Compared to the control, the IR increased under all tested treatments. It was recorded as 0.129 mg g-^{1}h$^{-1.}$ However, rather than tested treatments between 0.146 to 0.160 mg g-^{1}h^{-1}, there was a slight variation among different treatments; however, the highest IR was noticed under high temp conditions and the lowest under high salinity (Fig. 4).

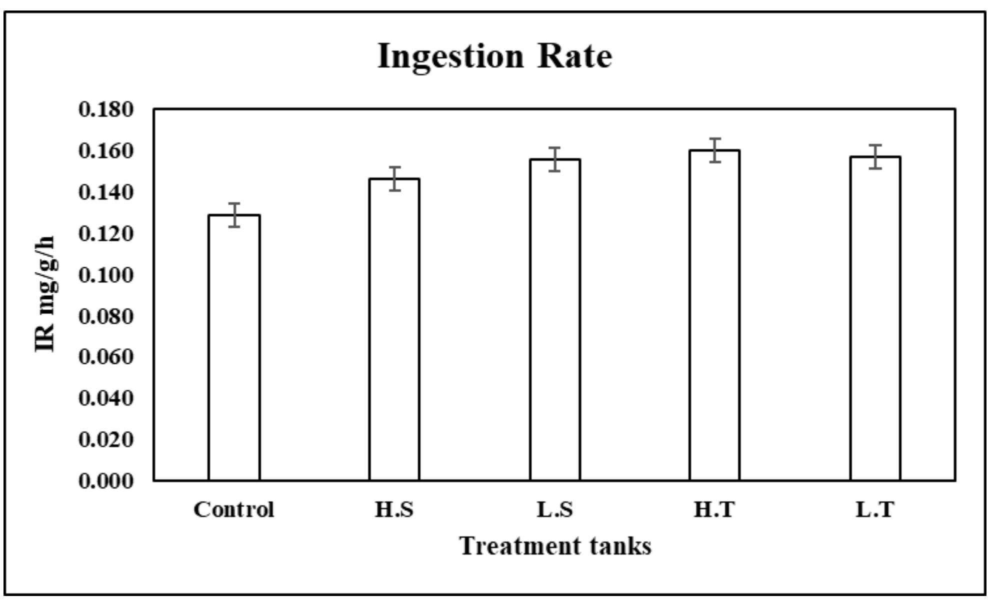

Fig. (4): The ingestion rate (IR) under different rearing conditions.

Oxygen Consumption and Ammonium Excretion Rates (OCR) and (AER)

The oxygen consumption rate (Fig. 5) was 0.76 $mgO_2/g/h$ in the control tank. The highest OCR of 1.15 $mgO_2/g/h$ was recorded in high-temperature tanks. There is a remarkable decrease in OCR in each of high salinity, low salinity, and low temp, with the lowest recorded value of 0.43 $mgO_2/g/h$ in high-salinity tanks. Low-salinity and low-temperature tanks showed almost the same OCR values of 0.63 and 0.66 $mgO_2/g/h$, respectively.

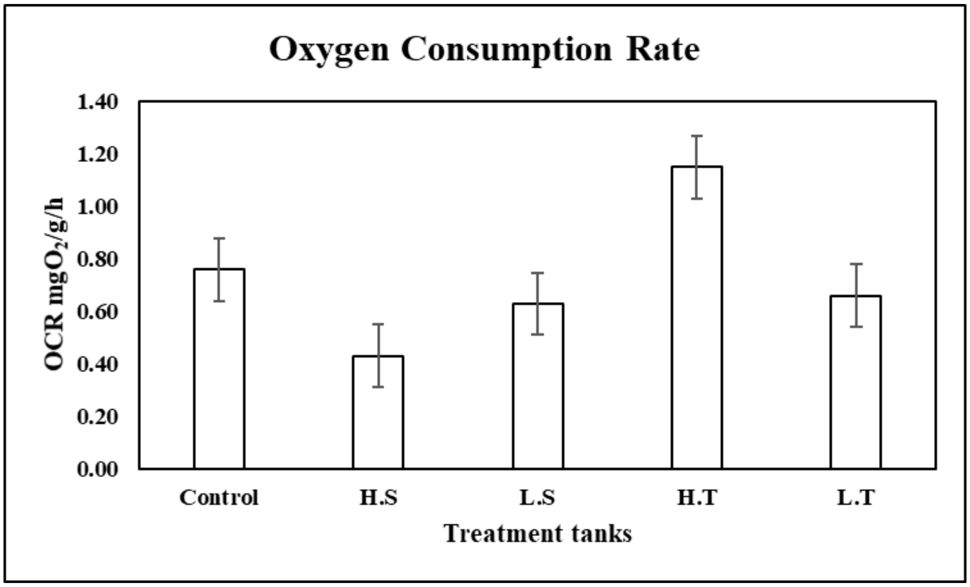

Fig. (5): The oxygen consumption rate under different water treatment conditions.

The ammonia excretion rate (Fig.6) was recorded as 47.44 μg/(g·h) in the control tank, while showed moderate elevation in the high-temperature tank at 55.57 μg/(g·h). There is a severe decline in low-salinity tanks, high-salinity, and low-temperature treatments with values of 15.5, 24.43, and 30.14 μg/(g·h), respectively.

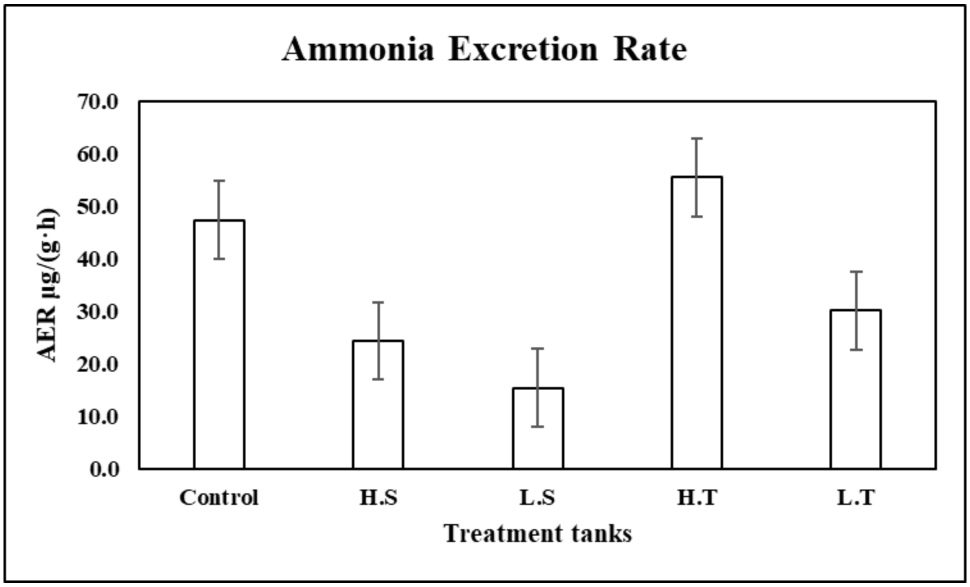

Fig. (6): The Ammonia excretion rate under different water treatment conditions.

The O/N ratio was estimated in animals under control conditions as 15.96 %. A slight increase in this ratio was recorded in high salinity, high-temperature, and low-temperature conditions (17.70, 20.76, and 22.01%, respectively). However, the animals reared under low salinity underwent a sharp increase in the O/N ratio, which was recorded as more than twice the value of the control condition (Fig. 7).

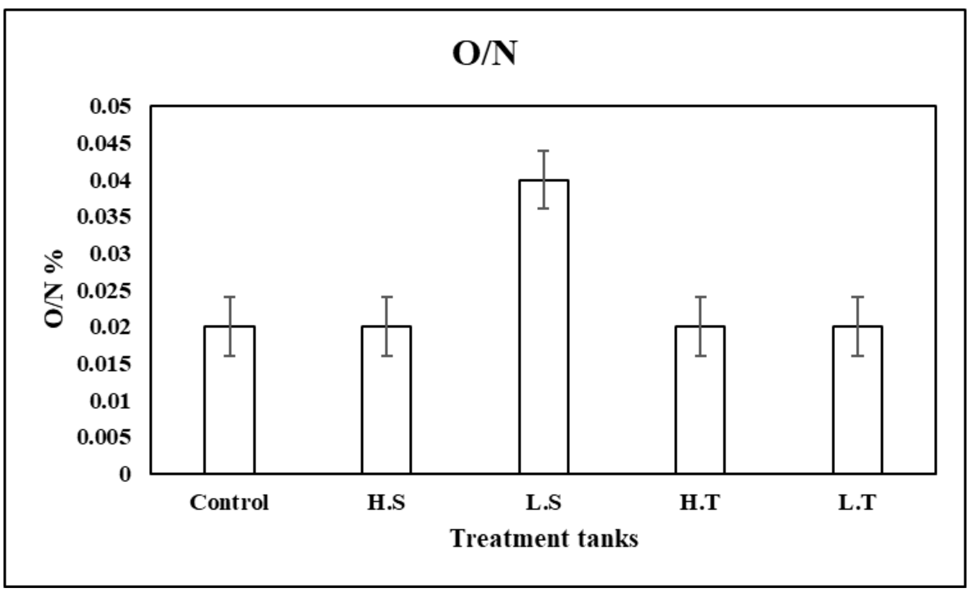

Fig. (7): The O/N ratio under different water treatment conditions.

DISCUSSION

The evisceration of sea cucumbers is common in normal, stressful, or experimental conditions (**Okada and Kondo, 2019**). *Holothuria sp*. is known for its low tolerance to salinity change (**Fankboner, 2002**); the high salinity stress was the cause of evisceration and death of *Holothuria scabra* at concentration 47 PSU (**Tuwo *et al*., 2021**). High temperature is a common stress that leads to the evisceration of *Holothuria sp*. High temperature is a common stress that leads to the evisceration of *Holothuria sp*. In an experimental study to record the gametes release under thermal stress at +10 and +12 °C above normal temperature, the *Holothuria scabra* showed evisceration of the digestive system (**Kautsari *et al*., 2020**). Regeneration of the digestive tract after the evisceration process is a unique feature in sea cucumber species, which enhances their survivability in any uncommon environmental change (**Su *et al*., 2022**). Thus, due to the high regeneration ability of sea cucumbers in the present study, the survival rate was 100% in all types of stressors except in high salinity.

In the same way, salinity and temperature influence the distribution and survival of aquatic species and impact their physiological responses. Temperature is one of the most important environmental factors that affect growth and physiological performance in aquatic animals such as sea cucumber *H. atra*. In addition, the effect of salinity on the growth and survival of different species of sea cucumber has been studied by many authors at different locations and has shown different results. However, sea cucumber *H. atra* is a euryhaline creature that can resist considerable fluctuations in salinity (**Wang *et al*., 2004**). The present study is considered the first attempt to study the effect of temperatures and salinity on the cucumber *H. atra* collected from Hurghada, Red Sea, under Aquaculture conditions. Stocking density is also a main factor affecting aquaculture survival and growth rate, according to Seeruttun *et al*. (**2008**). Since *H. atra* growth might be affected by density, a low stocking density five individual/tank neglected stress released from high density. The present study demonstrated that all individuals under thermal or salinity stresses showed a decline in the growth rate. This means that the used range of both salinity and temperature can support survival but not growth. This coincides with Wang *et al*. (**2008**), who stated that water temperature could significantly influence feeding and feed conversion efficiency and, thus, the growth of aquatic organisms.

On the other hand, Rhodes-Ondi and Turner (**2009**) stated that changes in salinity would cause changes in osmotic pressure in coelomic fluids and protein synthesis in sea cucumbers (**Niu *et al*., 2008**). Then, according to Abdel-Raheem (**2015**), optimal growth in sea organisms will be attained at iso-osmotic salinity conditions, where the organism does not require much energy for the osmoregulator process, allowing more energy to be utilized for growth. This coincides with our findings, as the highest SGR was recorded for specimens under control conditions (0.30±0.1 %). Overall, temperature and salinity variations have negative effects. *H. atra* growth showed a noticeable decline under any deviation from their typical temperature and salinity conditions. Recently, there has been a growing interest in utilizing sea cucumbers for the consumption of aquaculture waste, and the ingestion rate (IR) is commonly used as a key indicator to assess the bioremediation potential of sea cucumbers (**Zhou *et al*., 2006; Slater and Carton, 2007; Yu *et al*., 2011**). Like other sea cucumber species, *Holothuria sp* has also demonstrated its effectiveness as a deposit feeder, actively processing significant amounts of sediment through its digestive system in its natural habitat (**Bonham and Held, 1963; Che, 1990**).

Salinity is recognized as a significant factor influencing deposit feeders' ingestion rate (IR) (Yu *et al*., 2013). Some echinoderms, such as asteroids, were reported to have a feeding activity negatively impacted by hypo-osmotic conditions (**Shirley and Stickle, 1982; Forcucci & Lawrence, 1986**). The IR of sea cucumbers, including *Holothuria sp*, has also been observed to be inhibited by low salinity (**Mercier *et al*., 1999; Yuan *et al*., 2010**). The primary consequence of reduced ingestion is decreased energy for growth and normal functioning. However, the present study revealed different findings, as fluctuations in salinity and temperature were found to affect the IR of *Holothuria atra* positively.

Nevertheless, this increase in the ingestion rate did not appear to have positively affected the growth that was inhabited under all treatments, as mentioned before. Hence, the net result is the same, and this can be revealed as these fluctuations in salinity and temperature may increase the energy required for maintenance or disrupt the efficient utilization of energy at the cellular level (**Forcucci and Lawrence, 1986**). In other words, this means that it is reflected in terms of survival or regeneration (autotomy), not growth.

Moreover, OCR and AER are usually used as markers for energy measurements in sea cucumbers (**Yang *et al*., 2006**). OCR indicates energy production in sea cucumber *Apostichopus japoniscus* (**Yuan *et al*., 2010**). Although most echinoderm species can adapt to a wide range of salinity (**Yuan *et al*., 2006**), it has been recorded that the OCR level is affected by the low levels of salinity as in a study of *Holothuria leucospilota*, OCR decreased in low salinity which suggested decreasing the energy production in low salinity level **(Yu *et al*., 2013**). In another study of green, white, and purple morphs *of A. japonicus*, it was recorded that the three color morphs showed the minimum OCR in salinity 30 compared with higher and lower salinity degrees used in the experiment (**Bai *et al*., 2015**). In the current study, a decrease in OCR was recorded in both high and low salinity levels, suggesting that the energy production of *H. atra* decreases in any shift from their normal salinity. Additionally, AER has an unstable complex relation to the water salinity; in a study of *A. japonicas*, the AER increased in both low and high salinity levels (**Yuan *et al*., 2006**). In the case of *H. leucospilota*, the AER decreased in high salinity stress, and they suggested that this was due to the effect of water salinity on nitrogen catabolism (**Yu *et al*., 2013**). In the present study, the AER showed a marked decrease in the case of hypo salinity and hypersalinity levels. Sea cucumbers are ticklish to the environmental temperature; their OCR level is closely related to the change in the temperature (**Dong *et al*., 2011**). In an experiment to record the effect of aestivation on sea cucumber, *A. japonicus*, the OCR level showed marked fluctuation during the time of the experiment (**Ji *et al*., 2008**); in another study, the *Holothuria mobile* sea cucumber showed that the OCR levels decreased in winter and increased in high temperature (**Yu *et al*., 2018**) which agreed with this study as the OCR level was directly proportional to the water temperature. Temperature variations directly affect AER in different developmental stages of sea cucumbers (**Wei *et al.*, 2010**); understanding the relation between sea cucumber responses to the temperature helps understand its physiological reactions (**Sun *et al*., 2018**). Sea cucumber *A. japonicus* is considered nocturnal as the optimum digestive activities were recorded at the lowest temperature (**Sun *et al*., 2018**), and the AER in both mature and immature sea cucumbers were increased in the lower temperature (**Yang *et al*., 2006**). During this study, *H. atra* species showed that the AER activities were directly proportional to the temperature, which may reflect the temperature-dependent physiological activities of *H. atra*. Not only that, but also the O: N atomic ratio, determined by measuring oxygen uptake and ammonium nitrogen excretion, was employed to assess the proportion of proteins, lipids, and carbohydrates utilized as energy substrates by the organisms in various experimental conditions (**Wang and Li, 2020**). In the present study, the O/N ratio slightly increased under all treatment conditions. However, the low salinity condition sharply increased (>30), 40.71. This is explained as an indication of metabolism dominated by carbohydrates or lipids (**Xing *et al*., 2019**). Conversely, the O/N ratio below 10 indicates protein metabolism with ammonium as the primary nitrogen end product in teleost fish, which is not applied in the current case (**Yang *et al*., 2006**).

CONCLUSION

The current study provides crucial information about the potential of sea cucumber aquaculture in the Red Sea. It emphasizes the significance of careful environmental management for preserving optimum health and growth. Overall, temperature and salinity changes have a detrimental impact on the growth of *H. atra*, as it manifested a discernible drop under any departure from their normal temperatures and salinities. Contrarily, IR increased under all tested treatments, but this rise did not favor growth. It may have impacted survival or regeneration (autotomy) rather than growth. Both OCR and AER decreased with the fluctuations in salinity and showed a direct relationship with any change in temp. Additionally, the O/N ratio indicates that animals under low salinity utilized carbohydrates or lipids as energy substrates. As a result, *H. atra* may have reduced physiological health if exposed to an environment that deviates from their typical temperature and salinity settings.

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REFERENCES

Abdel Razek, F. A.; Abdel-Rahman, S.H.; El-Shimy, N. and Omar. H. A. (2005). Reproductive biology of the tropical sea cucumber *Holothuria atra* in the Red Sea coast of Egypt. Egyptian Journal of Aquatic Research 31(2): 383-402.

Abdel-Raheem, S.T. (2015). Developmental responses to fluctuations in environmental conditions in echinoid echinoderms. Thesis. College of William and Mary W&M Publish. 111 p.

Ahmed, M. I. (2015). A Decade of Sea Cucumber Fishing in Egypt, a Boom and Bust Case Study. CATRINA 12(1): 81 -85.

Ahmed, M. I. and Lawrence, A. J. (2007). The status of commercial sea cucumbers from Egypt's northern Red Sea Coast. Beche-de-Mer Information Bulletin 26: 14–18.

Aknaf, A.; Akodad, M.; Layachi, M.; El Madani, F.; Jaddar, A.; Mesfioui, A. and Baghour, M. (2017). Study of the spatial and temporal variation of physical-chemical parameters characterizing the quality of surface waters of the lagoon Marchica–North-East Morocco. J. Mater. Environ. Sci. 8: 3216–3225.

Al-Yaqout, A.; Nithyanandan, M; Al-Yamani, F.; Al-Kandari, M.; Al-Roumi, M. and Al-Baz, A. (2021). Sea cucumbers of the Arabian Peninsula and Iran – A review of historical and current research trends. Saudi Journal of Biological Sciences 28 (11): 6116-6126.

APHA, A. (2012). Standard methods for the examination of water and wastewater. American Public Health Association, Washington.

Bai, Y.; Zhang, L.; Liu, S.; Ru, X.; Xing, L.; Cao, X. and Yang, H. (2015). The effect of salinity on the growth, energy budget, and physiological performance of green, white, and purple color morphs of sea cucumber, *Apostichopus japonicus*. Aquaculture 437:297–303.

Battaglene, S.C.; Seymour, J.E. and Ramofafia, C. (1999).Survival and growth of cultured juvenile sea cucumbers, *Holothuria sabra*. Aquaculture 178:293-322.

Bell, J. D., Ganachaud, A., Gehrke, P. C., Griffiths, S. P., Hobday, A. J., Hoegh-Guldberg, O., ... & Waycott, M. (2013). Mixed responses of tropical Pacific fisheries and aquaculture to climate change. Nature Climate Change, 3(6): 591-599.

Bonham, K. and Held, E. E. (1963). Ecological observations on the sea cucumbers *Holothuria atra* and *H. leucospilota* at Rongelap Atoll, Marshall Islands. Pacific Science 17: 305–314.

Broom, M.; Gabr, M. H.; Al-Harbi, M. and Satheesh, S. (2021). Effect of different diets on growth of the sub-adult sea cucumber *Holothuria scabra*. Aquaculture International 29(6): 2403–2414.

Che, R.G.O. (1990). Aspects of the feeding biology of *Holothuria leucospiolota* Brandt (Echinodermata: Holothuroidea) in Hong Kong. Asian Marine Biology 7:133–146.

Chen, J. X. (2004). Present status and prospects of the sea cucumber industry in China. In: Lovatelli A, Conand C, Purcell S, Uthicke S, Hamel JF, Mercier A (eds) Advances in sea cucumber aquaculture and management. FAO, Rome, pp 25–38. Chimie.

Conand, C. (2004). Present Status of World Sea Cucumber Resources and Utilization: As International Overview. In: Lovatelli, A., Conand, C., Purcell, S., Uthicke, S., Hamel, J.-F. and Mercier, A., Eds., Advances in Sea Cucumber Aquaculture and Management, FAO, Rome, Fisheries and Aquaculture Technical Paper, pp. 13–24.

Dong, Y. W.; Yu, S. S.; Wang, Q. L. and Dong, S. L. (2011). Physiological responses in a variable environment: relationships between metabolism, hsp and thermotolerance in an intertidal-subtidal species. *PLoS One*, *6*(10):e26446.

Dong, Y.; Dong, S.; Tian, X.; Wang, F. and Zhang, M. (2006). Effects of diel temperature fluctuations on growth, oxygen consumption and proximate body composition in the sea cucumber Apostichopus japonicus Selenka. Aquaculture, 255(1-4): 514–521.

Effendie, M. I. (1997). Fisheries biological method. Yayasan Dewi Sri. Bogor. 112 ham.

Fankboner, P. V. (2002). Seasonal visceral atrophy and response to salinity by *Parastichopus californicus* (Stimpson): Osmoregulation. *SPC Beche-de-mer Inf Bull*, *17*, 22-26.

Forcucci D. and Lawrence J .M. (1986). Effect of low salinity on the activity, feeding, growth, and absorption efficiency of *Luidia clathrate* (Echinodermata: Asteroidea). Marine Biology 92: 315–321.

González-Durán, E.; Hernández-Flores, Á.; Headley, M.D. and Canul, J.D. (2021). On the effects of temperature and pH on tropical and temperate holothurians. Conserv. Physiol. 9(1):coab092.

Grasshoff, K., Ehrhardt, M., Kremling, K. and Almgren, T. (1983). Methods of Seawater Analysis 2nd rev. and extended edn Verlag Chemie. Weinheim, 28, p419.

Okada, A. and Kondo, M. (2019). Regeneration of the digestive tract of an anterior-eviscerating sea cucumber, Eupentacta quinquesemita, and mesenchymal–epithelial transition involvement in digestive tube formation. Zoological Letters, 5(1): 1-13.

Ha, N.T.K.; Huong, D.T.T.; Phuong, N.T.; Bayley, M. and Jensen, F.B. 2,19. Impact and tissue metabolism of nitrite at two acclimation temperatures in striped catfish (*Pangasianodon hypophthalmus*). Aquat. Toxicol. 212:154-161.

Hasan, M. H. (2019). Destruction of sea cucumber populations due to overfishing at Abu Ghosoun area, Red Sea. The Journal of Basic and Applied Zoology. 80 (1): 5.

Huisman, E.A. (1987). The principles of fish culture production. Department of Fish Culture and Fisheries. Netherland (NL). Wageningen University.

Ip, Y. K. and Chew, S. F. (2018). Air-breathing and excretory nitrogen metabolism in fishes. Acta Histochemica, 120(7): 680–690.

IPCC. (2007). Summary for Policymakers. In Climate Change 2007: The Physical Science Basis, SD Solomon, M Qin, Z Manning, M Chen, KB Marquis, M Averyt, HL Tignor (eds). Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.

Ji, T.; Dong, Y. and Dong, S. (2008). Growth and physiological responses in the sea cucumber, *Apostichopus japonicus* Selenka: aestivation and temperature. Aquaculture, 283(1-4), 180-187.

Kang, J. H.; Cho, S. M; Kim, T. O. and Park, K. S. (2015). Sanitary assessment of the oyster rack culture waters in Wando, Korea. Korean J Malacol. 31:129–36.

Kautsari, N.; Riani, E.; Lumbanbatu, D. T. F. and Hariyadi, S. (2020). Effect of temperature increase on gametes release of *Holothuria scabra*. AACL Bioflux, 13(4), 2375-2387.

Kühnhold, H.; Kamyab, E.; Novais, S.; Indriana, L.; Kunzmann, A.; Slater, M.; *et al*. (2017). Thermal stress affects energy resource allocation and oxygen consumption rate in the juvenile sea cucumber, *Holothuria scabra* (Jaeger, 1833). Aquaculture 467, 109–117.

Lawrence, A.; Ahmed, M.; Hanafy, M.; Gabr, H.; Ibrahim, A. and Gab-Alla, A.F.(2004). Status of the sea cucumber fishery in the Red Sea – the Egyptian experience. In: Advances in Sea Cucumber Aquaculture and Management. FAO Fisheries Technical Paper 463. Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 79– 90.

Lawrence, A.J.; Afifi, R.; Ahmed, A.M.; Khalifa, S. and Paget, A.T. (2009). Bioactivity as an option's value of sea cucumbers in the Egyptian Red Sea. Conservation Biology 24: 217–225.

Mahdy, A.; Omar H. A.; Nasser S. A. M.; Abd El-Wakeil, K.F. and Obuid-Allah, A.H. (2018). Community structure of Echinoderms in the littoral zone of the Red Sea Coast of Egypt. Egyptian Journal of Aquatic Biology and Fisheries, 22 (5): 483-498.

Mercier, A.; Battaglene S.C. and Hamel J. F. (1999). Daily burrowing cycle and feeding activity of juvenile sea cucumbers *Holothuria scabra* in response to environmental factors. Journal of Experimental Marine Biology and Ecology 239:125–156.

Niu, C.; Rummer, J.; Brauner ,C. and Schulte, P. (2008). Heat shock protein (HSP 70) induced by mild heat shock inhibits sharp plasma osmolarity increases upon seawater transfer in rainbow trout (Oncorhynchus mykiss). Comp Biochem Physiol 148C: 460–461.

Purcell, S. W. (2004). Rapid growth and bioturbation activity of the sea cucumber *Holothuria scabra* in earthen ponds. Proceedings of Australasian Aquaculture 2004, p. 244.

Rhodes-Ondi, S. E. and Turner, R. L. (2009). Salinity tolerance and the osmotic response of the estuarine hermit crab, *Pagurus maclaughlinae* in the Indian River Lagoon, Florida. Estuar Coast Shelf Sci 86: pp. 189–196.

Romin, M.; Azad, S. and Saleh, E. (2021). Variations in the Physicochemical Water Parameters and Phytoplankton Community in Coastal Water of Kudat, Sabah, Malaysia. Journal of Geoscience and Environment Protection. 9: 86-99.

Sabourin, T. D. and Stickle, W. B. (1981). Effects of salinity on respiration and nitrogen excretion in two species of echinoderms. Marine Biology, 65: pp. 91-99.

Seeruttun, R.; Appadoo, C.; Laxminaraya, A. and Codabaccus, B. (2008). A study on the factors influencing the growth and survival of juvenile sea cucumber, *Holothuria atra*, under laboratory conditions. University of Mauritius Research Journal. 14(1): 1–15.

Sewell, M.A. (1990). Aspects of the ecology of *Stichopus mollis* (Echinodermata: Holothuroidea) in north-eastern New Zealand. New Zealand Journal of Marine and Freshwater Research 24: 97-103.

Shirley, T.C. and Stickle, W.B. (1982). Responses of *Leptasterias hexactis* (Echinodermata: Asteroidea) to low salinity. Marine Biology 69: 155–163.

Slater, M. J. and Carton, A. G. (2007). Survivorship and growth of the sea cucumber *Australostichopus* (Stich-opus) *mollis* (Hutton, 1872) in polyculture trials with green-lipped mussel farms. Aquaculture 272: 389–398.

Su, F.; Yang, H., and Sun, L. (2022). A review of histopathological events and molecular mechanisms involved in intestine regeneration in holothurians. Biology, 11(8): 1095.

Sun, J.; Zhang, L.; Pan, Y.; Lin, C.; Wang, F. and Yang, H. (2018). Effect of water temperature on diel feeding, locomotion behavior and digestive physiology in the sea cucumber *Apostichopus japonicus*. Journal of Experimental Biology, 221(9), jeb177451.

Svobodova, Z.; Machova, J.; Kroupova, H. K. and Velisek, J. (2017). Water Quality–Disease Relationship on Commercial Fish Farms. In G. Jeney (Ed), Fish Diseases, (Pp167-185), Academic Press.

Taylor, A. L., Nowland, S. J., Hearnden, M. N.; Hair, C. A. and Fleming, A. E. (2016). Sea ranching release techniques for cultured sea cucumber *Holothuria scabra* (Echinodermata: Holothuroidea) juveniles within the high-energy marine environments of northern Australia. Aquaculture, pp. 465, 109–116.

Tuwo, A. (2004). Status of sea cucumber fisheries and farming in Indonesia 463 (Rome: FAO).

Tuwo, A.; Yasir, I.; Bestari, A. D.; Yanti, A.; Aprianto, R. and Tresnati, J. (2021). High salinity effect on mortality of sandfish *Holothuria scabra* (Jaeger, 1833). In IOP Conference Series: Earth and Environmental Science (Vol. 763, No. 1, p. 012019). IOP Publishing.

Wang, F. Y.; Yang, H. S.; Gao, F. and Liu, G. B. (2008). Effects of acute temperature or salinity stress on the immune response in sea cucumber, *Apostichopus japonicus*. Comparative Biochemistry and Physiology, Part A, 151: 491–498.

Wang, T. and Li, Q. (2020). Effects of temperature, salinity, and body size on the physiological responses of the Iwagaki oyster *Crassostrea nippon*a. Aquaculture Research 51(2): 728-737.

Wang, Y. G.; X. J. Rong; C. Y. Zhang and H. L. Sui. (2004). Primary problems in the culture of *Apostichopus japonicus* Selenka and techniques of disease prevention. Shandong Fish. 21(10): 29–31.

Wei, Z.; Jia-shen, T.; Bi, W.U.; *et al*. (2010). The respiration and excretion in sea cucumber *Apostichopus japonicus* with various body weights. Journal of Dalian Ocean University, 25(3): 281-284.

Xing, D.; Song, X.; Peng, L.; Cheng, Y. and Zhai, J. (2019). Effects of temperature and salinity on oxygen consumption and ammonium excretion rate of ♀ *Epinephelus fuscoguttatus* ×♂ *E. lanceolatus* juveniles. Journal of Ocean University of China, 18, 177-184.

Yang, H.; Zhou, Y.; Zhang, T.; Yuan, X.; Li, X., Liu, Y. and Zhang, F. (2006). Metabolic characteristics of sea cucumber *Apostichopus japonicus* (Selenka) during aestivation. Journal of Experimental Marine Biology and Ecology, 330 (2): 505–510.

Yu, Z.; Hu, C.; Zhou, Y.; Peng, P. and Li, H. (2011). Survival and growth of the sea cucumber Holothuria leucospilota Brandt: a comparison between suspended and bottom cultures in a subtropical fish farm during summer. Aquaculture Research (doi:10.1111/j.1365-2109.2011. 03016. x).

Yu, Z.; Luo, P.; Liu, W.; Huang, W. and Hu, C. (2018). Influence of water temperature on physiological performance of the sea cucumber *Holothuria moebii*. Aquaculture Research, 49(11): 3595–3600.

Yu, Z.; Qi, Z.; Hu, C.; Liu, W. and Huang, H. (2013). Effects of salinity on ingestion, oxygen consumption, and ammonium excretion rates of the sea cucumber *Holothuria leucospilota*. Aquaculture Research, 44(11), 1760-1767.

Yuan X.; Yang, H.; Wang, L.; Zhou, Y. and Gabr, H. R. (2010). Effects of salinity on energy budget in pond cultured sea cucumber *Apostichopus japonicus* (Selenka) (Echinodermata: Holothuroidea). Aquaculture 306, 348 –351.

Yuan, X. T.; Yang, H. S.; Zhou Y.; Mao, Y. Z.; Zhang, T. and Liu, Y. (2006). Salinity affects the respiration and excretion of sea cucumber *Apostichopus japonicus* (Selenka). Oceanologia Et Limnologia Sinica 37: 348– 354. (in Chinese with English abstract).

Yuan, X.; Yang, H.; Wang, L.; Zhou, Y. and Gabr, H. R. (2010). Effects of salinity on energy budget in pond-cultured sea cucumber *Apostichopus japonicus* (Selenka) (Echinodermata: Holothuroidea). Aquaculture, 306(1-4), 348-351.

Yun, L..; Zonghe, Y.; Yinyin, L.; Peng, L.; Xiao, J.; Yushun, T. and Xiongqi, D. (2019). Ammonia nitrogen and nitrite removal by a heterotrophic *Sphingomonas* sp. strain LPN080 and its potential application in aquaculture, Aquaculture 500: 477-484.

Zhou, Y.; Yang, H.; Liu, S.; Yuan, X.; Mao, Y.; Zhang, T.; Liu, Y. and Zhang F. (2006). Feeding on biodeposits of bivalves by the sea cucumber *Stichopus japonicus* Selenka (Echinidermata: Holothuroidea) and a suspension co-culture of filter-feeding bivalves with deposit feeders in lantern nets from long lines. Aquaculture 256: 510–520.