

Restoration of Stocks of the Sea Cucumber *Holothuria fuscogilva* in the Red Sea with Transplanted Wild Juveniles

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Abstract

Over the last decade, holothuroid sea cucumbers in the Gulf of Aqaba of the Red Sea have been the target of continuous fishing. This has severely depleted sea cucumber stocks, especially the high-value species such as *Holothuria fuscogilva*. The present work demonstrates that restocking populations of *H. fuscogilva* that are at critically and chronically low levels by transplanting wild-captured juveniles can be effective. Juveniles were translocated from a robust population at Pharoan Island and released into two sites (Wadi Quny and Hidden Bay). Population density, growth rate and mortality at the original and two release sites were monitored for 2 yrs. The Pharoan population density was highest, with *H. fuscogilva* showing a strong preference for sandy habitat (21.3 - 18.4 ind./100m²), over seagrasses (3.6 - 2.5 ind./100m²) and corals (0.9 - 1.7 ind./100m²). The restocked population at Wadi Quny increased from 2.6 to 9.8 ind./100m² from 2013-2015. In contrast, density at Hidden Bay decreased from 2.8 to only 0.1 ind./100m² in the first year. Sea cucumbers in the restocked population at Wadi Quny had higher growth rates (0.65 - 1.29 cm/month) compared to the original population at Pharoan Island (0.21 - 0.45 cm/month), while Hidden Bay showed a negative growth rate. Mortality was low at Pharoan Island (1% - 2%) and Wadi Quny (0.5% - 0.75%), but high at Hidden Bay (49% - 100% in the first year). There was a negative relationship between mortality and size ($P = 0.003$). The restocking of *H. fuscogilva* populations using wild-captured juveniles was very successful at Wadi Quny but a failure at Hidden Bay.

Keywords

Beche-de-Mer, Growth Rate, Gulf of Aqaba, Mortality, Restocking

1. Introduction

Due to the accelerating overexploitation of holothurians worldwide [1]-[8], management strategies have been adopted to face their stock depletion. Conservative management should be the key to sustainable sea cucumber fisheries, especially at locations with severely depleted stocks. Restocking is an important procedure used for sea cucumber population management that can be employed when sites are depleted by overfishing [9]. Releases of juveniles can be used for stock enhancement [10] [11] and can improve the productivity at sites with relatively low natural recruitment or increase access of species to isolated habitats [12]. Most workers consider restocking by introducing hatchery-produced juveniles to a certain location, a very important procedure in increasing holothurians populations [13] [14] [15]. However, an alternative approach is to transfer wild natural holothurians juveniles from one area to another, especially from areas with high densities (full carrying capacity) and more suitable environmental conditions for juvenile sea cucumber growth. Restocking success of sea cucumbers depends on many factors such as predation [16], adequate genetic diversity [17], diseases [18] [19], habitat quality [20], depth [21] and food availability [22]. This report documents an example of successful restocking using wild juveniles of *Holothuria fuscogilva*, from an area of high density to two other areas of low densities at the Gulf of Aqaba.

The Gulf of Aqaba is valued as a unique environment with a wide range of habitats and outstanding marine biodiversity [23] [24]. In spite of the suitable environmental conditions provided at the Gulf for sea cucumber species, populations currently have low densities and diversity. High fishing pressure exerted on the sea cucumber populations at the Gulf, which began in the mid-1990's, caused severe depletion in holothurid stocks, especially high-value species as *H. fuscogilva* [25] [26] [27].

Holothuria fuscogilva is a deep water species usually found at depths ranging from 10 - 40 m on clean sand or seagrasses, the preferred habitat for this species [1] [28]. Like many other commercial species at the Gulf of Aqaba, overharvesting of *H. fuscogilva*, combined with poor management caused a severe depletion in its population over the last few years except for few sites, where high densities still occur. Pharos Island as a protected area has a high abundance and diversity of sea cucumber species [26]. Presumably at some point, intraspecific competition would limit the number of individuals that could grow and survive to reproductive age [9] [10]. Transplanting juvenile *H. fuscogilva* to other locations with suitable habitat may be an effective way to enlarge this species' populations at the Gulf of Aqaba. This strategy, coupled with the protection of remnant wild sea cucumber populations through the use of marine protected areas in this respect could be a sustainable solution for holothurid management [29].

This study reports on the success of transferring juvenile *H. fuscogilva* from area of full carrying capacity (Pharos Island) to two areas where stocks have been depleted from overfishing. Population densities at the three localities were

monitored for two years, before and after restocking. The growth and survival of individually marked individuals at each locality were assessed to determine success or the failure of the recolonization efforts, and to identify habitat that may limit this approach in the future at new localities.

2. Materials and Methods

2.1. Study Sites

Of the three sites selected for this study, Pharoan Island served as the control or original site, and Wadi Quny and Hidden bay as the restocked sites. All three sites had similar physical habitat (benthic substrate), food resource availability and exposure to natural enemies.

2.2. Pharoan Island

Pharoan Island is a small island south of Taba at the Gulf of Aqaba, separated from the coast by a small (about 200 m wide) but deep (about 30 - 70 m) channel. It is small, with an area of approximately 3.9 hectares (9.6 acres). The island is a popular tourism site as it has an ancient citadel and spectacular coral reefs. The open sea side of the island has much greater biodiversity than the coast side. The site has a small reef flat that extends about 30 m and is composed of dead and live corals. The reef slope is steep and drops for about 15 m. The slope is composed of coral patches and white clean sand from the coral origin. The seabottom drops again for approximately 30 m, and is composed of sand and seagrasses. The island has a very flourishing marine life with a high species index for major taxonomic groups, particularly coral and fishes. It also has a high abundance of mollusks and echinoderms (personal observations). The algal cover is moderate at the island with high seagrass patches. The island is protected from fishing and other detrimental activities by a coast guard station.

2.3. Wadi Quny

The site located north of the city of Dahab on the coast of the Gulf of Aqaba. This site has a typical fringing reef, in which the reef flat area is divided into 3 zones. The "back reef" is composed of a fossil reef with very high algal cover. The "mid-reef" is composed of rocky patches and sand patches over a rocky basement from fossil reef. The "fore reef" is mainly composed of live corals with a small percentage of dead corals. The reef slope also has a well-developed coral formation that ends at depths around 20 m deep and transitions to a sandy bottom. This site supports a high density and diversity of fishes, corals and invertebrates. Benthic algae and seagrasses make up a high percentage of cover especially at the reef flat and in the sand of the seabed. The site has excellent conditions for sea cucumber existence [25] [26]. After the collection pressure on sea cucumbers was stopped by a governmental decree to ban its fishery, some species had the opportunity to reestablish and increase in density.

2.4. Hidden Bay

This site located inside Ras Mohamed National Park, located 12 km from the city of Sharm El Sheikh at the Southern tip of the Gulf of Aqaba. The park spans an area of 480 km², including 135 km² of surface land area and 345 km² area of water. The site is a semi-closed inland bay, with low wave and current action. The site has a shallow depth ranging between 1 and 2.5 m, with extensive sandy habitat. Benthic algae and dense seagrass beds mainly composed of *Halophilla stipulacea* provide cover with large sandy patches of coarse coral origin sand interspersed. The site has high biological diversity and density of invertebrates that preferred the sandy bottom areas, with fewer fish species. Inside the national park, this site has full protection from fishing or harvesting.

2.5. Field Surveys and Sampling

Field surveys of sea cucumber faunal composition and population densities of *H. fuscogilva* were conducted at Pharoan Island, Wadi Quny and Hidden Bay, at the Gulf of Aqaba over a period of 25 months, from April 2013 to April 2015. Faunal surveys were conducted twice a year, *H. fuscogilva* densities were quantified at (approximately) 3-month intervals. For each survey, a number of transects were made starting from the highest watermark (HWM), parallel to the shore and covering different zones and habitats. Transects were made at the back reef, mid reef and fore reef at the reef flat, then at depths 5, 10, 20, 30, 40 and >40 meters. The length of each transect was 150 m, with 2 - 5 replicate transects at each zone. Along each transect 10 quadrats were established, each 10 m × 10 m (100 m²). Reef flats were surveyed by snorkeling, while deep water was surveyed by SCUBA diving. All species of sea cucumbers were recorded based on visual observation. Population densities of *H. fuscogilva* were determined from counts in quadrats and expressed as number of individuals/100m². At each quadrat, visual surveys were used to describe different biotopes of the reef and composition of substrate in terms of percent of sand, small stones, seagrasses, algae, rocks, dead and live corals. At each site, the water temperature was also recorded.

2.6. Collection, Transport and Release of Wild Sea Cucumbers

In April 2013, 240 juveniles and 100 adults of *H. fuscogilva* were collected by SCUBA diving from Pharoan Island. The specimens were collected from depths ranging from 25 - 30 m. Juveniles ranged in body length from 3 - 10 cm, while lengths of the adults ranged from 28 - 36 cm. Most were collected from habitat defined as clean sand from the coral origin at the reef slope and sea bed.

There are two commonly used methods for transporting sea cucumbers: dry and wet. The former is used for short distances within three hours of the destination [30]. We used the wet method for transporting the collected individuals, which involves a canvas tank of 50 × 50 × 80 cm³ half full of aerated seawater. 60 juveniles or 20 adults were put into each tank. Animals were transported in the

early morning to avoid exposure to direct sunlight. The temperature inside the tanks maintained below 20°C. The first group of animals was released at Wadi Quny, which is 90 minutes (100 km) from Pharoan Island. 120 juveniles and 50 adults were released into sandy habitat by SCUBA diving at a depth of about 23 m. The second group of animals was released at Hidden Bay at Ras Mohamed National Park, which is approximately three hours (250 km) from Pharoan Island. 120 juveniles and 50 adults were released into sandy habitat at depths ranging from 2 - 2.5 m by snorkeling.

Animals were released into 10 m × 10 m (100 m²) quadrats along 150 m-transects at each site. Quadrats were spaced 5 m apart along the transect and there were 5 replicate transects. The total area of release at each site was slightly more than 1000 m².

2.7. Tagging

In order to distinguish individuals, before their release 100 juvenile *H. fuscogilva* were tagged at each site to monitor the growth. All juvenile body lengths ranged from 3 to 10 cm. Adults were not marked or monitored in this part of the study.

Tagging was performed by using the heat branding technique commonly used to individually mark sea cucumbers, as described in [3]. At each of the three study sites, individuals were given the numbers from 1 to 100 by branding. The branded numbers were visible on the animals' skin throughout the study period, with only a little blurring due to the growth of the animals. The branded marks provide an ideal way to monitor the growth of juveniles and the disappearance of the marks is slow; animals can be re-branded if necessary [3].

2.8. Growth Measurement

After a two-week acclimation period, tagged animals were measured bi-monthly. Body lengths were measured using a measuring tape held above the animals but without touching it to avoid its contraction. Although there are other methods of measuring body size using wet or dry mass, these methods require considerably more transport and handling of animals. We chose to carry out less intrusive body length measurements when the animals were in a relaxed (e.g. non-locomotory, unexcited) state. If done carefully, body length measures are repeatable and have low standard errors. Animals were recorded as present/absent. If animals were not relocated in subsequent surveys they were considered to have either died or dispersed from the study area.

2.9. Statistical Analysis

Absolute growth rates and body lengths for the three populations (two released populations and the original source population) were compared at two-month intervals for 2 years after establishment with ANOVA's and t-tests (when only two populations remained) computed in R version 3.5.0 [31]. The initial body sizes of juveniles assigned to the three sites did not differ.

Absolute growth rates were also calculated for animals in different size classes. Animals were allocated to seven size classes that differed by 1 cm and the growth rate for each month was computed as current-previous body length. Absolute growth rates allowed for better visualization of the growth of juveniles in different size classes and across different seasons.

3. Results

3.1. Faunal Composition

Bi-annual surveys carried out at the three sites (Pharoan Island, Wadi Quny and Hidden Bay), documented a total of 22 species of holothurian sea cucumber species (Table 1). Sea cucumber species richness at Pharoan Island was much greater than the other two sites. At the beginning of the survey (April 2013), 22 species were found at Pharoan Island, but only 4 at Wadi Quny and 3 at Hidden Bay. *H. fuscogilva* was observed only at Pharoan Island. The release of *H. fuscogilva* at the other two sites increased the number of species to 5 at Wadi Quny and 4 at Hidden Bay. After transplantation, *H. fuscogilva* became established at Wadi Quny and was recorded during subsequent surveys (Sep. 2013, Feb. 2014, Jul. 2014 and Apr. 2015), but in Hidden Bay it disappeared after few months (from Feb. 2014 onwards).

Table 1. Comparison of holothurian species recorded from the three investigated sites during the period from April 2013 to April 2015.

Species	Pharoan Island				Wadi Quny					Hidden Bay					
	Apr. 2013	Sep. 2013	Feb. 2014	Jul. 2014	Apr. 2015	Apr. 2013	Sep. 2013	Feb. 2014	Jul. 2014	Apr. 2015	Apr. 2013	Sep. 2013	Feb. 2014	Jul. 2014	Apr. 2015
<i>Actinopyga miliaris</i>	+	+	+	+	+										
<i>A. echinites</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>A. mauritiana</i>	+	+	+	+	+										
<i>A. crassa</i>	+	+	+	+	+										
<i>A. serratidens</i>	+	+	+	+	+										
<i>Bohadschia drachi</i>	+	+	+	+	+										
<i>B. cousteaui</i>	+	+	+	+	+										
<i>B. marmorata</i>	+	+	+	+	+										
<i>B. tenuissima</i>	+	+	+	+	+										
<i>B. vitiensis</i>	+	+	+	+	+										
<i>Holothuria atra</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>H. edulis</i>	+	+	+	+	+										
<i>H. hilla</i>	+	+	+	+	+	+	+	+	+	+					
<i>H. arenicola</i>	+	+	+	+	+										
<i>H. impatiens</i>	+	+	+	+	+										
<i>H. leucospilota</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>H. nobilis</i>	+	+	+	+	+										
<i>H. fuscogilva</i>	+	+	+	+	+		+	+	+	+		+			
<i>Pearsonothuria graeffei</i>	+	+	+	+	+										
<i>Stichopus horrens</i>	+	+	+	+	+										
<i>S. monotuberculatus</i>	+	+	+	+	+										
<i>Thelonota ananas</i>	+	+	+	+	+										

3.2. Population Density and Habitat Distribution of *H. fuscogilva*

The population densities of *H. fuscogilva* differed widely across the three study sites and habitats. Very high densities occurred at Pharoan Island in all surveys with no marked variations between seasons. However, densities at the receiving sites (Wadi Quny and Hidden Bay) were more variable (Table 2). After transfer *H. fuscogilva* to Wadi Quny, densities increased during the study period, from an average of 2.6 ± 1.5 ind./100m² in April 2013 to 9.8 ± 3.1 in April 2015. Conversely, the density of *H. fuscogilva* dramatically decreased at Hidden Bay from 2.8 ind./100m² at April 2013 to only 0.1 ind./100m² at January 2014, and then it completely disappeared from the site (Table 2).

Populations of *H. fuscogilva* at Pharoan Island, Wadi Quny and Hidden Bay showed a distinct preference for sandy habitats. Individuals were more concentrated on sandy substrate either on or near the reef slope and among the coral patches. Although average densities were greatest on sandy substrate (ranged from 21.3 to 18.4 ind./100m² at Pharoan Island, 9.8 and 2.6 ind./100m² at Wadi Quny and 2.8 to 0.1 ind./100m² at Hidden Bay), substantial densities were also observed in the seagrass (ranged between 3.6 and 2.5 ind./100m² at Pharoan Island, 0.4 and 0.2 ind./100m² at Wadi Quny and no individuals from seagrass at Hidden Bay). Densities on corals ranged from 1.2 to 0.9 ind./100m² at Pharoan Island, while no individuals were observed on corals at Wadi Quny and Hidden Bay.

3.3. Growth Rates of *Holothuria fuscogilva*

Individuals of *H. fuscogilva* exhibited different growth patterns at the three sites. Table 3 lists the ANOVA and t-test comparisons of body size and growth rates

Table 2. Seasonal variation in densities of *H. fuscogilva* (ind./100m²) found in different habitats at the study sites from April 2013 to April 2015 (values shown are means \pm SE of replicate transects).

Sample date	Pharoan Island			Wadi Quny			Hidden Bay		
	Sand	Seagrass	Coral	Sand	Seagrass	Coral	Sand	Seagrass	Coral
Apr. 2013	19.5 \pm 2.5	3.2 \pm 1.6	1.0 \pm 0.2	2.6 \pm 1.5	0	0	2.8 \pm 1.9	0	0
Jul.	21.3 \pm 3.1	2.5 \pm 1.3	1.7 \pm 0.2	2.7 \pm 1.5	0.2 \pm 0.1	0	1.1 \pm 1.0	0	0
Oct.	18.6 \pm 2.3	2.7 \pm 1.4	1.0 \pm 0.3	2.8 \pm 1.6	0.3 \pm 0.1	0	0.6 \pm 0.2	0	0
Jan. 2014	18.4 \pm 2.3	3.1 \pm 1.6	1.0 \pm 0.2	3.1 \pm 1.8	0.2 \pm 0.1	0	0.1 \pm 0.03	0	0
Apr.	19.1 \pm 2.5	3.6 \pm 1.7	1.2 \pm 0.2	4.0 \pm 1.9	0.2 \pm 0.1	0	0	0	0
Jul.	20.5 \pm 2.8	3.4 \pm 1.7	1.1 \pm 0.2	4.2 \pm 1.9	0.3 \pm 0.1	0	0	0	0
Oct.	19.4 \pm 2.9	3.1 \pm 1.6	0.9 \pm 0.1	5.6 \pm 2.1	0.4 \pm 0.1	0	0	0	0
Jan. 2015	20.3 \pm 2.8	3.0 \pm 1.5	1.0 \pm 0.1	7.5 \pm 2.7	0.4 \pm 0.1	0	0	0	0
Apr.	20.7 \pm 2.8	2.8 \pm 1.4	1.1 \pm 0.1	9.83 \pm 0.1	0.6 \pm 0.2	0	0	0	0

Table 3. ANOVA results from monthly comparisons of body lengths and growth rates among the three populations (from month 0 to 12), and t-test comparisons between Pharoan and Wadi Quny after 12 months.

Body length					
Month after transplant	Calendar date	df	F value	Pr (>F)	significance
0	Apr 2013	2	1.890	0.153	ns
2	Jun 2013	2	3.219	0.0417	*
4	Aug 2013	2	7.835	0.0005	**
6	Oct 2013	2	11.12	<0.0001	***
8	Dec 2013	2	24.9	<0.0001	***
10	Feb 2014	2	26.4	<0.0001	***
12	Apr 2014	2	17.78	<0.0001	***
14	Jun 2014	1	22.27	<0.0001	***
16	Aug 2014	1	35.46	<0.0001	***
18	Oct 2014	1	36.35	<0.0001	***
20	Dec 2014	1	35.19	<0.0001	***
22	Feb 2015	1	35.89	<0.0001	***
24	Apr 2015	1	40.83	<0.0001	***
Growth Rate					
0	Apr 2013	2	2322	<0.0001	***
2	Jun 2013	2	131.9	<0.0001	***
4	Aug 2013	2	54.43	<0.0001	***
6	Oct 2013	2	1.382	<0.0001	***
8	Dec 2013	2	412.8	<0.0001	***
10	Feb 2014	2	305.7	<0.0001	***
12	Apr 2014	1	1.382	0.241	ns
14	Jun 2014	1	35.7	<0.0001	***
16	Aug 2014	1	127	<0.0001	***
18	Oct 2014	1	0.373	0.542	ns
20	Dec 2014	1	0.962	0.328	ns
22	Feb 2015	1	0.166	0.684	ns
24	Apr 2015	1	5.795	0.017	*

at different time points during the 24 months after transplantation. Both Pharoan Island and Wadi Quny sea cucumbers increased in body size during the period of study (Figure 1, Table A1 and Table A2). In contrast, Hidden Bay showed no sign of growth, or at least average body size increased only slightly. The increases in body lengths of *H. fuscogilva* at Wadi Quny were much higher than those of the original population at Pharoan Island. For example, individuals

that began with a mean length of 3.51 cm on April 2013 reached 12.54 cm after two years at Pharoan Island (Table A1). At Wadi Quny, individuals that began with mean length of 3.58 cm at April 2013 reached 25.87 cm after the same period (Table A2). There were significant differences ($P < 0.0001$) in lengths of *H. fuscogilva* between the two sites from month 4 onwards.

When growth rates of the three populations were compared, it became evident that individuals at Hidden Bay initially had negative growth, and lost body mass during the first months (Figure 2). This was followed by an apparent modest increase in body length due to the disappearance of smaller individuals, which caused the average body length to appear to remain constant or increase slightly.

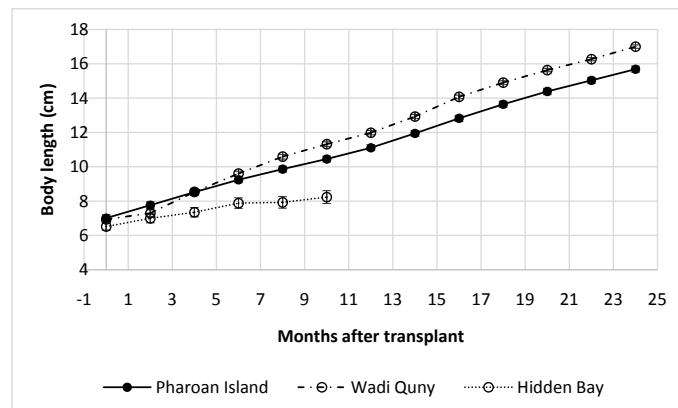


Figure 1. Average size (body length) of *H. fuscogilva* at Pharoan Island compared to those transplanted to Wadi Quny and Hidden Bay from April 2013 (month 0) to April 2015 (24 months later). Symbols indicate mean values \pm SE. Some SE bars are smaller than symbols. Initial sample sizes consisted of $N = 100$ marked juvenile individuals.

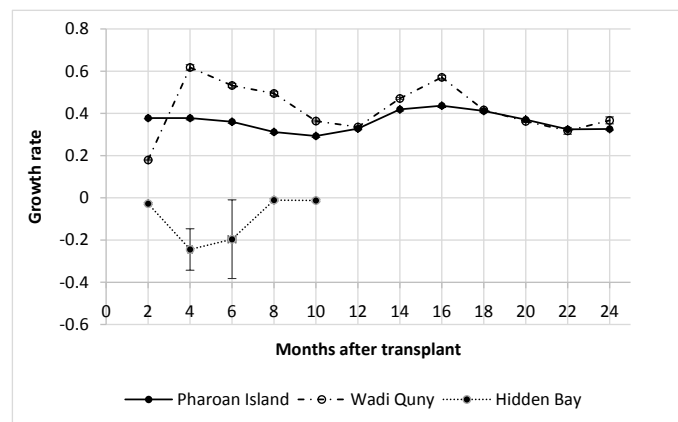


Figure 2. Comparison of growth rates of *H. fuscogilva* between the restocked juveniles at Wadi Quny and Hidden Bay with the original population at Pharoan Island during the period from April 2013 to April 2015. Symbols indicate mean values \pm SE. Some SE bars are smaller than symbols. Initial sample sizes consisted of $N = 100$ marked juvenile individuals.

When growth rates were examined relative to body size, it was apparent that smaller individuals had slightly higher growth rates than larger individuals. At Pharoan Island, growth rate ranged between 0.27 to 0.45 cm/month for size class 3 - 3.9 cm and from 0.21 to 0.30 cm/month for size class 9 - 9.9 cm (Figure 3), the same pattern was observed at Wadi Quny (Figure 4) although absolute growth rates were higher.

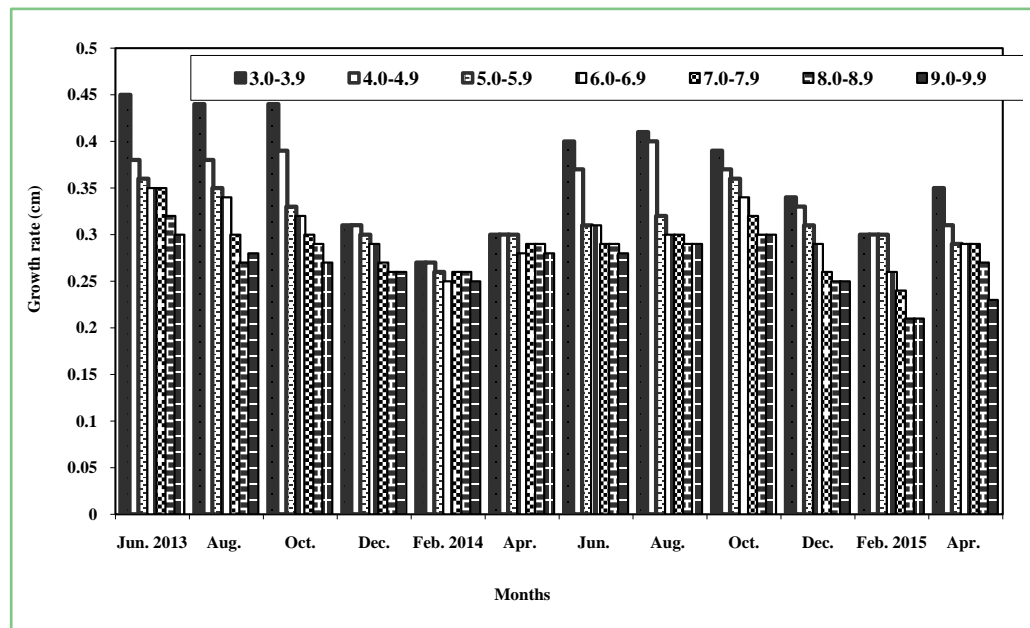


Figure 3. Growth rates of different size classes of the original population of *H. fuscogilva* at Pharoan Island during the period from April 2013 to April 2015.

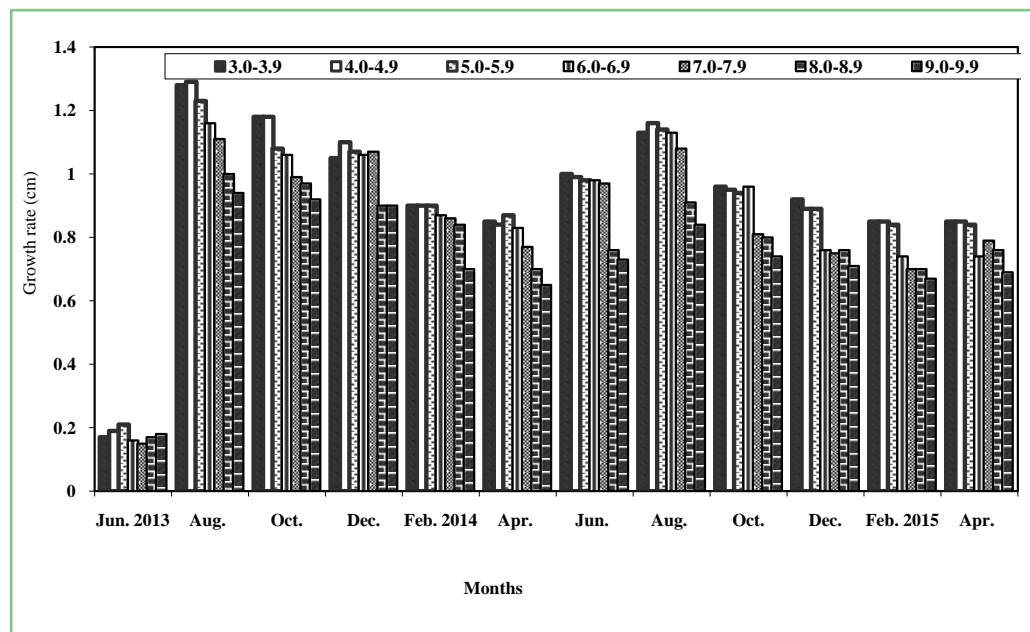


Figure 4. Growth rates of different size classes of the restocked population of *H. fuscogilva* at Wadi Quny during the period from April 2013 to April 2015.

Seasonal differences in growth rate were also observed. At both high-quality sites, the minimum growth rate occurred during winter between February and April (months 10 - 12 and 22 - 24), while the maximum growth rate was recorded between June and October (months 2 - 6 and 14 - 18). Comparison of growth rates between the two sites revealed that the restocked *H. fuscogilva* juveniles at Wadi Quny increased body length faster (ranged between 1.29 and 0.65 cm/month) than the original population at Pharoan Island (ranged between 0.45 and 0.21 cm/month) (Figure 3). There were also significant differences in growth at the two sites during the summer months (Table 3). Growth rates at Wadi Quny were low after the first two months after transplantation (by June 2013), ranging between 0.21 and 0.15 cm/month, but increased from August 2013 onwards.

3.4. Mortality

Natural mortality is an important factor in determining the success of any given population to disperse and establish at a new area. Results from this study showed that natural mortality (disappearance) of *H. fuscogilva* juveniles ranged from 1% to 2% at Pharoan Island (original population) and ranged between 0.5% and 0.75% at Wadi Quny (released population) during the two-year study period. At Wadi Quny mortality occurred (0.75%) shortly after the restocking process, but after two months only a few juveniles died. Conversely, Hidden Bay exhibited high mortality (Figure 5). By two months after introduction to the site, 49% of the restocked *H. fuscogilva* juveniles were dead. The mortality rate increased to 59% by August 2013; 76% by October 2013; 81% at December 2013; 96% at February 2014 and all animals had completely disappeared by April 2014 (Figure 5).

The size of individuals played an important role in mortality. There was a correlation ($P = 0.003$) between the size of the individuals and mortality at Hidden Bay, with smaller sizes suffering a higher mortality rate. For example, in size class 3 - 3.9 cm, 84.6% of the individuals were dead after two months. The mortality decreased to 55.56% for size class 4 - 4.9 cm, 53.86% for size class 5 - 5.9 cm, and 26.67% for size class 9 - 9.9 cm (Figure 5). Larger individuals survived for a longer time than smaller ones; whereas all individuals in size class 3 - 3.9 cm were dead at October 2013, size class 4 - 4.9 persisted until December 2013, size classes 5 - 5.9, 6 - 6.9 and 7 - 7.9 cm survived until February 2014 and individuals in both size classes 8 - 8.9 and 9 - 9.9 did not disappear until April 2014 (Figure 5).

3.5. Assessing Restocking Success

The present study generally followed a BACI (Before-After-Control Impact) approach [32] [33] to assess the success of the restocking of *H. fuscogilva* juveniles from the original population at Pharoan Island to the released populations at Wadi Quny and Hidden Bay. Surveys conducted at the original (the control site)

before, during and after the restocking effect (e.g. after 1 and 2 years) allowed better assessment of the effect of restocking from natural variability arising from other environmental factors.

Our findings indicate that the restocking of *H. fuscogilva* was successful at Wadi Quny, where the population density increased from 2.6 ind./100m² at the releasing time to 4.0 ind./100m², one year after the release (April 2014) and to 9.8 ind./100m² after two years (April 2015). In contrast, Hidden Bay failed to support the restocking. Initial densities were 2.9 ind./100m² at the release time but all animals had completely disappeared before the end of the first year (Figure 6). Densities at the original population, Pharoan Island, remained unchanged during the study period.

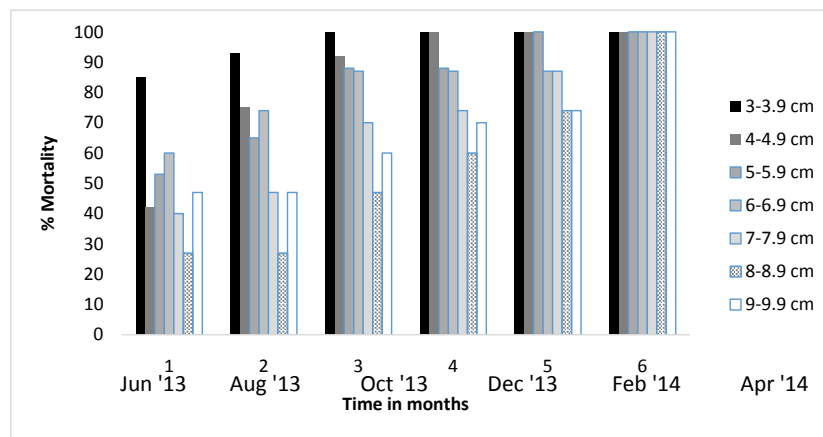


Figure 5. Mortality (or disappearance) of different size classes of the released juveniles of *H. fuscogilva* at Hidden Bay during the period from April 2013 to April 2014. Initially, ten animals in each size class were transplanted.

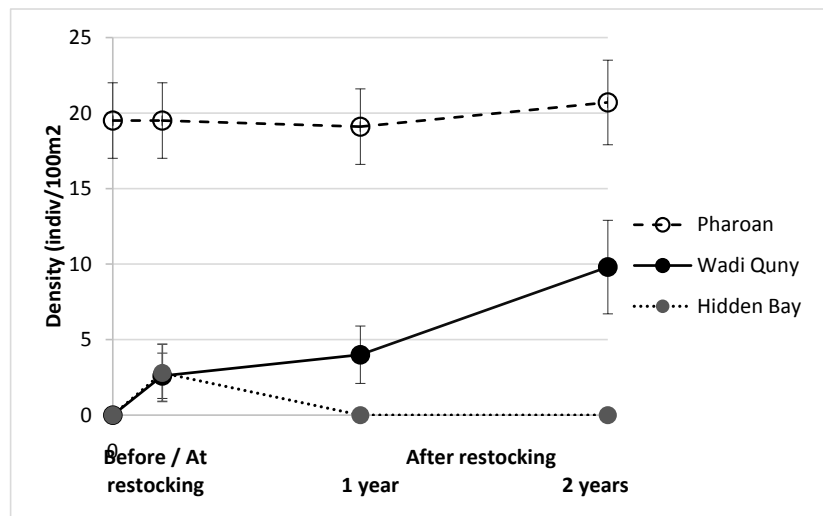


Figure 6. Assessment of the restocking success (density of animals) of *H. fuscogilva* juveniles before, at and after (1 year and 2 years) transplanting from the original population at Pharoan Island (Control) to the two released populations at Wadi Quny and Hidden Bay using BACI (Before-After-Control Impact) design.

4. Discussion

During the past few years, over-exploitation of sea cucumber in the Gulf of Aqaba has caused a severe decline in the population densities of almost all species [25] [34] at most locations monitored [26]. The high fishing pressure leads to the disappearance of many commercial species and reduction of the others. The recovery of over-fished sea cucumber stocks is a lengthy process and can take several years [35] [36] [37]. The enhancement of natural populations at over-fished areas with new wild stocks combined with an adequate management scheme is recommended as a management strategy. In the present study, we translocated *H. fuscogilva* juveniles ranging in size from 3 to 10 cm from a flourishing population at Pharoan Island to two sites (Wadi Quny and Hidden Bay). All three sites have favorable environmental conditions for sea cucumbers in terms of food availability and suitable substrate. After two years, the introduced population of *H. fuscogilva* showed a marked success in Wadi Quny, with sustained densities, high survival and even higher growth rates than the original population at Pharoan Island. In contrast, Hidden Bay individuals exhibited negative growth rates and very low survival, especially among smaller sized individuals.

4.1. Species Ecology

Favorable environmental conditions are no longer the only factor controlling the existence of sea cucumber at the Gulf of Aqaba; over-exploitation from commercial harvests strongly influences sea cucumber densities and distribution [26]. Even though the chosen sites (Pharoan Island, Wadi Quny and Hidden Bay) all appeared to have good environmental conditions for the existence and well-being of sea cucumbers, their sea cucumber fauna varied. Pharoan Island supported a very high species richness (22 species) and densities, most likely due to the protection of the site with a coast guard station, which prevents fishing. The same number of species was recorded at Pharoan a decade ago [26]. On the other hand, Wadi Quny supported very low taxonomic richness (4 species) and density, illustrating a dramatic decrease in sea cucumber density and diversity in just the past few years due to fishing pressure and weak protection and management. The site supported 12 species in 1995, 8 species in 2002 [25] [35], and only 4 species in 2003 [26]. After the sea cucumber population was decimated, fishermen moved to other areas, providing the opportunity for restocking of *H. fuscogilva* at the site on April 2013. The species not only re-established at the site but also grew well due to suitable natural conditions and the absence of sea cucumber fishing. In contrast, at Hidden Bay in spite of the existence of apparently favorable environmental conditions and no fishing pressure, there was low sea cucumber richness (3 species) and *H. fuscogilva* transplanted to the site did not succeed.

Habitat suitability is a critical factor that can limit the number of individuals that can recruit successfully without retarded growth and survival [9]. High

population densities can be achieved when the number of individuals and/or species at the site utilize the habitat efficiently but do not hamper the growth of the individuals from stress or lack of food, substrate, space or other resources needed for established and growth of the species [10]. The greatest densities of *H. fuscogilva* were observed at Pharoan Island, where the values were almost on order of magnitude higher than those at the other two sites. *H. fuscogilva* showed negligible temporal variation in density at Pharoan Island during the period of study (April 2013-April 2015). An earlier study [38] also reported that there was no significant difference in temporal abundance measured on a monthly basis over a 14-month period. This suggests that Pharoan Island may be near its full carrying capacity. In contrast, at Wadi Quny, sea cucumbers increased rapidly in density, especially in the second year (April 2014-April 2015). The rapid increase suggests the high suitability of habitat at this site could sustain many more individuals and perhaps additional species.

Hidden Bay initially appeared to have favorable conditions that could sustain a large sea cucumber population. However, the density of *H. fuscogilva* decreased from the beginning of the study and disappeared after January 2014. The failure of *H. fuscogilva* to establish at this site may be attributed to the shallow depth and high water temperature. In addition to substrate [39] [40], food availability [22] [41], a variety of niches and small numbers of natural predators [42] [43], depth is one of the most important factors controlling the distribution and well-being of sea cucumber species [44] [45] [46]. *H. fuscogilva* is a deep water species, usually found at depths ranged between 10 to 40 meters [45] on clean sand of coral origin on the reef slopes of fringing, patch or barrier reef [28]. Temperature plays a very important role in the well-being of the sea cucumber. The movement and feeding activity of young sea cucumbers sharply decreased when water temperature exceeds 23°C [47]. When environmental factors at the two release sites (Wadi Quny and Hidden Bay) are compared, it appears that Wadi Quny has high availability of food, suitable substrate, high variety of niches and a low number of natural enemies. In addition, water depth ranges between 20 to 25 m, and temperatures at this depth do not exceed 22°C during the summer season. On the other hand, Hidden Bay also appears to have high food availability, suitable substrate, a high variety of niches and low numbers of natural enemies. However, the shallow depth of the site, which ranges between 1.2 and 2.5 meters, accompanied by higher water temperatures, which can exceed 36°C during the summer season, cause the site to be unfavorable. Subsequently, transplanted animals failed to establish at the site and disappeared after 10 months.

The analysis of benthic habitat in the current study suggests that *H. fuscogilva* prefers sandy habitat, followed by seagrass. Similar findings were reported at New Caledonia [1]. Low densities of the species were recorded from the coral habitat only from Pharoan Island, indicating that coral is not the favorable habitat for the species and it occasionally exists on corals. The study never recorded

H. fuscogilva from dead corals or rocks.

The right habitat features to release juveniles are critical for restocking. In optimal habitats, the survival of juveniles can exceed 90%, whereas survival in unsuitable habitat is much lower and highly variable [16] [48] [49]. The best habitat for releasing juveniles may not be the same as the habitats where adults are found [9] [50]. Adult habitat may offer better foods but a higher risk from predators. Moreover, juveniles may survive and grow better in certain microhabitats within the general habitat in which they occur with adults. The presence of very a small density of *H. fuscogilva* in seagrass habitat at Wadi Quny suggested that juveniles preferred the same habitat of the adult (sandy habitat).

4.2. Growth Rate

Growth rates of *H. fuscogilva* showed marked differences between sites and seasons. The released population at Wadi Quny showed a higher growth rate than the original population at Pharoan Island during the two years of study period except for the first two months when the growth rate at Wadi Quny was very low. This may be due to the need for released juveniles to acclimatize on the new site. The released population at Hidden Bay showed a negative growth until animals completely disappeared after a few months from introducing to the site. Growth rates of holothuroids are affected by population density [51] and the availability of food, which is determined by the number of animals feeding upon the resource [52]. From the results obtained it seems clear that Wadi Quny had more suitable habitat and food resources for the density of animals located there compared to Pharoan Island.

The high growth rates of *H. fuscogilva*, especially at Wadi Quny, were greater than rates reported in the literature for *H. scabra* and *Actinopyga echinites* (0.5 cm/month) [28]. Generally, at Wadi Quny the commercial length of *H. fuscogilva* was achieved within 10 to 12 months after transplantation. This is shorter than the period taken by wild juveniles of *Apostichopus japonicas* when reared in ponds (10 - 18 months) at Dalian [47]. *H. fuscogilva* at Pharoan Island needed a longer period (22 - 24 months) to reach similar lengths. When food is plentiful, animals can grow faster. *A. echinites* in aquaria grow to sexual maturity within one year and full size in another year [28].

The growth rates of sea cucumber are closely related to water temperature, with the highest growth occurring in spring and winter months [47]. The optimum water temperature for sea cucumber growth is 17°C, but juveniles can maintain high growth rates at 24°C to 25°C [53]. The present study recorded higher growth at both Pharoan Island and Wadi Quny during summer and autumn, where *H. fuscogilva* populations were at depths of 20 to 25 m and water temperatures of 22°C in summer and 20°C in autumn, favorable temperatures for the species to achieve high growth rates. This temperature decreased to 12°C in winter, which slowed growth rates. At Hidden Bay, the negative growth may be attributed to the unfavorable shallow depth (1.2 to 2.5 m) and high fluctua-

tion in water temperature across different seasons.

4.3. Mortality

One concern arising from the restocking of natural juveniles of *H. fuscogilva* is the relatively high mortality rates that could occur. The success of *H. fuscogilva* at any particular habitat depends on several factors such as substrate, depth, settlement, distance from the shoreline, availability of food and water temperature [40] [53] [54] [55] [56]. Depth is particularly important for *H. fuscogilva*; the species is more abundant at the deeper waters ranged from 10 to 40 m [45]. In our study, mortality was very low at both Pharoan Island and Wadi Quny. The high mortality rate at Hidden Bay may be attributed to the shallow depth and higher water temperatures. The depth at the site ranged between 1.2 and 2.5 m, which is not suitable for *H. fuscogilva*. The water temperature ranged between 30°C and 32°C in summer and 7°C to 10°C in winter. The juveniles of *H. fuscogilva* likely could not tolerate the extreme summer temperatures at Hidden Bay and died a few months after release.

This study also revealed that the small juveniles at Hidden Bay had a higher mortality rate than the bigger ones. The same conclusion was reached in another study that reported higher survival in larger sized juveniles; they also found that individuals larger than 2 cm had a survival rate of at least 20% - 30% under favorable conditions [47].

4.4. Restocking Success

There is much recent interest worldwide in restocking sea cucumbers in the wild using hatchery-produced juveniles [13] [14] [15] [57]. However, the habitat preferences and ecology of many species of juvenile sea cucumber is poorly understood [48] [49] [50] [58] [59]. No study to date has evaluated the restocking of native juveniles by transferring them from sites of high density to a new site that would allow the juveniles to survive, grow and increase in density. Our study demonstrates that transplanting can be a viable alternative to restocking with hatchery juveniles to help rebuild damaged sea cucumber populations while maintaining the genetic diversity of wild populations, which is sometimes a concern [15] [17] [60]. Not only did the restocking of *H. fuscogilva* succeed at Wadi Quny, it resulted in a higher density of animals than the original population at Pharoan Island after two years. Wadi Quny appears to have a higher carrying capacity (food availability and space) that permits strong growth, survival and increase in *H. fuscogilva* population density.

Sea cucumbers are among the most fishery-targeted marine organisms due to their high demand in the fish market. The overfishing exerted on these species has severely depleted stock worldwide. Once depleted, fishermen move and seek new fishing grounds. The high-value and easy collection of sea cucumber due to their slow movement have caused the severe depletion of stocks all over the world. The need for new techniques to compensate and regenerate depleted stocks is very important worldwide.

Ethical Approval

All applicable international, national and/or institutional guidelines for the care and use of animals were followed by the authors.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Appendix

Table A1. Increase in length (cm) of different size classes of *H. fuscogilva* during the period of study from April 2013 to April 2015 from the original population at Pharoan Island (values in bold are S.D.).

Apr. 2013	Jun. 2013	Aug. 2013	Oct. 2013	Dec. 2013	Feb. 2014	Apr. 2014	Jun. 2014	Aug. 2014	Oct. 2014	Dec. 2014	Feb. 2015	Apr. 2015
3.51	4.44	5.32	6.20	6.98	7.52	8.12	8.92	9.74	10.52	11.24	11.84	12.54
0.24	0.28	0.26	0.28	0.25	0.21	0.29	0.36	0.33	0.41	0.36	0.36	0.36
4.51	5.28	6.04	6.82	7.44	7.98	8.58	9.32	10.12	10.86	11.58	12.18	12.80
0.36	0.27	0.33	0.35	0.41	0.44	0.34	0.33	0.36	0.35	0.42	0.44	0.43
5.51	6.23	7.11	7.77	8.37	8.89	9.49	10.11	10.75	11.47	12.13	12.73	13.31
0.28	0.28	0.32	0.31	0.33	0.36	0.40	0.62	0.60	0.44	0.40	0.46	0.48
6.52	7.32	7.90	8.54	9.32	9.62	10.18	10.80	11.40	12.08	12.72	13.24	13.87
0.31	0.37	0.53	0.51	0.36	0.32	0.32	0.30	0.32	0.41	0.40	0.36	0.37
7.56	8.31	8.91	9.51	10.05	10.57	11.15	11.73	12.33	12.97	13.59	14.47	15.05
0.32	0.33	0.19	0.13	0.18	0.16	0.12	0.09	0.09	0.11	0.16	0.17	0.17
8.64	9.28	9.82	10.40	10.92	11.44	12.02	12.60	13.18	13.78	14.36	14.78	15.32
0.26	0.26	0.26	0.25	0.25	0.23	0.27	0.24	0.25	0.26	0.24	0.21	0.20
9.39	9.99	10.55	11.09	11.61	12.11	12.67	13.23	13.81	14.41	14.95	15.37	15.83
0.34	0.31	0.36	0.40	0.43	0.42	0.40	0.43	0.38	0.38	0.37	0.37	0.37

Table A2. Increase in length (cm) of different size classes of *H. fuscogilva* during the period of study from April 2013 to April 2015 from the restocked population at Wadi Quny (values in bold are S.D.).

Apr. 2013	Jun. 2013	Aug. 2013	Oct. 2013	Dec. 2013	Feb. 2014	Apr. 2014	Jun. 2014	Aug. 2014	Oct. 2014	Dec. 2014	Feb. 2015	Apr. 2015
3.58	3.93	6.49	8.85	10.95	12.75	14.45	16.45	18.71	20.63	22.47	24.17	25.87
0.31	0.28	0.35	0.35	0.43	0.43	0.40	0.40	0.39	0.38	0.38	0.37	0.37
4.57	4.94	7.52	9.88	12.08	13.88	15.56	17.54	19.86	21.76	23.54	25.24	26.49
0.24	0.22	0.21	0.23	0.21	0.21	0.23	0.23	0.21	0.21	0.23	0.23	0.21
5.46	5.88	8.34	10.49	12.63	14.43	16.17	18.13	20.41	22.29	24.07	25.75	27.43
0.29	0.33	0.35	0.31	0.31	0.27	0.29	0.30	0.30	0.31	0.29	0.29	0.27
6.46	6.79	9.11	11.23	13.35	15.09	16.75	18.71	20.97	22.89	24.41	25.89	27.37
0.29	0.27	0.25	0.20	0.16	0.18	0.15	0.11	0.13	0.15	0.15	0.12	0.12
7.55	7.85	10.07	12.05	14.19	15.91	17.45	19.39	21.55	23.17	24.67	26.07	27.65
0.27	0.31	0.30	0.27	0.29	0.35	0.34	0.33	0.29	0.31	0.29	0.33	0.30
8.45	8.81	10.81	12.75	14.55	16.23	17.63	19.15	20.97	22.57	24.09	25.49	27.01
0.28	0.28	0.23	0.20	0.14	0.12	0.13	0.17	0.16	0.16	0.14	0.15	0.20
9.51	9.88	11.76	13.60	15.40	16.80	18.10	19.56	21.24	22.72	24.14	25.48	26.86
0.28	0.30	0.29	0.30	0.33	0.33	0.37	0.40	0.36	0.31	0.25	0.36	0.24