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The potential for propagation of the commercial sea cucumber *Isostichopus fuscus* (Ludwig, 1875) by induced transverse fission

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HIGHLIGHTS

- *Isostichopus fuscus* asexual reproduction was induced by fission and body parts regenerated in 90 days.
- A high protein diet with brown macroalgae powder produced a rapid growth of posterior body parts.
- This technique might be used for restoring wild populations of sea cucumbers.

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ABSTRACT

This study evaluated the potential to propagate asexually the brown sea cucumber *Isostichopus fuscus* by induction of transverse fission, and its ability to survive, grow and regenerate body parts into a whole animal. Two independent experiments were performed. Experiment 1: sixty-two adult animals (18.8 ± 0.2 cm and 368.1 ± 7.2 g) were cut six centimeters from the rear, and during this process they eviscerated. Survival of body-parts (anterior and posterior) of animals and regeneration times were evaluated, until all individuals showed complete regeneration in terms of its morphology (lasted 13-wk). Animals were maintained in starved condition and had high survivorship (100%). Complete regeneration occurred within 84 to 95 days. Experiment 2: 48 completely regenerated posterior body-parts of *I. fuscus* (with mouth and anus well developed) were used (lasted 13-wk) and animals were fed *ad libitum* four diets in powder and two controls: diet A with *Ascophyllum nodosum* and *Sargassum* spp; diet B was a commercial shrimp feed with a mix of proteins from marine animals and vegetal material; diet C with *Padina durvillaei* and *Sargassum ecuadoreanum*; and diet D with a mixture of diet C with calcium citrate and Vitamin D. Two controls were used. Survival was not affected by diet but this significantly affected somatic growth rate in length and weight. The fastest growth rates (in length and weight) were for diet B (0.50 ± 0.10 cm month^{-1} and 0.57 ± 0.11 g month^{-1}) and the lowest for diet A (0.15 ± 0.10 cm month^{-1} and 0.11 ± 0.07 g month^{-1}). No growth was detected in controls. *I. fuscus* had a high potential for regeneration. Our results encourage further research to explore the feasibility of mariculture and/or restoration programs of wild sea cucumber populations in Ecuador, using asexual propagation techniques for *I. fuscus*.

1. Introduction

The brown sea cucumber *Isostichopus fuscus* (Holothuroidea: Aspidochirotida) is an epibenthic large-size (19–25 cm and 100–410 g) deposit-feeder that inhabits sheltered (low-medium energy) rocky shores associated with foliate and crustose algae from the shallow subtidal to 39 m in rocky coasts from Baja California, México (including the Gulf of California) to northern Perú (Islas de Lobos de Afuera), including Galápagos, Socorro, Cocos, Malpelo and Revillagigedos islands (Maluf, 1988).

The global marketing for sea cucumber shows a recent increasing trend in response to a growing demand in Asia, but distribution channels are multilayered and inefficient, with a proliferation of redundant players and unsustainable fisheries. The potential for aquaculture has also been explored (Perez and Brown, 2012).

Ecuador reports six holothurian species with commercial value: *I. fuscus*, *Stichopus horrens*, *Holothuria atra*, *H. kefersteini*, *H. pardalis*, and *H. theeli*, but *I. fuscus* has a superb commercial importance to meet the increased demands of the "bêche-de-mer" or "trepang" (final dried product). It is the third highest-value species of sea

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cucumber in the market, reaching a maximum of USD $ 1200 per kg (dry weight) (Purcell et al., 2014).

The brown sea cucumber (*I. fuscus*) has a high potential to diversify mariculture (Hamel et al., 2003; Mercier et al., 2007, 2012; Purcell et al., 2012a,b) but paradoxically, private initiatives for culturing this sea cucumber species in Ecuador developed slowly and paused in 2000. Mercier suggests that disease may have been a bottleneck. This species is currently listed as endangered in the IUCN Red List of Threatened Species (IUCN, 2013; Mercier et al., 2013). However, since 2013, aquaculture of this valuable sea cucumber species has been advancing in larval rearing and juvenile production in captivity with a new validated technology at the Centro Nacional de Acuicultura e Investigaciones Marinas of the Escuela Superior Politécnica del Litoral of Ecuador, which might be helpful to restore wild populations. Within this perspective, hatchery is feasible (sexual reproduction), but is not the only option. Although there is no information indicating that fission has been used to rebuild populations elsewhere; this study addresses this new alternative exploring the asexual reproductive potential of this species.

The increasingly high demand for sea cucumber has impacted natural populations in Ecuador. At present, there is a ban on capture of *I. fuscus* in the mainland coast in Ecuador. This fishery was closed in 1992 (Acuerdo Ministerial N°147, RO 26, del 15 de septiembre de 1992), but populations are severely depleted and have not recovered, largely due to illegal fishing (Toral-Granda, 2008). However, in the Galápagos Islands, the fishery of *I. fuscus* is managed based on natural recruitment pulses (throughout its sexual reproduction) without a complete ban on fishing activities. However, this resource has diminished due to overharvesting, reaching densities lower than 0.1 ind.m^{-2} (Toral-Granda and Martínez, 2004; Toral-Granda, 2008; Aguilar et al., 2010; Castrejón and Charles, 2013; Purcell et al., 2013; Castrejón et al., 2014; Purcell et al., 2014; DPNG, 2015). According to Shepherd et al. (2004) a minimum of 1.2 ind.m^{-2} is required for a 50% fertilization success in *I. fuscus* which means that when density of adults is not high enough the 'Allee Effect' is likely to reduce recruitment levels below those necessary to sustain both the fishery and the population (Lundquist and Botsford, 2004; Uthicke et al., 2009).

Some holothurians are notable because they possess diverse regeneration abilities after asexual reproduction by fission (Dolmatov, 2014). Therefore, studies on fission and its associated regeneration are essential for understanding the reproduction of holothurians and its potential to restore wild holothurian populations (Thorne et al., 2013). However, the relationship and synchrony between sexual and asexual reproduction in holothurians has still to be explored.

Research on the reproductive biology of *I. fuscus* is limited (Toral-Granda, 1996; Herrero-Pérezrul et al., 1998; Sonnenholzner et al., 2013) and this does not allow rigorous population studies directed toward its recovery. Although it is well known that most populations of *I. fuscus* (like many other sea cucumber species) are maintained by sexually produced progeny (Mercier et al., 2007), nothing is known about their ability to reproduce asexually by transverse binary fission. If body parts regenerate to form complete adult individuals with full sexual reproduction capabilities (Uthicke, 1997, 2001; Lee et al., 2008) important implications on the structure and dynamics of *I. fuscus* populations would be expected. To date, asexual reproduction has been confirmed in 16 other species of sea cucumbers (Dolmatov, 2014).

Recovering sea cucumber populations by using regenerated artificial fission animals might become a successful management practice for *I. fuscus* and other fissiparous sea cucumber species, but to date no previous studies are available to support this alternative, mainly because this is not a common mariculture intervention. It has been determined that gonad regeneration apparently occurs in all fissiparous holothurians, as even populations with a high degree of fission continue to reproduce sexually, and the proportion of asexual individuals (or clones) does not increase (Emson and Mladenov, 1987; Uthicke, 1997; Conand et al., 2002). Kille (1942) studied the development of the reproductive system after fission in *Holothuria parvula*, and histological analysis showed that the primordium of the reproductive system was observed only in those individuals which had completely formed all other organs. These studies are important and useful to learn more about regeneration abilities to develop cultivation technologies and to restock holothurian populations (Dolmatov, 2014). Indeed, more studies are needed focusing on this issue for fissiparous tropical holothurians.

Further, studies on dietary requirements are needed for inducing a rapid growth rate after fission in *I. fuscus* before releasing them in the sea. Feeding is a key element for advancing aquaculture techniques for high-value commercial sea cucumbers (Liu et al., 2010; Shi et al., 2015; Yanfeng et al., 2015; Zacarías-Soto and Ólvera-Novoa, 2015; Qinzeng et al., 2016).

I. fuscus preferentially inhabits subtidal areas with macroalgae and rich detrital food source. They feed primarily on benthic biota: small organisms (e.g., copepods); microorganisms (e.g., bacteria, protozoans, diatoms, and fungi), and decaying material in the sediment (e.g., seaweed, shell fragments, sponge spicules, etc.) (Sonnenholzner and Cajas, 1997; Sonnenholzner, 2014). Sea mud and powdered seaweed are used as the main components of formulated feeds for sea cucumber farming, mixed with commercial enriched products for shrimp, tilapia, and rabbit (Liu et al., 2010; Zacarías-Soto and Ólvera-Novoa, 2015). Considerable research efforts have been made to understand the feeding ecology and physiology of various sea cucumber species with high commercial value and aquacultural interest (Zhou et al., 2006; Slater and Carton, 2009; Slater et al., 2011; Sicuro and Levine, 2012; Zacarías-Soto and Ólvera-Novoa, 2015), but to date, not for *I. fuscus*.

To address these subjects, the goals of this study were to assess the potential of *I. fuscus* to be propagated asexually by (1) estimating the regeneration time of individuals after forced transverse fission; (2) measuring survival of body parts in captivity conditions; and (3) comparing the effect of different diets on growth rate (in length and weight).

2. Materials and methods

2.1. Ethic statement

Specific permits were required from the Environmental Ministry and Fisheries Resources of Ecuador for collecting specimens from the marine protected area *El Pelado* Island (MpA-EP), Santa Elena, Ecuador. Ethical approval was not required for this study because no endangered animals were involved. Specimen collection and maintenance were performed in accordance with standard protocols (Elwood, 2011).

2.2. Sample collection and acclimation process

One hundred adult sea cucumbers *I. fuscus* (20.1 ± 0.8 cm and 400.2 ± 7.2 g, in length and body weight, respectively) were collected and held at the Centro Nacional de Acuicultura e Investigaciones Marinas, Escuela Superior Politécnica del Litoral, San Pedro de Manglaralto, Santa Elena, Ecuador (1°57′20.84″S, 80°43′47.54″O) in March 2014. Length and body weight were determined using a plastic ruler in centimeter units and a spring scale with a 0.1-g resolution, respectively.

Sixty sea cucumbers were transferred to three rectangular 1000 l concave-base fiberglass tanks (2.2 m × 1.4 m × 0.6 m) at similar stocking densities (20 holothurians per tank). These animals were

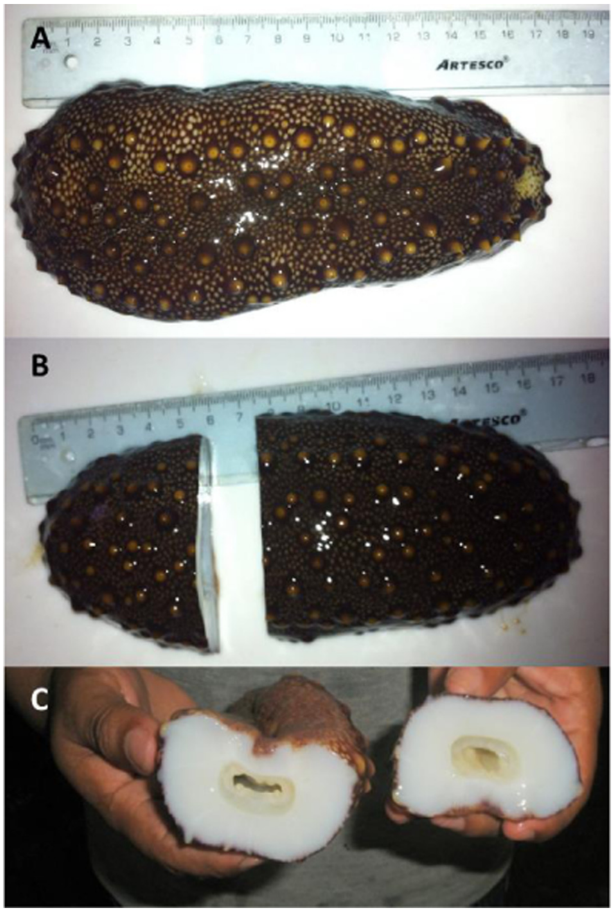

Fig. 1. *I. fuscus* (25 cm in length and 432 g in weight). (A) Anterior body part (19 cm in length and 210 g in weight); (B) posterior body part (6 cm and 120 g in weight); (C) transversal view of a cut eviscerated animal. Scale in centimeters.

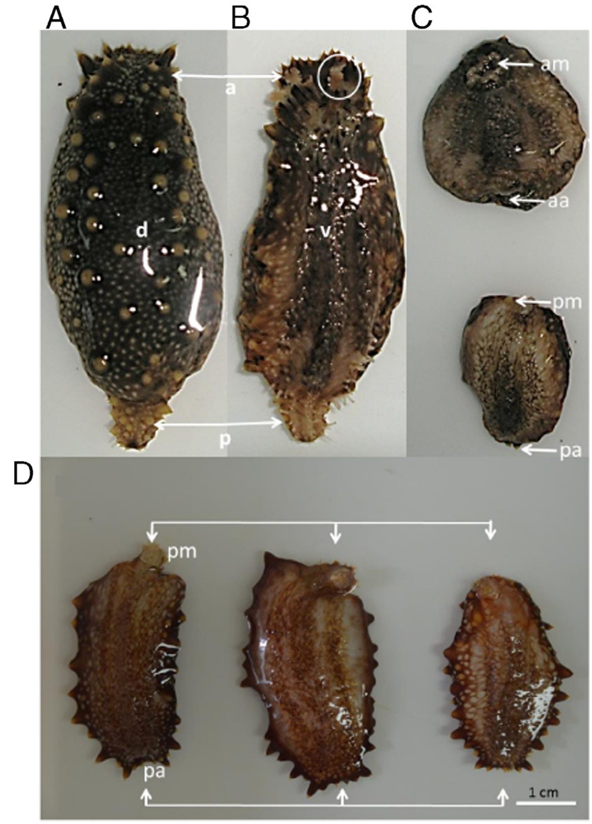

Fig. 2. Regeneration of body-parts of *I. fuscus*. (A) Dorsal view and (B) ventral view of a posterior body-part of a relaxed individual; (C) ventral views of anterior and posterior body-parts of two contracted individuals; (D) ventral view of posterior body-parts of three relaxed individuals. Letters indicate: (a) anterior body part, (p) posterior body part, (d) dorsal region, (v) ventral region, (am) mouth in anterior body-part; (aa) anus in anterior body-part, (pm) mouth in posterior body-part, (pa) anus in posterior body-part.

acclimated to the laboratory conditions for two weeks, they were not fed one-week prior to experiment 1, and feces were daily removed from the tanks. Experiment 1 was performed during the season in which sexual reproductive activity has not been reported. Previous observations indicate that *I. fuscus* in the MpA-EP mainly reproduces sexually during the equatorial summer season (between July to October 2014) when colder sea surface temperature occurs.

During the experiments seawater was filtered (5 and 1 μm), exchanged once a day, and aeration was provided continuously. Temperature was 25.8 ± 1.5 °C; salinity 34 psu; pH 7.9–8.2; Dissolved Oxygen (DO) > 5.2 mg l^{-1}; ammonia < 0.10 mg L^{-1} and photoperiod 12: 12 (L/D).

2.3. Experiments

2.3.1. Experiment 1: Induced transverse cutting and regeneration

Sixty adult sea cucumbers (mean 18.8 ± 0.2 cm and 368.1 ± 7.2 g, in length and weight, respectively) were cut in two on March, 22nd 2014. The cut was done six centimeters from the rear of the body, and during this process animals eviscerated (Fig. 1(A–C)). Length and weight of the anterior and posterior body-parts of the sea cucumbers were recorded after their induced transverse fission at the beginning and at the end of the experiment.

Anterior and posterior body-parts were transferred to six rectangular concave-base fiberglass tanks of 1-m^3 (2.2 m × 1.4 m × 0.6 m) at similar stocking densities (three tanks with 20 anterior each and three tanks with 20 posterior body parts each). Sea cucumbers were reared indoors in darkness condition with open flow of seawater at relatively constant temperature (averaging 23.6 °C). This experiment lasted 13-wk (March–June, 2014) and animals were maintained in starved condition. At the end of experiment 1, animals were fed two times a week for two weeks with a mix of algae powder and clay soil, to complete body regeneration (anterior and posterior body-parts).

Survival (%) was calculated combining the data from both anterior and posterior body-parts for each individual, and status of regeneration were surveyed. For the first month, observations were done weekly and thence fortnightly (two times a day: in the morning and evening, 08:00–09:00 to 15:00–16:00 hours, respectively). During the regeneration period, detached tissue (hard tissue and mucous) from the body-parts of animals were collected with a mesh to register total weight of lost tissue.

The success of fission of anterior and posterior body-parts of *I. fuscus* was determined by the functional responses of their body parts as follows: (1) anterior parts = animals can gently squeeze water from the cloaca by their dilated anus (evidence of pumping water of a functional anus and presence of respiratory tree), and fecal pellets production near of animals (evidence of functional tentacles and intestine) (Fig. 2(A, B)); and (2) posterior parts = a functional mouth is noted with the presence of oral tentacles and fecal pellets observed near of animals (evidence that animals had eaten) (Fig. 2(C, D)).

Table 1
Initial and final lengths (cm) and body weights (g) of *I. fuscus* in Experiment 2 (mean ± se, $n = 8$). See text for the description of the four diet treatments (A–D) and two controls (C1, C2). Different letters indicate significant difference among different diet treatments ($P \leq 0.05$). Experiment lasted 13 wk.

	Treatments					
	Diets				Controls	
	A	B	C	D	C1	C2
Length (cm)						
Initial	5.7 ± 0.1	5.3 ± 0.8	5.2 ± 0.6	5.5 ± 0.6	6.0 ± 0.3	5.6 ± 0.2
Final	6.5 ± 0.2 b	7.5 ± 0.9 a	6.7 ± 0.3 bc	6.7 ± 0.4 bc	5.6 ± 0.6 d	5.7 ± 0.3 d
Weight (g)						
Initial	32.1 ± 14.3	22.7 ± 7.7	23.3 ± 8.7	23.9 ± 13.2	21.8 ± 11.5	30.5 ± 4.8
Final	25.1 ± 3.0 c	33.3 ± 2.9 a	30.7 ± 1.9 ab	26.9 ± 4.7 bc	16.7 ± 4.7 d	21.6 ± 0.9 cd

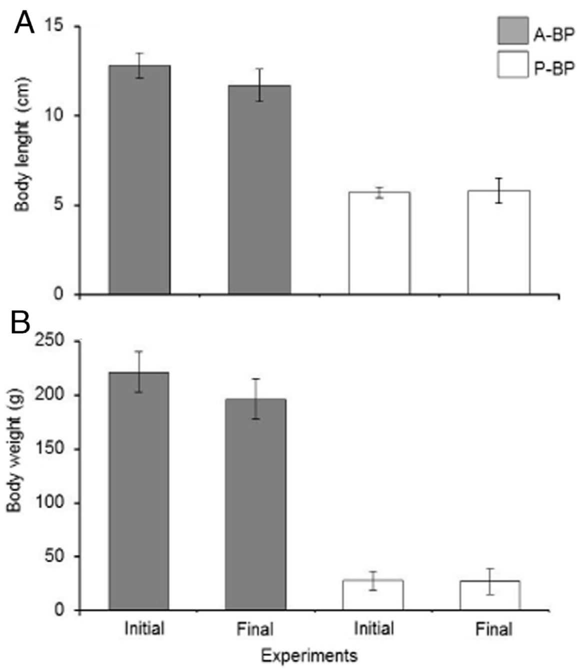

Fig. 3. Initial and final length and weight of anterior and posterior body-parts (A-BP and P-BP, respectively) of *I. fuscus* during experiment 1 which lasted 13 wk (means ± se).

2.3.2. *Experiment 2: Effects of different diets on regenerated I. fuscus body parts*

The posterior body-parts of *I. fuscus* were used in experiment 2, because these showed less variability in length and weight, than the anterior body-parts at the end of experiment 1 (Fig. 3(A, B)).

Forty-eight completely regenerated posterior body-parts of *I. fuscus* (with mouth and anus well developed, Fig. 2(D)) were randomly allocated to 40 L plastic boxes (40 cm × 25 cm × 40 cm). Each box contained one individual, under the same conditions as in the acclimation period. Prior to the start of the experiment, sea cucumbers were starved for 24 h, and the initial length and wet weight of posterior parts were determined individually (mean 5.8 ± 0.14 cm and 25.7 ± 1.80 g in length and weight, respectively). Six diet treatments and eight replicates per treatment were used in the experiment and there were no significant initial differences in length and weight between the experimental groups (ANOVA, $P > 0.05$, Table 1).

The six treatments included four diets and two controls. Diets A, C and D were based on powdered seaweed. Diet A was a mixture of *Ascophyllum nodosum* (L.) and *Sargassum* spp (Algatec WP, Lignoquim); diet C was a mixture of *Padina durvillaei* Bory and *Sargassum ecuadoreanum* Taylor; and diet D was a mix of diet (C) with calcium citrate 1.500 mg (equivalent to 315 mg of calcium D3 vitamin 200 IU). Diet B was a commercial formulated product for shrimp maturity, based on animal and vegetal proteins (EZ Mate, Zeigler). Two controls were included: (C1) only sediment (sandy and clay soil) and (C2) no sediment or diet. Proximate analysis of the four diets is summarized in Table 2. All diets were well-mixed with prepared sediment, except controls. Sediment was composed of sandy and clay soil. Ratio of sand and clay in sediments was 1:1. Diets A–D (30%) were mixed with sediment (70%) based on dry weights. The sandy and clay soil was ground and sieved through a 0.30-mm mesh. Sea cucumbers were fed once every two days. The amount of food supplied to sea cucumbers was between 5% and 7% of their average weight. Uneaten food was removed every 3 days.

Table 2
Proximate analysis of experimental diets fed to *I. fuscus*.

Nutrient content (%)	Diets			
	A	B	C	D
Protein	9	55	5	5
Lipid	3	12	7	7
Ash	65	13	35	35
Carbohydrates	18	10	45	45
Humidity	5	10	8	8

Sea cucumbers were measured and weighed individually every 20 days. During the experiment growth rates, fecal production rates and uneaten feed of *I. fuscus* were determined. Feces were separately collected three times a week by siphoning, and then dried at 60 °C to constant weight. Wet weight measurements were taken within 1 min of removal from seawater after blotting specimens on paper towels. This experiment lasted 13-wk (June–September, 2014).

Sea cucumbers were fed *ad libitum* three times a week at 16:00 h and kept in a semi-closed recirculating system of seawater. Water temperature was relatively constant, averaging 23.6 °C.

2.4. *Data calculation*

Specific growth rates (SGR % d^{-1}) and fecal production rate (FPR % d^{-1}) were calculated in length and body weight of each individual:

$$\text{SGR (in length)} = 100\,(\ln L_2 - \ln L_1)t^{-1} \quad (1)$$

$$\text{SGR (in weight)} = 100\,(\ln W_2 - \ln W_1)t^{-1} \quad (2)$$

$$\text{FPR} = 100 \times F/[t\,(W_1 + W_2)/2] \quad (3)$$

where L_1 and L_2 and W_1 and W_2 are the initial and final lengths (in centimeters) and the initial and final wet body weights (in grams) of the sea cucumber individuals, respectively, t is the duration of the experiment (in days), F is the average dry weight of feces (g). The coefficients of variation (CV) were calculated for growth rates in length and weight.

Table 3
Results of one-way ANOVAs for *I. fuscus* in experiment 2 for measuring the effects of different diets on regenerated *I. fuscus* posterior body parts obtained by fission. Experiment lasted 13 wk.

Factors	SS	DF	MS	F	P
Survival	1.900	5	0.400	0.500	0.7938
Error	34.000	42	0.800		
SGR (in length)	1.638	5	0.328	12.464	0.0000
Error	1.104	42	0.026		
SGR (in weight)	2.389	5	0.478	5.305	0.0007
Error	3.783	42	0.090		
FPR (in feces)	296.491	5	59.298	21.877	0.0000
Error	113.841	42	2.710		

2.5. Statistical analysis

The difference in the SGR (in length and weight) and FPR between the diets were compared with one-way analysis of variance (ANOVA) followed by Tukey *post hoc* test for multiple comparisons with a significance level of 0.05. Prior to analysis, raw data were diagnosed for homogeneity of variance with Cochran's test. Differences between means of survival, regeneration time, and length and weight, between anterior and posterior body-parts at the beginning and at the end of experiment 1 were analyzed using t-Student analysis. Percentage data were subjected to the square root arcsine transformation. Results are expressed as mean values $\pm$ standard error (se). Statistical analyses were performed using the software Statistica for Windows, Release 10.0. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Experiment 1

3.1.1. Regeneration time

During the experimental period, sea cucumbers lost part of their body tissue. Anterior body-parts of *I. fuscus* lost a high quantity of tegument tissue (average of 25 g per animal) during the first 3 weeks. In contrast, posterior body-parts of *I. fuscus* lost less tissue (average of 6 g per animal) during the first four weeks. Anterior and posterior body parts of *I. fuscus* were completely regenerated within three months (84–95 days) (Fig. 2(A–D)). The average regeneration times for anterior and posterior body parts were 89.7 $\pm$ 2.0 days and 89.3 $\pm$ 1.8 days, respectively, and there were no significant differences (t-student $= -0.312$, $df = 4$, $P = 0.3854$).

3.1.2. Survival rates

Survival rates of anterior and posterior body-parts of adults of *I. fuscus* were 100% and 98%, respectively, and this difference was no significant (t-student $= -0.530$, $df = 4$, $P = 0.3119$).

3.1.3. Length and weight

The anterior body-part of *I. fuscus* significantly decreased in length and weight (averages of 3% and 25%, respectively) at the end of the experiment (length: t-Student $= 5.377$, $df = 4$, $P = 0.0029$; and weight: t-Student $= 35.693$, $df = 4$, $P = 0.0000$, Fig. 3(A, B)). Contrasting with this, the loss in length and weight (averages of 2% and 8%, respectively) of the posterior body-part of *I. fuscus* was not significant at end of the experiment (length: t-Student $= -1.360$, $df = 4$, $P = 0.1227$; and weight: t-Student $= -0.070$, $df = 4$, $P = 0.4737$, Fig. 3(A, B)).

3.2. Experiment 2

3.2.1. Survival rates

Survival rates of posterior body parts was high (85%–90%) and did not differ significantly among diet treatments ($F = 0.500$ $P = 0.7938$, Table 3).

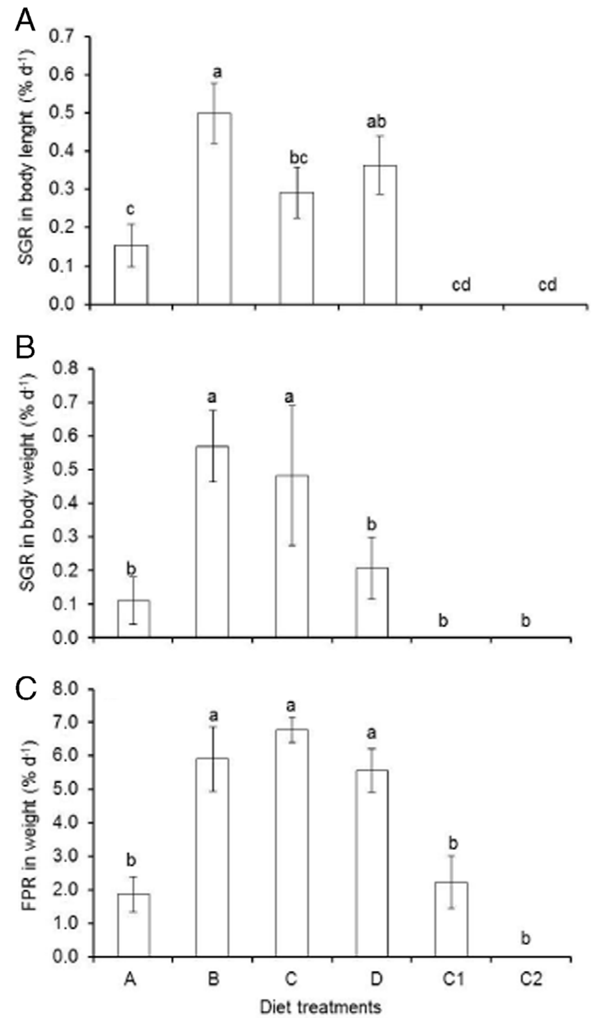

Fig. 4. Experiment 2: Effects of different diets on regenerated posterior body-parts of the sea cucumber *I. fuscus*. Specific growth rates in (A) length and (B) body weight (SGR $\%d^{-1}$), and (C) fecal production rates (FPR $\%day^{-1}$). Different letters indicate significant differences ($n = 8$; $P < 0.05$) and bars represent standard errors of the means.

3.2.2. Growth rate (in length)

There was no significant difference in the initial body length ($F = 2.207, P = 0.0714$, Table 1) between sea cucumbers allocated to the diet treatments. There was a significant effect of the diet on SGR in length ($F = 12.463$, $P = 0.0000$, Table 3). The mean SGR across all treatments was 0.30 ± 0.04 cm month^{-1}. The fastest growth rate was determined for diet B (0.50 ± 0.10 cm month^{-1}, CV = 44%, Fig. 4(A)). The lowest growth rates were determined for diet A (0.15 ± 0.10 cm month^{-1}, CV = 164%) and controls 1 and 2 in which no growth was detected.

3.2.3. Growth rate (in weight)

There was no significant difference in the initial body weight ($F = 1.092, P = 0.3789$, Table 1) between sea cucumbers allocated to the diet treatments. There was a significant effect of the diet on SGR in weight ($F = 5.305$, $P = 0.0007$, Table 3). The mean SGR across all treatments was 27.0 ± 1.5 g month^{-1}. The fastest growth rates were determined for diets B (0.57 ± 0.11 g month^{-1}, CV = 53%, Fig. 4(B)), and C (0.48 ± 0.21 g month^{-1}, CV = 125%, Fig. 3(B)). The lowest growth rates were determined for diet A (0.11 ± 0.07 g month^{-1}, CV = 183%), and control 1 and 2, in which no growth was detected.

3.2.4. Fecal production rate (FPR)

A significant effect of diet on fecal production rate ($F = 21.877$ $P \leq 0.0000$, Table 3). The mean FPR across all treatments was 4.25 ± 0.46 g d^{-1}. The highest production rate was determined for diet C (6.78 ± 0.38 g d^{-1}, respectively, Fig. 4(C)), but this was not significantly different from diets B and D. The lowest values were for the sea cucumbers in diets A and controls. In control 1, sediment was swallowed with production of feces (Fig. 4(C)).

4. Discussion

4.1. Asexual reproduction and induced fission

In the present study the artificial division by transverse cutting was documented for the first time in *I. fuscus*. This invasive technique was effective, yet simple. Nonetheless, this procedure is very far from natural simulations of asexual reproduction (Dolmatov, 2014). This study was performed in March, when *I. fuscus* does not have sexual reproductive activity, with no mortality of newly split individuals, which suggests that a segment of the population might be able to reproduce asexually during the equatorial warm season (December to May) when animals are not in their sexual reproductive season, according to our data collected from April 2013 to March 2016.

During a population study on March 2002, before opening the fishery of *I. fuscus* at the Galapagos Islands (Sonnenholzner et al., 2002), lengths and densities were determined in 17 sites within the Bolivar Channel (at the west coast of Galapagos Archipelago, between Isabela and Fernandina islands). In four sites several sea cucumbers (ca. 10%) were collected with evidence that natural fission had occurred a few weeks before (anterior body parts without an anus and posterior body parts without a mouth).

Sonnenholzner (2003) observed in the field that the sea cucumber *Holothuria theeli* reproduces asexually. A high seasonal variability in abundance of *H. theeli* was reported in May, but the number of individuals increased by the end of August (three months later) as a result of asexual reproduction by fission, when sea temperature is low. During the sampling period *H. theeli* was observed without a mouth (a recent fission event). The fluctuations in number of individuals following a massive fission event could be related to the variable survival rates of posterior and anterior parts (Reichenbach and Holloway, 1995; Reichenbach et al., 1996).

H. theeli reproduces sexually between February and April, when sea temperature is warm in the Ecuadorian sea. This contrasts with observations on the sea cucumber *I. fuscus*, which mainly reproduces when sea temperature is typically cold. In both cases, sexual and asexual reproductions are apparently synchronized by the sea temperature and both events might not occur simultaneously.

Our results show that transverse induced fission might become an alternative to restore wild populations of *I. fuscus*, and other commercial fissiparous holothurian species. This technique might be helpful within those alternatives provided in Reichenbach et al. (1996): (1) producing seed stock for grow-out operations in locations where hatchery facilities would be difficult to establish; (2) offsetting the mortality in sexual breeding programs which to date have had high mortality in the larval stages; and (3) producing animals for juvenile release programs where a simple, low-cost, on-site propagation technique is required.

4.2. Survival and regeneration time

Echinoderms are well known for their remarkable regenerative capabilities following injury, self-induced autotomy or fission (García-Arrarás and Dolmatov, 2010). Holothurians are especially capable of rapid and complete regeneration of their digestive tube after evisceration when fission occurs (Emson and Wilkie, 1980; García-Arrarás and Greenberg, 2001).

In this study, *I. fuscus* reached a stable weight condition 21 and 30 days after cutting for posterior and anterior body-parts, respectively. Therefore, they may initiate their regenerating phase without continuously losing more body-tissue from the body-wall, and particularly from their ambulacral feet area. Sea cucumbers need to maintain their ability to obtain oxygen via their body-wall and ambulacral feet. These are critical areas that may influence their survival due to oxygen diffusion until the respiratory tree is completely regenerated in both parts (Smith, 1971). The duration of regeneration of internal organs in holothurians forced to undergo fission may range from 1.5 to 3.5 months (Vuki and Viala, 1990; Reichenbach and Holloway, 1995; Purwati et al., 1999; Dolmatov et al., 2012). Here, the duration of the regeneration of body-parts in *I. fuscus* was 3 months.

The high survival of fission products (anterior and posterior body parts) and its regeneration ability, confirm that *I. fuscus* is a notable successful fissiparous tropical sea cucumber species in the Ecuadorian coast. This asexual reproductive mode is widely spread in the family Stichopodidae (e.g., *Stichopus chloronotus*, *Stichopus variegatus*, *Stichopus monotuberculatus*, *Stichopus naso*, *S. horrens*, and *Parastichopus californicus*) (Reichenbach et al., 1996; Dolmatov, 2014).

Our results suggest that asexual reproduction of *I. fuscus* is possible and this might be applied along with other management criteria for rebuilding populations of this valuable species.

4.3. Asexual reproduction and growth

Asexual reproduction by fissiparity and regeneration in wild populations of holothurians may produce high densities of smaller individuals, (Conand, 1996; Sonnenholzner et al., 2002; Miethe et al., 2009). Nonetheless, the effects of length, weight and density on growth and survival of sea cucumbers under natural conditions (at coastal areas) have received limited research attention (Qin, et al., 2009; Liang, et al., 2010). Our results show a high survival rate of large and small fission body-parts under laboratory conditions.

At present, there is insufficient knowledge on the reproductive biology of *I. fuscus* in Ecuador. Nevertheless, in concordance with previous observations in other Ecuadorian locations (Sonnenholzner et al., 2002), our results suggest that the populations where *I. fuscus* were collected might present asexual processes during the warm season. Sexual reproduction of *I. fuscus* along the coast of Ecuador may occur throughout the year; but there is a peak of reproduction when low temperatures of the sea occur, between July and October. Therefore, these reproductive modes probably do not occur simultaneously at the same intensity, which contrasts with reports for *H. atra* in which both modes may peak during the same season (Chao et al., 1993, 1994; Lee et al., 2008).

4.4. Diets

Granulated powders from different types of seaweed species, such as *Sargassum thunbergii*, *S. polycystum*, *Undaria pinnafitida*, *Laminaria japonica*, *Zostera marina* and *Ulva lactuca* have been suggested as essential ingredients in the artificial diets for cultured sea cucumbers (Zhou et al., 2006; Slater and Carton, 2009). The results of this study indicate that the mix of *P. durvillaei* and *S. ecuadoreanum* are adequate seaweed diets for use in the culture of *I. fuscus*. Those two seaweeds promoted a considerable SGR (in length and weight) of *I. fuscus* than the mix of *Sargassum* spp and *Ascopyllum nodosum*.

The sea cucumbers remained in good health condition in controls with and without substrate (clay and sand), but animals fed continuously when substrate was provided. This suggests the importance of keeping the organisms in tanks with clay and sand, as an inert element for supporting their feeding behavior. This is in agreement with reports by Robinson et al. (2013) and Qiu et al. (2014) who highlighted the importance of substrate for sea cucumber feeding, because this promotes growth in length and weight.

Our results showed that *I. fuscus* was able to assimilate better diet B with the highest protein content (Table 2), which was based on ingredients of animal and vegetal origins. This diet is capable of inducing a rapid growth (in length and weight) and healthy conditions in this commercially valuable sea cucumber. Diets C and D also promoted good growth, probably because of their high carbohydrate content (Fig. 4(A–B)). Length and weight gain of *I. fuscus* significantly decreased with the increasing ash content in diet A (Huiling et al., 2004; Slater et al., 2011). Our results suggest using diet B for growing regenerated body parts of *I. fuscus*, although diet C might be an alternative for the general maintenance and feeding in captivity, since the organisms are kept in good conditions and because this diet is cheap and easy to acquire. More research is required on this topic.

5. Conclusions

Asexual reproduction of the sea cucumber *I. fuscus* was successfully induced by transverse fission. There were no significant differences between the survival of the anterior and posterior animal body parts. Moreover, all surviving parts regenerated in ca. 90 days. This suggests that *I. fuscus* might have a good potential for mariculture, and encourages further research based on the results described here.

Asexual reproduction should be considered for restoring populations of this valuable species given that fisheries management has failed, and hatchery production of juvenile sea cucumbers is not well developed.

This study suggests that a diet based on a brown macroalgae powder of *P. durvillaei* Bory and *S. ecuadoreanum* Taylor can be used to promote adequate growth (in length and weight) and healthy conditions in regenerated body parts of *I. fuscus*.

Additional studies should be carried out on this species, to explore the application of the methods developed here to intensive asexual propagation plans to restore the natural adult populations of *I. fuscus* in Ecuador.

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