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Role of sand as substrate and dietary component for juvenile sea cucumber *Holothuria scabra*

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Abstract

The sea cucumber Holothuria scabra, or sandfish, is a commercially valuable aquaculture species; however viable intensive tank-based aquaculture techniques have not yet been developed. The study aimed to assess the role of sand as a substrate and/or dietary component in intensive tank culture of sandfish in recirculating aquaculture systems (RAS) in South Africa. A control experiment was conducted to confirm the reported positive effect of sand as a substrate on sandfish growth and a sand-in-diet experiment was conducted to determine if incorporation of sand into formulated diets could improve sandfish growth in bare tanks. In the control experiment, mean growth rate of juvenile sandfish in the bare tanks was significantly lower than that of juveniles reared in tanks with a sand substrate (-0.12 ± 0.16 g day-1 SE and 0.03 ± 0.01 g day-1 respectively; $F(1,2) = 1.91$, $p < 0.001$). However in the sand-in-diet experiment, mean growth rate of juvenile sandfish in bare tanks, fed a formulated diet containing 20 % sand was not significantly different to juveniles fed a standard formulated diet (-0.13 ± 0.01 and -0.12 ± 0.16 g day-1 respectively; $F(1,2) = 1.26$, $p > 0.05$). Results confirmed the reported positive effect on sandfish growth when sand is provided as a substrate, however sand in diets did not promote growth in the same way, indicating that inclusion of sand in formulated diets is unlikely to compensate for lack of sand as a substrate. Future research should therefore aim to identify optimum parameters of sand substrate and develop tank holding systems capable of maintaining favourable substrate conditions for intensive sandfish culture.

Key words: sandfish, intensive aquaculture, substrate, recirculating aquaculture system, formulated diet, digestion.

The sea cucumber *Holothuria scabra* (Jaeger), or sandfish, is a commercially valuable aquaculture species, cultured extensively in tropical regions. Viable intensive tank-based techniques for post-nursery culture of this species have not yet been developed. Current extensive methods for sandfish grow-out, including sea ranching, pen farming and pond culture, all require sandfish holding on sandy-muddy sediment substrates which mirror the deposit-feeders' natural diet (Duy, 2012; Hair, 2012; Juinio-Meñez et al., 2012; Robinson and Pascal, 2012). In tank-based culture of sandfish, substrate provision can be impractical and incur significant management cost. For the first time, the current study investigates the necessity of sand substrate for intensive tank culture of sandfish in recirculating aquaculture systems (RAS) and the potential for replacing in-tank substrate with formulated diets containing sand.

To date, intensive farming techniques have only been developed for the temperate sea cucumber, *Apostichopus japonicus* (Selenka) in ponds (Chen, 2004). No methods or data are available for tank-based post-nursery culture in RAS or flow-though systems for any sea cucumber species. Intensification of sandfish culture techniques, in closed or semi-closed land-based tanks, would offer greater control over the production cycle, with the potential to increase production efficiency.

One difficulty associated with tank-based sea cucumber culture is maintenance of optimum substrate conditions. Development of unfavourable substrate conditions, often exacerbated by the addition of high organic content in formulated diets, is common during tank-based conditioning of adult broodstock and during the nursery phase for small juveniles (Morgan, 2000; pers. obs.). Current management requiring regular substrate exchange is expensive, labour intensive and can induce handling stress which negatively affects sandfish behaviour by increasing their burial frequency (Eeckhaut – pers. comm. James, 1996; Purcell, 2010). Thus, avoiding the need for substrate and substrate exchange in tanks is desirable from an economic, management and health perspective. However, studies to date of sandfish cultured in bare tanks and fed commercially available diets have reported poor growth performance (Battaglene et al., 1999; Pitt et al., 2001; Watanabe et al., 2012). Sand may be an essential part of sandfish diets: Watanabe et al. (2012) suggested diet digestion was facilitated by the presence of sand available for consumption, while Battaglene et al. (1999) suggested that sand substrate in tanks may function as a source of both food and shelter. These hypotheses remain untested.

The current study, aimed to assess the role of sand as a substrate and/or dietary component for sandfish. A control experiment aimed to confirm the reported effect of sand as a substrate on sandfish growth, by comparing growth of sandfish juveniles:

(A) held in tanks with sand substrate and fed a standard formulated diet; and

(B) held in tanks without sand substrate (i.e. bare tanks) and fed the same standard formulated diet.

A sand-in-diet experiment aimed to determine if including sand in the diet improved growth of sandfish when sand as a substrate was absent from tanks, by comparing growth of juvenile sandfish in bare tanks:

(B) fed the standard formulated diet; and

(C) fed the formulated diet containing 20 % sand (dry weight).

The three treatments A, B and C (Table 1) were allocated to tanks using a randomised block design, with four tank replicates per treatment. Two experimental diets were formulated (Table 2) based on a commercially available abalone weaning diet Abfeed ® (1 mm pellet) (Marifeed Pty Ltd., Hermanus, South Africa). Sand was sourced from a commercial sand dune quarry (SSB Mining, Macassar, South Africa) and sieved to a particle size of 125 – 500 μm.

Trials were conducted at HIK Abalone Farm Pty (Ltd), Hermanus, South Africa between December 2011 and January 2012. Juvenile sea cucumbers were imported from a private hatchery (Madagascar Holothurie S.A., Madagascar) and acclimated to rearing conditions in the RAS for six weeks.

Juvenile sea cucumbers were suspended in mesh bags for 24 h to ensure gut contents were evacuated prior to weighing. They were then drained on a damp cloth for one minute, weighed to the nearest 0.0001 g and photographed for individual photo-identification to permit tracking of individual growth rates (Raj, 1998; Slater and Carton, 2007). Forty-eight individuals with a mean weight of 8.33 ± 0.2 g individual^{-1} (mean ± SE) were allocated to 12 groups of four individuals per group. Each group was randomly allocated to one of the 12 polyethylene experimental tanks (455 x 327.5 x 175 mm) supplied with seawater (24 L min^{-1}) filtered through a re-circulating system comprising a composite sand filter, protein skimmer and biofilter. Aeration was provided continuously except during feeding which occurred once daily at 16:00 hours. Animals in treatments A and B were fed the formulated diet at approximately 1 % body weight, however animals in treatment C were fed the diet including sand at 1.2 % body weight per day, in order to standardise the amount of food fed across treatments. Decaying uneaten food was removed by siphoning every 48 hours. An artificial photoperiod of 12:12 L:D (07:00 to 19:00) was maintained to approximate natural habitat conditions. After 28 days, sea cucumbers were gut evacuated as above and individuals were identified and weighed as previously described. Water quality parameters (mean ± SD) during the experimental period were: temperature 27.7 ± 0.4 °C; salinity 35 ± 0.0 g L^{-1}; dissolved oxygen 6.4 ± 0.4 mg L^{-1}; pH 7.9 ± 0.4; total ammonia nitrogen 0.05 ± 0.4 μg L^{-1}.

Weight (g), weight gain (%) and growth rate (g day^{-1}) data were tested for normality (Shapiro-Wilk) and homogeneity of variance (Levene's test). Mean initial weights per tank were compared using a one-way analysis of variance to ensure no significant differences between the three treatments. A Student's t-test was used to compare final mean weight, growth rate and weight gain between treatments A and B in the control experiment and between treatments B and C in the sand-in-diet experiment Means were considered significantly different at $p < 0.05$.

Survival was 100 % in all treatments. In the control experiment 1, mean growth rate of juvenile sandfish in the bare-tank treatment (B) was significantly lower than that of juveniles in the sand-in-tank treatment (A) (-0.12 ± 0.16 g day^{-1} SE and 0.03 ± 0.01 g day^{-1} respectively; $F_{(1,2)}$ =1.91, $p < 0.001$; Table 3). Mean final wet weight and weight gain were also significantly lower in the bare-tank treatment (B) ($p < 0.001$; Table 3). In the sand-in-dietexperiment there was no significant difference in final wet weight, weight gain or mean growth rate between treatments B and C ($p > 0.05$; Table 3). Sandfish in both bare-tank treatments suffered considerable weight loss, namely -41.20 ± 2.04 % for animals fed the standard formulated diet (B) and -42.72 ± 1.74 % for animals fed the formulated diet containing 20 % sand (C). The mean growth rate of juvenile sandfish from the diet including sand treatment (C) was comparable to growth of sandfish in the bare-tank treatment (B) (-0.13 ± 0.01 and -0.12 ± 0.16 g day^{-1} respectively; $F_{(1,2)}$ =1.26, $p > 0.05$).

The negative growth rates obtained in the bare tank treatments in the current study are consistent with weight loss of both large and small juvenile sandfish held in bare tanks and fed commercially available diets reported by Pitt et al. (2001) and Watanabe et al. (2012; Table 4), however growth rates may differ due to variations in animal size in previous studies. Juvenile sandfish (mean weight 8.26 ± 0.38 g) in bare tanks in the current study exhibited growth rates of -0.12 g day^{-1} comparable to the -0.13 g day^{-1} reported by Watanabe et al. (2012) for sandfish of a similar size (mean weight 7.74 ± 0.59g) fed powdered shrimp feed in bare tanks kept free of natural food production. In comparison, Battaglene et al. (1999) and Lavitra et al. (2010) reported poor, but positive sandfish growth rates in bare tanks, possibly due to exposure to strong natural light conditions promoting growth of natural food sources such as diatoms and epiphytic algae in tanks. All treatments in the current study were subject to controlled environmental conditions to isolate the factor that was tested, hence reported growth is comparable within this study; furthermore, these results were similar to studies which excluded supplementary food sources (Watanabe et al., 2012).

Results show a positive effect on sandfish growth when sand is provided as a substrate in tanks compared to sandfish reared in bare tanks. However, sand incorporated in formulated diets for sandfish did not promote growth in the same way, indicating that sand made available for consumption via inclusion in formulated diets is unlikely to compensate for lack of sand provided as a substrate. This result suggests that sand may not function as a digestive aid as suggested by Watanabe et al. (2012) when consumed with or in a diet for juvenile sandfish reared in RAS. Improved growth when sand was present in the tank may instead be due to increased surface area for the development of natural food for holothurians, particularly bacteria and/or microphytobenthic primary production; this remains to be tested in future research. Alternatively, behavioural effects and resulting stress may explain poor growth in bare tanks, as animals are unable to bury in accordance with their diurnal cycle, in response to stress from handling and/or fluctuation in environmental parameters (Hamel et al., 2001; Mercier et al., 1999; Purcell, 2010).

In conclusion, it appears that in tank holding, as elsewhere, sandfish need sand. Future research should therefore aim to identify the optimum parameters of sand substrate for sandfish culture in RAS, in addition to developing tank holding systems capable of maintaining favourable substrate conditions to avoid extensive and costly management.

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Table 1: A description of the treatments in the control (C) and sand-in-diet (S) experiments (4 replicates per treatment).

Treatment	Substrate	Diet	Experiment
A	4 cm sand (125 – 250 µm)	Abfeed ®	C
B	Bare tank	Abfeed ®	C & S
C	Bare tank	Abfeed ® + 20 % sand (125 – 500 µm)	S

Table 2: Nutritional composition of the formulated diets.

	Standard formulated diet [a]	Formulated diet [a] + 20 % sand
Crude protein (%)	33.0	33.0
Crude lipid (%)	3.0	3.0
Vitamins (%)	0.1	0.1
Sand (125-500 µm) (%)	0.0	20.0
Gross energy (kJ g^{-1})	15.6	12.3
Feed ration (% biomass)	1.0	1.2

[a] Proprietary formulation manufactured by Marifeed (Pty) Ltd, Hermanus, South Africa.

Table 3: Growth and survival of juvenile sandfish (mean ± SE, n = 4). Superscripts indicate significant differences between treatments A and B (Student t-test; $p < 0.05$) and between treatments B and C (Student t-test, $p > 0.05$). C = control experiment, S = sand-in-diet experiment.

Treatment	Initial weight* (g)	Final weight^ (g)	Survival (%)	Growth rate† (g day^{-1})	Weight gain‡ (%)	Experiment
A	8.17 ± 0.50	9.05 ± 0.32[a]	100	0.03 ± 0.01[a]	11.44 ± 4.23[a]	C
B	8.26 ± 0.38	4.87 ± 0.25[b]	100	-0.12 ± 0.01[b]	-41.20 ± 2.04[b]	C & S
C	8.57 ± 0.21	4.88 ± 0.04[b]	100	-0.13 ± 0.01[b]	-42.72 ± 1.74[b]	S

*Initial mean wet weight (g individual^{-1})

^Final mean wet weight (g individual^{-1})

†Growth rate (g day^{-1}) = (final wet weight – initial wet weight)/no. of days

‡Weight gain (%) = (final wet weight – initial wet weight) x 100/initial wet weight

Table 4: Growth rates (g day^{-1}) of sandfish juveniles of various sizes and developmental stages reared in bare tanks and on sand substrates as reported in previous studies

Substrate type	Initial wet weight (g)	Feed type	Food ration (% body weight day^{-1})	Growth rate (g day^{-1})	Length of trial (days)	Authors
Beach sand (3 cm, < 1 mm)	1.5	Algamac	10	0.39	57	Battaglene *et al*. (1999)
Beach sand (3 cm, < 1 mm)	1.5	Algamac	1	0.25	57	Battaglene *et al*. (1999)
Bare tank (concrete)	1.6	Algamac	10	0.09	57	Battaglene *et al*. (1999)

Bare tank (concrete)	1.7	Algamac	1	0.12	57	Battaglene *et al*. (1999)
Bare tank (concrete)	157.2	Juvenile shrimp feed (Betagro)	0.3	-1.81	33	Pitt *et al*. (2001)
Bare tank (concrete)	157.2	Unfed	0	-1.38	33	Pitt *et al*. (2001)
Micro-atoll sand (5cm, unsieved)	0.24	Unfed	0	0.23	56	Lavitra *et al*. (2010)
Bare tank (concrete)	0.24	*Sargassum sp*. extract	2.5 g m-2 day^{-1}	0.007	56	Lavitra *et al*. (2010)
Bare tank (fibreglass)	2.11	Powdered shrimp feed	5	-0.026 to -0.022	21	Watanabe *et al*. (2012)
Beach sand (5 cm, < 1 mm)	7.76	Powdered shrimp feed	0.5	0.068	14	Watanabe *et al*. (2012)
Bare tank (fibreglass)	7.74	Powdered shrimp feed	0.5	-0.13	14	Watanabe *et al*. (2012)

Highlights

- Sand in culture tanks required for sandfish growth in intensive culture
- Poor growth performance of sandfish in bare tanks
- Inclusion of sand in formulated diets does not promote growth
- Results suggest that sand does not function as a digestive aid