Allozyme variation as a tool for beche-de-mer fisheries management: A study on *Holothuria scabra* (sandfish)

by Sven Uthicke[1] *& John Benzie*[1]

Introduction

Information on the biology and ecology of the Sandfish (*Holothuria (Metriatyla) scabra*) is sparse, although it is one of the most valuable beche-de-mer species. As do most aspidochirotide holothurians, it feeds on sediments. It prefers muddy substrates (Baskar, 1994) from which it selects fine particles rich in organic matter (Wiedemeyer, 1993). *Holothuria scabra* is one of the few species that prefers coastal areas to coral reefs (Conand, 1989) and is often found in intertidal seagrass beds. This species burrows into the sediment for a part of the day (Wiedemeyer, 1993; James *et al.*, 1994). Sexual reproduction via broadcast spawning occurs in the warm months in the southern hemisphere (Conand, 1989 and 1993a), although a secondary spawning peak has been observed in June in Moreton Bay, Australia (Harriott, 1980). Some information exists on the population size of this species in the Northern Territories (Vail, 1989), Moreton Bay (Harriott, 1980), Papua New Guinea (Shelley, 1981) and the Torres Strait (Long *et al.*, 1996).

Along the Queensland coast, the Sandfish occurs in two distinct colour morphs. One of these is nearly entirely black, with a dark grey ventral surface (hereafter referred to as Black Sandfish, Figure 1). The second is of creamy colour underneath and the dorsal surface is greyish green, with black stripes in wrinkles of the epidermis (hereafter referred to as Grey Sandfish, Figure 1). The Grey Sandfish fits the general descriptions of *H. scabra* (Conand, 1989; Anonymous, 1994) whereas the black colour morph may be one colour variety of *H. scabra var. versicolour*, as described by Conand (1989) from New Caledonia. The extent of interbreeding between the two varieties, and hence whether they should be treated as different fishery stocks is not known.

Both Sandfish varieties are also reported in deep subtidal areas from where they are caught as bycatch in the prawn trawl fishery. In contrast to the intertidal mudflats, Sandfish are currently not commercially fished in deep areas. During a stock assessment in the Northern Territories, Vail (1989) observed that animals from seagrass beds shallower than two meters are distinctly smaller than deep animals and suggested that seagrass beds are nursery areas for the Sandfish.

Allozyme markers are a useful tool to describe gene-flow and between populations of holothurians (Uthicke *et al.*, 1998; Uthicke *et al.*, 1999). With the aid of allozyme genetic markers developed in this study for the Sandfish, we aimed i) to determine whether black and grey individuals of *H. scabra* are one species or are simply colour morphs, and ii) to investigate whether deep populations may be sources of recruits for intertidal areas.

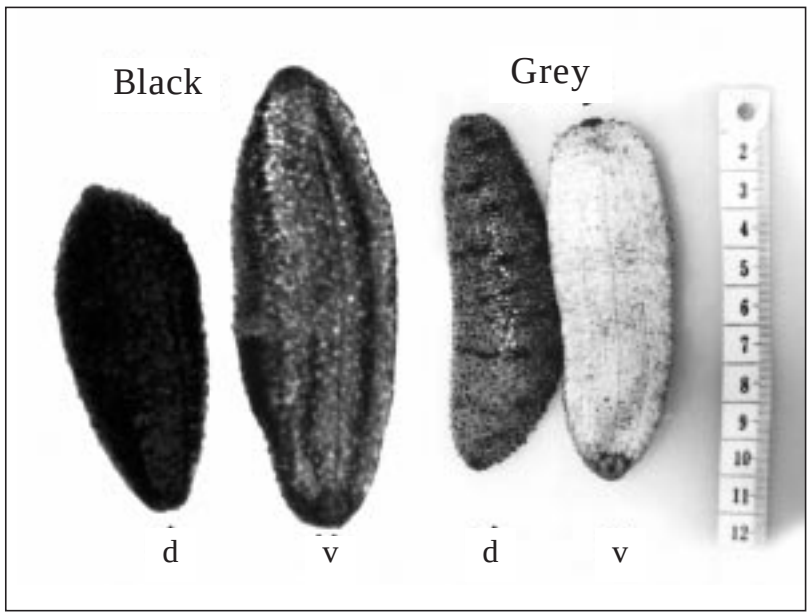

Figure 1. **Black and Grey Sandfish**

1. Australian Institute of Marine Science, PMB No. 3, Townsville MC, Qld. 4810, Australia

Material and methods

Sampling strategy

Two shallow populations of the Sandfish were sampled in the area of Hervey Bay (Urangan; Tin Can Bay) in south Queensland (Figure 2). An additional intertidal population was sampled ca. 450 nautical miles (nm) north (Upstart Bay). Samples were taken during low tides by walking on the mud flats. Animals from a deep population (18–20 m) were obtained during three trawling shots (Figure 2) using commercial prawn trawling equipment.

The length of all animals was recorded to the nearest centimetre. During dissection, the presence or absence of gonads was noted and a subsample of the gut (cleaned from sediments) was snap frozen in liquid nitrogen for later analyses.

Allozyme electrophoresis

Approximately 250 mg of frozen gut tissue was homogenised in the same volume of Tris HCl buffer (100 mM Tris adjusted to pH 8.0 with HCl) prior to electrophoresis. Electrophoresis for all enzymes was performed on 12% horizontal starch gels. In an initial screening process, 21 enzyme systems which appeared promising in a previous screening of two other holothurian species (details in Ballment *et al.*, 1997) were tested for 5 individuals of each colour morph on three buffer systems. This screening identified 7 polymorphic enzymes [*PGM** (5 alleles), *GPI** (3 alleles) and *HK** (2 alleles), *MDH** (2 alleles), *PEP-1** (3 alleles), *PEP-2** (2 alleles), *PEP-3** (2 alleles)]. Statistical analyses were performed with standard genetical software packages as described for example in Uthicke *et al.* (1998).

Results

General characteristics of the populations

The proportions of the two colour varieties were different at different sites ranging from significantly more black individuals in the trawl shots to only grey individuals in Tin Can Bay (see Table 1 on next page). The individuals sampled from 18–20 m depth by trawling were distinctly larger than those in all shallow populations (Table 1). The size frequency distributions of all populations appeared unimodal and were similar between the black and grey colour morphs in each population (data not shown). Gonads were present in nearly all of the individuals from the subtidal population at Hervey Bay. In contrast, none of the animals in

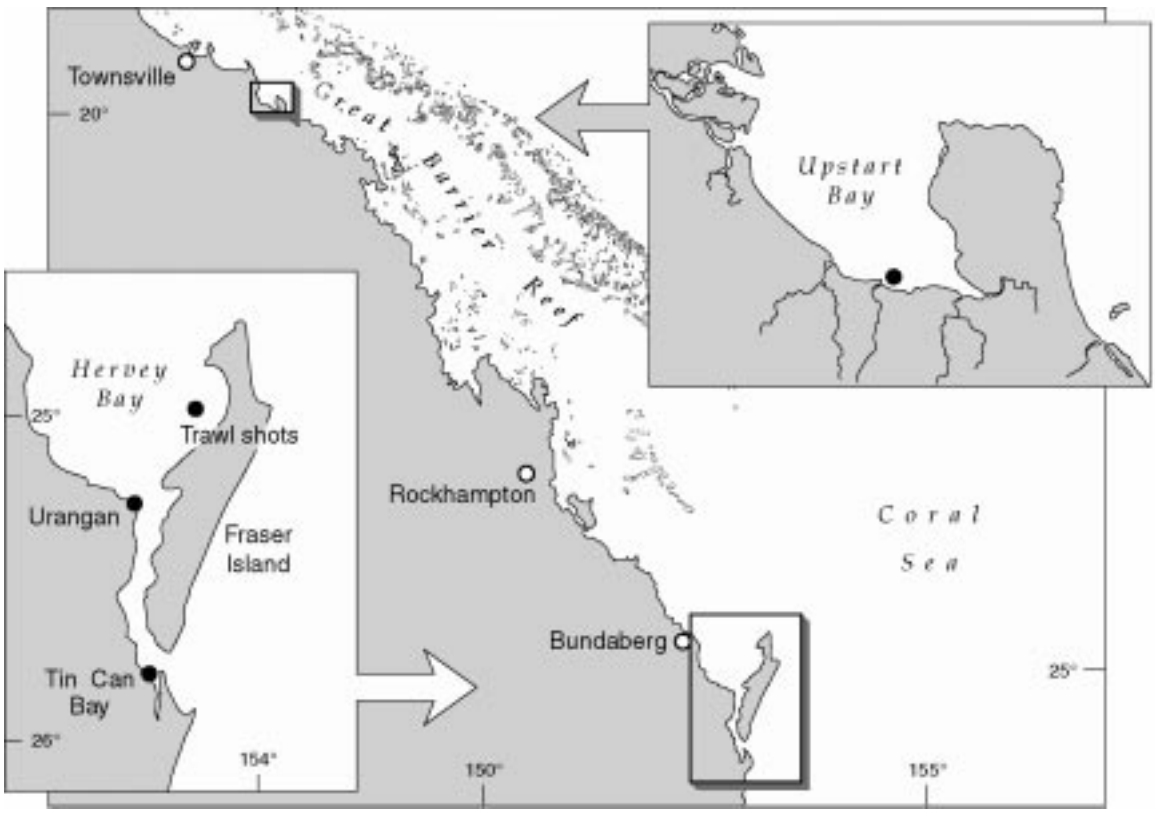

Figure 2. **Locality map of four sampling stations on the Queensland coast**

Table 1. **Average size (cm, standard deviation in parentheses), ratio of Black to Grey Sandfish (significant deviations from 1:1 ratio indicated, *: p < 0.05, NS: not significant) and the percentage of animals without gonads for 4 populations of *Holothuria scabra***

	Mean size	Black : Grey	Animals without gonads
Urangan	17.8 (2.5)	52: 48 NS	34%
Tin Can Bay	14.4 (1.8)	only grey, N = 17	53%
Upstart Bay	9.8 (1.5)	< 8 : 48 [1]	100%
Trawl shots	26.9 (4.0)	154 : 116*, [2]	12%

1. All black animals found were collected, but many more than 42 grey animals were present
2. Ratio only from shot 2 and 3

Table 2: **Probabilities (p) of exact test for differences in alletic frquencies between Black and Grey Sandfish at three populations in Queensland. No test is significant at p < 0.05 after corrections for multiple simultaneous tests**

Locus	Trawl shots	Urangan	Upstart Bay
*GPI**	1.000	1.000	0.660
*HK**	0.029	0.525	1.000
*MDH**	0.496	1.000	1.000
*PEP-1**	0.319	0.298	0.674
*PEP-2**	0.507	0.095	0.358
*PEP-3**	0.480	0.081	0.032
*PGM**	0.572	0.034	0.384
Total: χ^2	14.71	15.61	12.46
p	0.398	0.338	0.569

Table 3. **Pairwise FST values between four populations of Holothuria scabra. Values above the diagonal were derived from all 7 loci, those below the diagonal are derived from 6 loci (omitting PEP-3). Significance levels: *: p < 0.05; **: p < 0.01; ***: p < 0.001; ns: not significant.**

	Urangan	Trawl	Tin Can Bay	Upstart Bay
Urangan	-	0.005^{ns}	-0.006^{ns}	0.137^{***}
Trawl	0.006^{ns}	-	0.006^{ns}	0.172^{***}
Tin Can Bay	-0.015^{ns}	-0.003^{ns}	-	0.074^{***}
Upstart Bay	0.038***	0.081^{ns}	0.028^{ns}	-

Upstart Bay had detectable gonads. There appears to be a distinct correlation between the average animal size at each population and the number of animals with gonads (Table 1).

Population genetics

Genotype frequencies were not significantly different from those expected under Hardy-Weinberg equilibrium, irrespective of whether both colour morphs were analysed separately or pooled for each population with one exception (*PGM** showed significant heterozygote deficits at one population).

In the three populations where Black and Grey Sandfish occurred, we detected no difference in allelic frequencies between these two colour morphs.

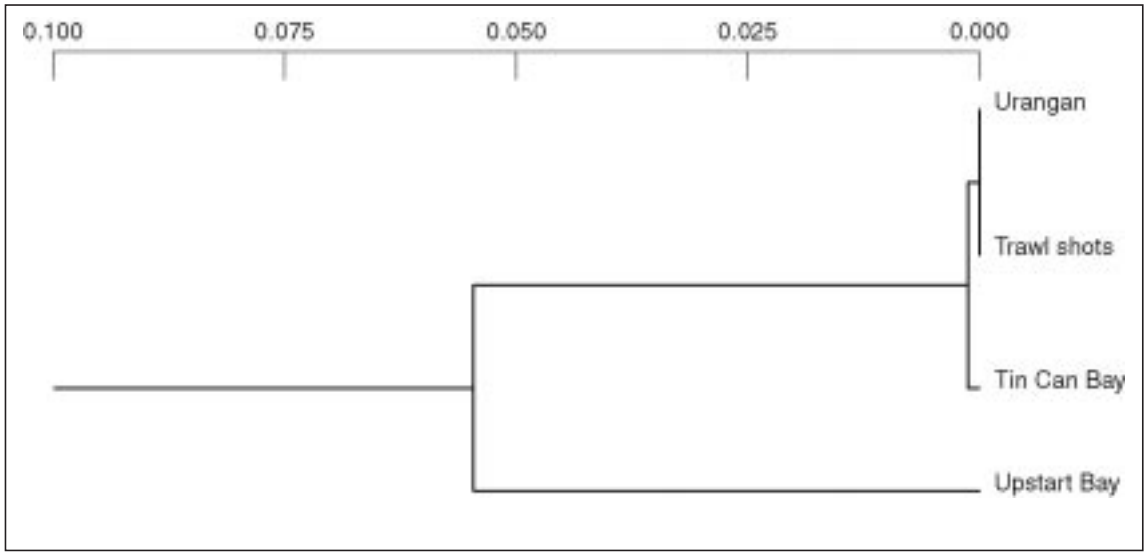

Figure 3. Dendrogram illustrating genetic relationships from four populations on the Queensland coast, using UPGMA cluster algorithm and Nei's unbiased genetic distance (Nei 1978)

(Table 2). We therefore pooled data from the two varieties at each population for subsequent analyses.

Several F_{ST} values for single loci and the mean (0.068) over all loci differed significantly from zero. Because the *PEP-3** locus showed extremely high values, we recalculated the average F_{ST} also without that locus. Although the value was lower (0.027), it was still significantly different from zero demonstrating significant differentiation among populations. F_{ST} values for single pairs of sampling stations revealed that there was no differentiation between the three southern populations near Fraser Island, but all of these show significantly restricted geneflow with the population in Upstart Bay (Table 3). Cluster analyses (Figure 3, cophenetic coefficient: 0.891) showed a similar trend. There was virtually no separation between the shallow population in Urangan and the trawled population, and the highest degree of separation was detected between the three southern populations and that of Upstart Bay.

Discussion

Black and Grey Sandfish

We detected no difference in allelic frequencies between the Black and Grey Sandfish colour morphs in any of the three populations in which they occurred together. The same alleles exist in both varieties and no fixed allele differences were detected. Genotype frequencies conformed to those expected under Hardy-Weinberg equilibrium, whether data for both colour morphs were pooled or kept separate. Additionally, Grey and Black Sandfish showed similar average lengths and length-frequency distributions at each population. Thus, there are no indications that Black and Grey Sandfish found off the Queensland coast are two separate species and we conclude that they are colour morphs of one species.

The factors causing this variation in colour is not known. It also remains unresolved why the colour morphs constitute varying proportions of the population, and whether the colour is environmentally or genetically determined. Although only 17 animals were sampled at Tin Can Bay it seems that the black variety is extremely rare at that location, and it also constitutes less than 10 % of the population in Upstart Bay. Thus, it appears that the high percentage of black individuals in Urangan and the trawl samples is a phenomenon specific to Hervey Bay.

Conand (1989) mentioned a colour variety of *H. scabra* in New Caledonia and tentatively termed this *H. scabra var. versicolour*. Although the coloration seems to be similar to the Black Sandfish investigated here, Conand noticed that the two varieties in New Caledonia occur in different areas, *H. scabra var. versicolour* being found in deeper habitats. There were also slight differences in spawning times between the two varieties (Conand, 1989 and 1993a), but the latter author concluded that the similar morphology of the calcareous ring and the spicules did not allow the erection of a new species. Colour seems to be a highly plastic feature in *H. scabra*. Apart from the variations observed in this study and in New Caledonia, specimens from India were reported to have yet another colour pattern, with a brown to black dorsal surface and white to yellow wrinkles (Sachithananthan 1994, see colour photography in

James *et al.*, 1994). It remains to be investigated whether differences in colour on a larger geographic scale represent species differences in *H. scabra.*

Deep and shallow populations

Neither the cluster analyses nor the variations of the F_{ST} values indicated any genetic subdivision between the deep population in Hervey Bay and the nearest shallow population in Urangan. Significant population subdivision was detected between the population in Upstart Bay and all southern populations. This allows sound inferences to be made concerning general levels of dispersal among populations.

It can be concluded that there is a large exchange of genetic material between deep and shallow populations. The higher number of large sized and sexually mature individuals in the deep area supports the hypothesis that there is a successive downward migration of growing individuals. In a study of a shallow population of *H. scabra* in Moreton Bay Harriott (1980) observed that all individuals had gonads. Since a fraction of the individuals from the seagrass beds in our study also had gonads, it can be assumed that spawning also takes place in the shallow areas.

James *et al.* (1994) reported that *Holothuria scabra* migrates to deeper areas for breeding, but does not mention how this was determined. It is a feature of many holothurian species that individuals in deeper areas are larger than shallow specimens (Conand, 1993b; Uthicke 1994), and the latter authors also suggested a migration to deeper areas during the life of these species.

Although we could show that the deep and shallow populations are closely linked, the genetic data alone cannot determine whether a migration of adults from shallow to deep water occurs. Similarly, the genetic data demonstrate that both deep and shallow populations derive recruits from the same larval pool. Presumably they both contribute to this pool but this requires other (non-genetic) research to confirm. Thus, the genetic data show that deep water animals could act as a source of recruits for the shallow water populations, but do not provide absolute proof that they do.

The occurrence of immature juveniles in all shallow populations supports the hypothesis that shallow seagrass beds are important settlement and nursery areas for *H. scabra* (Vail 1989).

This finds strong support from the samples at Upstart Bay, where all individuals were juveniles. If growth rates given by Shelley (1985) are used, it is possible that all of these animals had settled during the eight months prior to sampling. For sustainable management of the Sandfish stock it seems crucial to determine whether shallow seagrass beds are the only settlement area for juvenile *H. scabra*. If no juveniles can settle in the deep populations, this would indicate that these rely on recruitment of downward migrating adults. In that case, a careful management of the shallow populations and of the seagrass habitat *per se* is necessary to sustain both shallow and deep *H. scabra* populations.

The population at Upstart Bay was the only population that was genetically distinct from the other populations. This finding shows that the method developed is suitable to detect genetic distances, and it therefore confirms that the low degree of separation between deep and shallow southern populations is not simply due to methodological problems.

Since the population at Upstart Bay is approximately 450 nm further north, we assume that genetic differentiation of the Sandfish follows a separation-by-distance model. However, this has to be confirmed with a larger set of populations sampled.

In summary, both colour-morphs of the sandfish appear to belong to one species and it seems conservative to pursue the current practice to manage these together. Currently, deep populations of the Sandfish are not commercially fished, but a loss to these due to mortality caused by trawl-fishing cannot be excluded. These deep populations may constitute a buffer and a new source of recruits for the fished shallow populations. Due to the easy access by walking in the intertidal zone, shallow areas may be prone to overfishing if not appropriately managed.

We encountered two areas (Moon Point and Tin Can Bay) which were previously fished and now only contain very few animals. Although, apart from fishing also environmental factors may be the cause for reduced population size, this indicates that fished areas should be carefully monitored, especially because very few populations exist along the Queensland coast.

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