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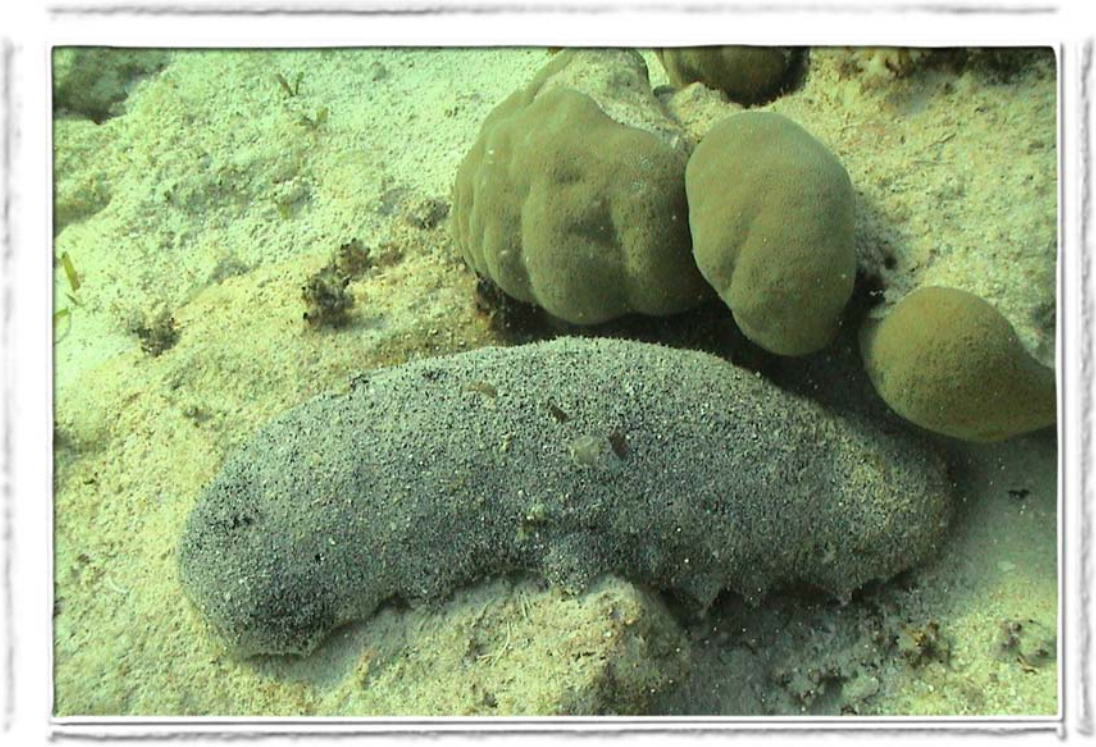

Stock size of bêche-de-mer, recruitment patterns and gene flow in black teatfish, and recovery of over-fished black teatfish stocks on the Great Barrier Reef

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Fisheries Research and Development Corporation

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OBJECTIVES: 98/133

1. To develop a survey methodology applicable for all shallow water bêche-de-mer species.
2. To adapt established techniques for enzyme electrophoretic analyses of holothurians to several bêche-de-mer species.
3. To determine the stock size of bêche-de-mer over a large geographic area in the GBR.
4. To determine the stock size and biomass of the black teatfish over a large geographic area in the GBR.
5. To establish the period of reproduction of the black teatfish on the GBR.
6. To measure dispersal and recruitment in black teatfish using genetic markers.
7. To identify and report the implications of these findings for management of bêche-de-mer fisheries.
8. To measure the recovery time for over-fished black teatfish stocks, (numbers and biomass).
9. To assess the likely source of recruits to recovering populations, including the role of protected reefs.
10. To estimate growth rates for black teatfish.
11. To describe large-scale gene flow and dispersal of sandfish among fished populations in NT and WA.

OUTCOMES ACHIEVED

Successful survey of several bêche-de-mer species, and of black teatfish (*Holothuria nobilis*) in particular, and the description of the population genetic structure of black teatfish has provided a basis for improved management of a productive and valuable fishery in the Great Barrier Reef (GBR) through improved stock definition, assessment of stock size and determination of levels of dispersal among populations. Surveys over time have shown low recruitment rates, slow growth rates and potentially long times required for the recovery of over-fished black teatfish stocks. The development of important biological information such as the time of reproduction of black teatfish, and background information on this and other species will assist further work on those species. The results will also help develop bêche-de-mer management in regions outside the GBR.

The main purpose of the project was to provide biological data urgently needed for a sustainable management of the black teatfish fishery in the Great Barrier Reef (GBR). The principal goals were 1) to estimate the standing stock of the black teatfish and estimate densities of other bêche-de-mer species by undertaking large scale surveys in the GBR and 2) provide further information required to determine annual harvest levels and proposed closed seasons for the black teatfish including reproduction period and likely sources of supply of recruits. Following closure of the fishery the project was extended to: 3) establish re-colonisation rates of holothurians on the fished reefs where fishing had ceased, 4) determine the sources of those recruits, and 5) estimate growth of holothurians using genetic fingerprinting to identify individuals. The project was also extended to include genetic work on sandfish (*Holothuria scabra*) in Northern Territory and Western Australia.

Using manta tows covering an area of 500 m^2 each, stock surveys on 72 reefs indicated that black teatfish is the only high quality bêche-de-mer species in shallow water areas of the GBR. Most abundant are low quality species such as greenfish and lollyfish. The highest densities of black teatfish occur north of Townsville, and the distribution of the species south of this is patchy. The only other locations with high densities are in some Whitsunday Reefs and in the Pompey region, which is difficult to access. A comparison between fished (“blue”) and un-fished ("green") reefs in the main fishing area between Townsville and Princess Charlotte Bay indicated that fishing has reduced density and biomass by at least 75% on fished reefs. Black teatfish stocks on over-fished reefs had not recovered two years after closure of the fishery, while the stocks on reefs previously protected from fishing remained at high densities. No recruits could be found in the two years after closure of the fishery, preventing the identification of the source of the recruits

using genetic techniques. Black teatfish reproduce on the GBR between April and August, but high gonad indices were observed on some reefs as late as October.

Allozyme electrophoretic markers, and more sensitive mitochondrial DNA markers, indicated no restrictions to gene flow in *H. nobilis* populations on the entire length on the GBR, suggesting that the GBR bêche-de-mer fishery can be managed as one stock. A novel technique to measure growth in bêche-de-mer using genetic fingerprinting revealed that medium sized black teatfish grew slowly, and some large individuals actually shrunk. Animals of an average size on the GBR reefs may be 10 years old. These data, in conjunction with a lack of observed recruits suggest that significant restrictions to recruitment may occur on ecological time scales. Low natural adult mortality rates in *H. nobilis* were inferred from indirect demographic data. Model calculations on black teatfish stocks indicated that the virgin biomass in the fished area of the GBR was in the order of 5,500 tonnes and about 5 million individuals and was now reduced to 1,103 t and 920,000 individuals in the areas open to fishing. These data show black teatfish vulnerable to fishing, with takes as low as 5% of standing stock being too great for sustainable fishing, and prolonged recovery times in the event of over-fishing.

In summary, most objectives, and the performance indicators for the project, were met through developing a methodology based on manta tows, applicable to all shallow water bêche-de-mer species; adapting techniques for enzyme electrophoresis for use in several species of bêche-de-mer; estimating the spatial patterns of abundance of several species of bêche-de-mer; calculating black teatfish stocks; estimating the main reproductive period, and growth rates of black teatfish on the GBR (between April and August); determining the genetic structure of black teatfish stocks, surveying stocks up to two years after closure of the fishery, specifically identifying the implications of these data for fishery management and maintaining the support of the Bêche-de-mer Industry and Management agencies The likely source of recruits to recovering reefs could not be assessed as no recruits were observed and geneflow of sandfish in NT and WA was not estimated because of lack of samples.

KEYWORDS: Bêche-de-mer, *Holothuria nobilis*, Genetics, Fisheries management, allozymes, DNA fingerprinting, black teatfish.

ACKNOWLEDGMENTS

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FINAL REPORT

1998/133. Stock size of bêche-de-mer, recruitment patterns and gene flow in black teatfish, and recovery of over-fished black teatfish stocks, on the Great Barrier Reef.

BACKGROUND

In the mid 1980s, a limited entry sea cucumber fishery was introduced to the Great Barrier Reef (GBR). At present, the fishery is performed by about 18 licence holders largely based near Cairns. The total annual allowable catch (TAC) of bêche-de-mer for Queensland was set at 500 tonnes, and most of this catch is exported and is valued at approximately 3 million dollars. Recent developments of the fishery on the GBR have shown that the level of 500 t was actually never fished, and catches of black teatfish drastically declined until the closure of its fishery in 1999. When this project started, 61% of the catch in the GBR was reported to be the black teatfish (*Holothuria nobilis*). The main emphasis of this project was on this sea cucumber species but a methodology for genetic surveys and some data on distribution was also to be provided for other bêche-de-mer species. Sandfish (*H. scabra*) is also part of the catch on the east coast, but is more important in NT and WA.

There was an urgent need to obtain basic biological information to assist management of this industry, as given by the highest research priorities identified by QFMA. The only information available on the black teatfish and other commercially important holothurians in the GBR were some data on densities in the central region of the GBR and Torres Strait (Harriott 1984, Hammond *et al.* 1985, Long *et al.* 1996). The sources of juvenile recruitment and basic population parameters such as reproduction period, population structure and mortality were unknown. In many holothurian populations, juveniles are scarce and replenishment by sexual propagules may be low and sporadic.

The main aims of this project were 1) to estimate the standing stock of the black teatfish and estimate densities of other bêche-de-mer species by undertaking large scale surveys in the GBR and 2) provide further information required to determine annual harvest levels and proposed closed seasons for the black teatfish including reproduction period and likely supply of recruits. Although focused on the GBR it was hoped that the knowledge

gained in achieving these goals will provide useful information to assess and develop similar industries in Northern Territory and Western Australia.

The only means to identify sources of recruits is by genetic markers because it is not possible to physically tag the small larvae, and microchemistry approaches are not practical in holothurians. Allozyme genetic markers were to be developed for at least another five bêche-de-mer species. Connectivity of black teatfish populations between single reefs of the GBR was to be investigated using these allozyme markers. In the case of high gene flow, populations should be resilient even to high levels of fishing pressure whereas in the case of low genetic exchange, management may have to monitor signals at very local scales.

The project (FRDC No. 98/133) began in July 1998, but in October 1999, the fishery on black teatfish was closed because low catch rates indicated over-fishing. First analysis of our survey data from 1999 indicated that black teatfish densities on fished reefs from the Townsville to the Cooktown sector of the GBR were approximately 25% of that found on reefs protected from fishing. The closure of the fishery provided a unique opportunity to 1) establish re-colonisation rates of holothurian on the fished reefs by repeating surveys in late '00 and '01, 2) provide information on the sources of recruits using genetic markers developed in 98/133 and, 3) provide information on growth of holothurians using genetic fingerprinting to identify individuals. To seize the opportunity to measure recovery rates of the black teatfish the project was extended to examine these questions.

The project was also extended to include work on sandfish in Northern Territory and Western Australia. The sandfish is the most important species in the bêche-de-mer fisheries in these states. In WA, there were clear indications that at least one population showed symptoms of over-fishing three years after the current fishery opened. In the NT, four different areas have been fished for several years, and NT fisheries planned a stock survey in 2000. Due to our current work on bêche-de-mer and a previous Environment Australia funded genetic project on the sandfish genetics (which demonstrated significant genetic differentiation among Queensland stocks) the fishery agencies from both NT and WA approached AIMS to assist in investigating geneflow and determining the sources of recruits for sandfish stocks using genetic tools. NT Fisheries has specific plans for collection, while interest from WA was for ad hoc assessments when collections could be made by fisheries officers.

NEED

There was an urgent need to provide basic information to assist management of the bêche-de-mer fishery in Queensland's GBR and to determine sustainable harvest levels. The proposed research directly addressed two of the highest priority research topics for harvest fisheries outlined by QFMA (Research needs and priorities for the management of Queenslands fisheries, QFMA, 1997, p 16), which were: 1) to estimate of standing stocks of bêche-de-mer (holothurians) off the east coast and, 2) to determine sustainable annual harvest levels of bêche-de-mer off the east coast. The project also addressed the lower priority research topic: to determine the ecology of the major bêche-de-mer species (black teatfish).

The project was later extended to examine three issues following the closure of the black teatfish fishery on the GBR, and because of interest in sandfish fisheries in the Northern Territory and Western Australia. Re black teatfish on the east coast - there was an urgent need to establish recovery times, re-colonisation rates and growth of bêche-de-mer on reefs of the GBR. The fishery has been closed but information on recovery of population numbers, biomass and individual growth would provide vital information for managers deciding when to open the fishery. In conjunction with the estimates on stock size and gene flow in project No. 98/133, the extension of the project potentially allowed the provision to QFMA of information on, 3) recovery rates of over-fished holothurian stocks, 4) sustainable harvest levels of bêche-de-mer on the east coast, 5) sources of recruits and, 6) growth rates of bêche-de-mer.

This information was needed to address QFMA priority issues "Estimate sustainable annual harvest levels" and "Monitor black teatfish stocks" (as determined on HarvestMac meeting in March 2000). The findings of the research will be discussed with QFMA, and data on recovery rates, sources of recruits, growth and stock size will directly influence the management decisions on when to re-open the fishery and at which harvest level.

Although the fishery on sandfish in NT and WA was only recently introduced, there were already concerns about over fishing. Management agencies in both states asked AIMS to provide in formation on: 1) genetic structure of sandfish stocks and, 2) likely sources of recruits. Surveys of the fishery were to be carried by NT fisheries in 2000, during which

time it was planned to obtain samples for genetic analysis. The results of this survey were to be used in the assessment of the fishery.

OBJECTIVES

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8. To measure the recovery time for over-fished black teatfish stocks, (numbers and biomass).
9. To assess the likely source of recruits to recovering populations, including the role of protected reefs.
10. To estimate growth rates for black teatfish.
11. To describe large-scale gene flow and dispersal of sandfish among fished populations in NT and WA.

METHODS

DEVELOPMENT OF SURVEY METHODOLOGY

During an initial field trip to Big Broadhurst Reef, 40 and 100 m^2 belt transects, and standardised manta tows, were used to survey holothurians on the reef flat to test the technique best suited for surveys of all species. Animals were counted only within 1 m on either side of the tow line to reduce the chance of overlooking animals which occurred further from the mid-line as has been reported for wider transects (Harriott 1984). A 2 m long aluminium bar attached to the front edge of the manta board facilitated the estimation of the 2 m width. Each tow lasted for four minutes, at a speed of 2 knots giving a survey area of 250 m x 2 m. Only boat drivers and snorkelers experienced in this work were involved in the surveys and the boat driver measured the speed with a hand held GPS.

SPATIAL SURVEYS AND STOCK SIZE OF BÊCHE-DE-MER OVER THE GBR

Prior to undertaking the quantitative surveys, swim searches and long manta tows were undertaken on a number of reefs to establish crude distribution patterns (all reefs surveyed are marked on Fig. 1). These initial surveys confirmed that mid and outer shelf reefs are the main habitat of *H. nobilis*, and that the species is generally not found on inshore reefs. The manta tow techniques described above were used to survey bêche-de-mer stocks on the GBR, but all reefs investigated in this way were deemed *a priori* to be suitable habitat for this species. The number of replicate locations per reef and tows per location varied with the size of the reefs (see Table 1). For statistical analysis, reefs were grouped into four arbitrarily chosen sectors, representing regions from north to south of the GBR, and these are also indicated in Table 1. Surveys concentrated on the reef flat area, and areas with > 60% sand cover were avoided because *H. nobilis* was not found there, as the surveys were mainly designed to estimate stock size of *H. nobilis*.

Although some other densities of holothurians are reported, it should be mentioned that many other commercial species have different habitats (white teatfish/*H. fuscogilva*: deeper areas, prickly red fish/*Thelenota ananas* and curry fish/*Stichopus variegatus*: more in backreef/lagoonal areas, Sandfish/*H. scabra*: more coastal and in seagrass beds). Possible exceptions are several *Actinopyga* (blackfish, deepwater redfish) species, the low value species *H. atra* (lollyfish), *S. chloronotus* (greenfish) and leopardfish (*Bohadschia argus*).

Table 1. Number and locations of manta tows or searches on reefs of the GBR.

Reef Name	Sector	Date	No. of Locations	No. of Tows	Latitude (S)	Longitude (E)
Nomad Rf.	1	28-08-99	2	7	12.10	143.31
Nomad Rf.	1	28-08-99	2	7	12.10	143.31
Rf. 13-050	1	29-08-99	1	14	13.33	143.98
Rf. 13-120	1	30-08-99	2	14	13.72	144.23
Pelican Rf.	1	01-10-98	1	search only	13.92	143.84
Stainer Rf.	1	01-10-98	1	search only	13.95	143.84
Davie Rf.	1	03-10-98	2	8	13.97	144.45
Corbett Rf.	1	30-09-98	1	search only	14.00	144.11
Grub Rf.	1	02-10-98	1	4	14.03	143.93
Clack Rf.	1	29-09-98	1	search only	14.06	144.26
Eden Rf.	1	02-10-98	1	search only	14.08	143.92
Wharton Rf.	1	30-09-98	1	search only	14.13	144.00
Flinders Isl.	1	29-09-98	2	search only	14.18	144.28
Swizer Rf.	1	28-09-98	1	7	14.37	144.76
Bewick Rf.	1	28-09-98	1	search only	14.43	144.81
Hicks Rf.	1	10-04-98	2	9	14.46	145.49
Ribbon Rf. No. 10	1	26-09-99	6	30	14.74	145.71
Linnett Isl.	1	27-09-98	1	6	14.79	145.34
Ribbon Rf. No. 8	1	29-09-99	3	21	15.07	145.73
Three Islands	1	27-09-98	1	6	15.11	145.42
Ribbon Rf. No. 7	1	25-09-99	4	30	15.19	145.74
Ribbon Rf. No. 2	1	06-01-00	3	18	15.55	145.80
Agincourt Rf. No. 4	1	28-09-99	2	12	15.95	145.83
Agincourt Rf. No. 1	2	28-09-99	2	14	16.05	145.87
St Crispin Rf.	2	29-09-99	3	21	16.09	145.85
Opal Rf.	2	29-09-99	2	20	16.22	145.90
Tongue Rf.	2	30-09-99	3	21	16.36	145.90
Hastings Rf.	2	30-09-99	2	20	16.51	146.02
Michaelmas Rf.	2	01-10-99	5	34	16.59	146.03
Arlington Rf.	2	02-10-99	6	42	16.72	146.09
Sudburry Rf.	2	24-09-99	5	30	17.02	146.23
McCulloch Rf.	2	03-10-99	4	24	17.29	146.44
Peart Rf.	2	05-01-00	2	12	17.48	146.41
Feather Rf.	2	05-01-00	3	18	17.54	146.39
Potter Rf.	2	07-01-00	3	12	17.70	146.56
Ellison Rf.	2	07-01-00	3	18	17.73	146.41
Rf. 17-065	2	08-01-00	3	18	17.87	146.74
Moss Rf.	2	08-01-00	3	18	17.94	146.80
Myrmidon Rf.	2	24-05-00	2	12	18.27	147.39
Slasher Rf. No. 1	2	25-05-00	1	12	18.49	147.18
Bowl Rf.	2	23-05-00	3	18	18.51	147.54
Cenetepede Rf.	2	16-10-98	1	12	18.74	147.53
Keeper Rf.	2	19-12-98	1	9	18.75	147.27
Wheeler Rf.	2	15-10-98	1	11	18.80	147.53
Davies Rf.	2	22-05-00	5	24	18.83	147.65
Big Broadhurst Rf.	2	27-05-00	6	88	18.91	147.74
Little Broadhurst Rf.	2	14-10-98	1	3	18.95	147.69
Stanley Rf.	3	25-02-00	5	28	19.30	148.11
Old Rf.	3	24-02-00	4	24	19.35	148.08
Stucco Rf.	3	17-05-99	2	16	19.56	149.60
Rf. 19-156	3	16-05-99	1	7	19.66	149.76
Rf. 19-159	3	16-05-99	1	14	19.67	149.92
Black Rf.	3	18-05-99	1	7	19.74	149.35
Hardy Rf.	3	19-05-99	1	21	19.74	149.21
Hyde Rf.	3	15-05-99	2	18	19.76	150.09
Hook Rf.	3	19-05-99	1	7	19.77	149.20
East-Black Rf.	3	18-05-99	2	9	19.79	149.43
Rebe Rf.	3	14-05-99	1	14	19.81	150.15
White-tip Rf.	3	13-05-99	1	18	19.92	150.27
James Rf.	3	12-05-99	2	13	19.96	150.22
Rf. 21-149	4	21-04-99	2	14	21.10	151.72
Rf. 21-151	4	20-04-99	2	14	21.12	151.76
East Cay	4	19-04-99	1	12	21.49	152.57
Rf. 21-433	4	21-04-99	1	3	21.56	151.48
Recreation Cay	4	18-04-99	1	search only	21.68	152.44
Turner Cay	4	18-04-99	1	18	21.72	152.56
Detour Rf.	4	17-04-99	1	search only	21.76	152.42
Half Tide Rf.	4	17-04-99	2	14	21.79	152.49
Rf. 21-551	4	15-04-99	2	18	21.96	152.06
Rf. 22-101	4	16-04-99	3	15	22.00	152.63
Chinaman Rf.	4	16-04-99	2	search only	22.01	152.66
Snake Rf.	4	13-04-99	2	12	22.03	152.18
Keppel Isl.	4	13-04-99	1	search only	23.16	150.96

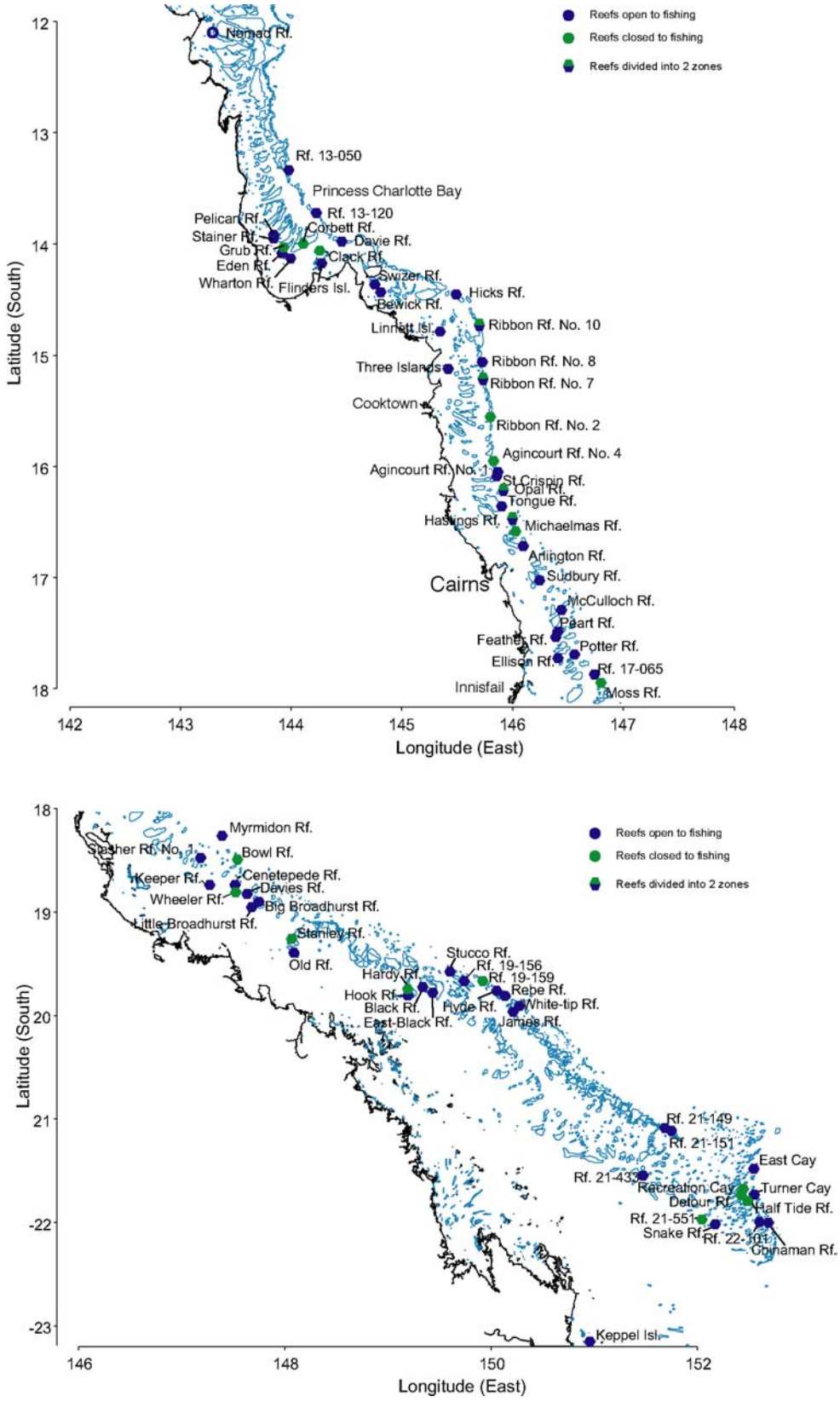

FIGURE 1. Sample and transect locations on northern (top map) and southern (bottom map) reefs of the Great Barrier Reef

A rough estimate of the total stock size in the main fished area of the GBR before and after the fishery on black teatfish was obtained as follows. A GIS coverage provided by the Great Barrier Reef Marine Park Authority gave the size of each reef in the GBR as a 'dry-reef area'. Comparison of that area with the reefs of our surveys indicates that this reef area is a reasonable estimate for the size of the habitat of *Holothuria nobilis* at each reef. In some instances, the area might be an overestimate because sandy backreef areas are included in these estimates. However, the fact that on some reefs *H. nobilis* were also detected in deeper habitats (and thus not included in the estimates) will balance this bias to some degree.

Using the GIS coverage, the total habitat area for black teatfish in the main fished area (between 12° and 19° S) of the GBR was calculated, summing areas across all mid shelf and outer shelf reefs. Inshore reefs were excluded because our field surveys indicated that they were not inhabited by *H. nobilis*. For estimates of the potential virgin (before fishing) biomass, it was assumed that natural densities corresponded to the average density currently found on green (protected) reefs. Using the upper and lower 95% confidence limit of the density estimates gives a 95% confidence range of the total number of individuals before fishing. However, it should be emphasized that this range only takes into account the variance due to errors in density estimates, and not potential errors in the estimate of the habitat size. The estimates of total numbers were transformed to biomass values by multiplying with the average gutted weight of all individuals dissected and weighed during this study. Gutted weight was chosen because it is the unit in which catch rates are reported.

Estimates of the actual biomass after fishery closure were obtained. The total habitat size over all fished and all protected reefs separately as described above. Standing stocks on these were estimated by multiplying the habitat areas with the respective densities measured for fished and unfished reefs (including confidence limits), and total biomass was estimated by subsequent multiplication using the total average gutted weight.

TEMPORAL SURVEYS OF BÊCHE-DE-MER AND RECOVERY AFTER CLOSURE TO FISHING

Twenty three of the reefs in the area between Townsville and Princess Charlotte Bay initially surveyed in 1998 and 1999, were resurveyed approximately one year after the closure of the fishery, and two years after the closure. Five of these reefs were protected from fishing (green reefs) prior to closure of the fishery, and 14 were open to fishing (blue reefs). Four reefs, which were divided into fished and un-fished areas, were also

investigated. On most of these reefs, manta-tow transects were repeated as during the original survey. On 11 reefs individuals of *H. nobilis* were also collected and weighed.

DISPERSAL AND RECRUITMENT

Dispersal and sources of recruitment were studied using genetic techniques to establish the amount of gene flow among populations, and infer the dispersal among populations.

Sampling in the GBR

Samples of *Holothuria nobilis* were obtained from shallow (0.5 to 4 m, depending on tide level) reef flat areas from 15 reefs of the GBR from all sectors. As a large area of each reef had to be searched, sub-samples from different areas within most reefs could not be obtained, with a few exceptions. The exceptions were Hicks Reef (frontreef and backreef), Ribbon Reef No. 10 (north and south), Michaelmas Cay (north and south), Big Broadhurst Reef (NE and SE-reef flat, West Bommie), and Reefs 21-149 and 21-151 (backreef and frontreef on both).

Samples were obtained during research cruises in September 1998 (Davie Reef, Hicks Reef and Ribbon Reef No. 10 south), April 1999 (East Cay, Turner Cay, Reef 21-149, Reef 21-151), May 1999 (White Tip and Stucco Reef), August 1999 (Reef 13-050), and September 1999 (Ribbon Reef No. 10 north, Opal Reef, Michaelmas Cay). Holothurian samples from Little Broadhurst and Davies Reef were obtained both in October and December 1998. In Big Broadhurst Reef, holothurians were sampled in October and December 1998, and in February, August and September 1999, providing samples for which tests of temporal stability of gene frequencies could be made.

After sampling, animals were kept for several hours on board the research vessel in 60 L containers with flowing seawater to allow the gut contents to be partially voided before processing. To obtain a sample of the gut lining, a cut approximately three cm long was made near the oral end of the ventral surface of the animal. The internal organs were removed, a length of the gut cut out and cleaned of any remaining sediment. Samples were immediately snap frozen in liquid nitrogen in small zip-lock bags. *Holothuria nobilis* was shown to survive this dissection procedure in most cases (Uthicke unpublished data, see also Reichenbach *et al.* 1996 on the ability for regeneration in *H. nobilis*). Therefore, to minimise the impact of the samples on the holothurian populations, the animals were usually returned to the reef flat or in the backreef area from which they were sampled.

In addition to the allozyme analysis, variation was analysed in a mitochondrial DNA fragment from *H. nobilis* collected from three reefs of the Northern Section of the GBR (Reef 13-050, Davie Reef, Hicks Reef), and two reefs in each of the Cairns Section (Michaelmas Reef and Opal Reef), the Central Section near Townsville (Big Broadhurst and Davies), the Central Section near the Whitsunday area (Stucco Reef and White Tip Reef), and the Southern Section (Reef 21-151 and Turner Cay).

Visual search for recruits

Intensive searches for the presence of small (<500g) *H. nobilis*, which are potentially juveniles, during the surveys described above and in additional field visits were undertaken. However only five such individuals were found in total. From these observations it is clear that recruitment was low. Due to the small number of individuals genetic analysis to identify the potential source population for recruits could not be conducted.

Sampling sandfish from NT and WA

Although considerable effort was made to obtain samples from NT and WA only one population was obtained from NT. In September 2001 FRDC agreed to the deletion of this objective, as it was unlikely that further populations could be obtained during the project and there was insufficient time remaining for their laboratory analysis.

Enzyme electrophoresis

Prior to the work described here, polymorphic enzyme systems for three holothurian species, *H. atra* and *S. chloronotus* (Ballment *et al.*1997) and *H. scabra*, had already been developed (Uthicke and Benzie 1999). These established techniques were tested in a wider range of species to determine their general applicability. The new species tested were *Actinopyga echinites*, *A. miliaris*, *H. nobilis*, *H. fuscogilva*, *H. fuscopunctata*, *T. ananas* and *S. variegatus*.

An initial screening of 27 enzyme systems followed the general procedures outlined in Ballment *et al.* (1997). Approximately 250 mg of frozen gut tissue was homogenised in the same volume of Tris HCl buffer (100 mM Tris to pH 8.0 with HCl) prior to electrophoresis. Electrophoresis was performed on 12% horizontal starch gels, testing three buffer systems: TEC7.9 (electrode buffer 135 mM Tris, 32 mM citric acid, 4 mM Na_2EDTA, pH 7.87; gel buffer 8.5 mM Tris, 2 mM citric acid, 0.27 mM Na_2EDTA, pH 7.87), TG8.4 (electrode buffer 25 mM Tris, 192 mM glycine pH 8.4; gel buffer same as electrode buffer) and HC6.5 (electrode buffer 65 mM Histidine, 7 mM Citric acid$\cdot H_2O$, gel buffer 16 mM Histidine, 2 mM Citric acid$\cdot H_2O$). Based on experience with previous

holothurian species, some enzymes were also tested on three buffer systems using cellulose acetate Gels (Cellogel™): CP6.4 (10 mM Citrate-phosphate), Phos7 (20 mM Na phosphate) and TM7.8 (50 mM Tris-maleate). Although several enzyme systems were only faintly active, a variety of enzymes were found to be suitable for population genetic screening of all the species tested (Table 2). Most enzymes working in one species appeared to work in the majority of the other species. Several enzyme systems were well resolved and variable in most of the species tested (e.g. GPI, PGM, HK).

After the initial tests described above, 20 individuals of *H. nobilis* were screened to optimise buffer systems and to test for polymorphisms in that species. Interpretable polymorphisms were detected in the following seven enzymes systems: FL-EST, GPI, HK, MDH, MPI, PGM and TPI. For GPI and PGM, best resolution was achieved on 12% starch gels using the TEC7.9 buffer. HK, MDH and TPI were resolved best on 12% starch gels using TG8.4 buffer. Cellulose acetate (Cellogel™) gels with TM7.8 (Tris-maleate pH 7.8) buffer were used to score FL-EST and MPI. Electrophoresis on Cellogel™ was performed for 75 min at 200 V, starch gels were run for 16 h (TEC7.9: 75 V, TG8.4: 230V). For details on buffer composition, staining and electrophoresis methods see Ballment *et al.* (1997). Alleles were labeled according to their mobility relative to the most common allele in the total sample, which was set at 100.

TABLE 2. Enzymes used in screening for activity gut tissue from seven bêche-de-mer species: *Actinopyga echinites* (AE), *A. miliaris* (AM), *Holothuria nobilis* (HN), *H. fuscogilva* (HF), *H. fuscopunctata* (ET), *Thelenota ananas* (TA) and *Stichopus variegatus* (SV). Except for the peptidases, which have been named according to the peptide used as substrate, enzyme names are those recommended by the International Union of Biochemistry's Nomenclature Committee (IUBNC, 1984). Bold print indicates the potential to use an enzyme for the respective species in further studies, Bold and Italics print indicates systems used for population genetic screening in *H. nobilis*. M, P = monomorphic and polymorphic, respectively, however only based on the study of three individuals.

Abbrev.	Enzyme	E.C. No.	Best buffer/Comments
AAT	Aspartate aminotransferase	2.6.1.1	no activity
ALAT	Alanine aminotransferase	2.6.1.2	no activity
ALPDH	Alanopine dehydrogenase	1.5.1.17	low activity in *Actinopyga* spp.
DIA	Dihydrolipoamide dehydrogenase, (Diaphorase)	1.8.1.4	no activity
ENOL	Enolase	4.2.1.11	low activity
FBP	Fructose bisphosphatase	3.1.3.11	Best on TEC, faint and blurry for most spp., also good on CP (Cellogel), good resolution (M) for HF
FL-EST	Carboxylesterase	3.1.1.1	General: good activity on TEC (later studies showed very good activity on Cello gels). AE, AM, TA: P (?). *HN*, HF, SV,ET: M
GA3PD	Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	only faint activity
G3PD	Glycerol-3-phosphate dehydrogenase	1.1.1.8	low activity on TG for AE, SV, ET
GPI	Glucose-6-phosphate isomerase	5.3.1.9	active on TG, TEC and HC, best resolution on HC. AE, AM, HF: M. *HN*, ET, TA SV: P
GCDH	Glocose dehydrogenase	1.1.1.47	no activity
HK	Hexokinase	2.7.1.1	General: good activity and resolution on TG, OK on TEC AE, AM, HF, ET: P. *HN*, TA, SV: M
IDH	Isocitrate dehydrogenase (NADP+)	1.1.1.42	TA, SV (on HC): M. AE, AM, HF (on TEC): M. HN, ET: only very faint
LDH	L-Lactate dehydrogenase	1.1.1.27	faint activity
LGG	Peptidase (leucylglycylglycine substrate)	3.4.11/13	TG, pH7. AE: P. HN: M. AM, HF, TA, ET, SV: only some samples show activity
LP	Peptidase (leucylproline substrate)	3.4.11/13	no activity
LT	Peptidase (leucyltyrosine substrate)	3.4.11/13	TG, pH 7: AE, AM, HN, ET, TA: P. HF, SV: M
VL	Peptidase (valylleucine substrate)	3.4.11/13	TG, pH 8: AE, AM, ET(?), TA, SV: P. HN, HF: M
MDH	Malate dehydrogenase	1.1.1.37	General: good activity on TG, 2 loci, lower one blurry with the exception of SV. upper locus: P for SV lower locus: AE, AM, HF, *HN*, ET, TA: M. SV: P(?)
ME	Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+) (Malic enzyme)	1.1.1.40	TEC, 2 loci in AE, TA, upper locus V in both (?), lower locus: AE, AM, ET(?), TA, SV: P. HN, HF
MPI	Mannose-6-phosphate isomerase	5.3.1.8	best on TM (Cello). AM, HF(?), *HN*, TA(?): V. AE, ET, SV: M
PGD	Phosphogluconate dehydrogenase(decarboxylating)	1.1.1.44	best on TEC, also OK on HC, only faintly active in ET. AE, AM, HN, HF, TA, SV: M
PGK	Phosphoglycerat kinase	2.7.2.3	only tested on Cellogel, best on Phos, but low activity
PGM	Phosphoglucomutase	5.4.2.2	good activity for most on Starch. AM, AE: clearly P. TEC and TG. *HN*, HF: TEC, TG, some alleles better on HC. ET: TEC, P(?). TA: TEC, HC P(?). SV: TEC, but blurry
SOD	Superoxide dismutase	1.15.1.1	some activity on some stains, but not interpretable
STRDH	Strombine dehydroxinase	-	low activity, works faintly on TG for AE
TPI	Triose-phosphate isomerase	5.3.1.1	TEC, TG, often faint and slow TA: V. SV: 2 loci (M) AE, AM, *HN*, HF, ET: M

DNA extraction

Frozen gut samples were removed from the freezer and kept on liquid nitrogen until processing. The frozen samples were broken into chips and 50-80 mg of each sample was weighed and placed into a 1500 µL microcentrifuge tube. 500µL of hot (60º C) CTAB extraction buffer (100 mM Tris-Cl pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 2% polyvenyl pyrolidone) and 12.5 µL of proteinase K (Promega, 20 mg/ml) were pipetted directly on the sample. The sample was briefly ground with a cleaned plastic pestle, vortexed and incubated over night at 60º C. After addition of 5µLRNase A (10 mg/ml) the samples were incubated for 60 min at 37ºC. After cooling to room temperature, 500 µLphenol : chloroform : isoamyl alcohol (25 : 24 : 1) was added, samples were vortexed and centrifuged (15900 g) for 5 min. The supernatant was carefully removed, 500 µLchloroform was added and samples vortexed and centrifuged again. The supernatant was transferred to another vial, and DNA was precipitated by adding 30 µLsodium acetate (3 M, pH 5.2) and 600 mL100% ethanol. Samples were allowed to precipitate for 15 min at room temperature and than centrifuged (15900 g) for 15min. The supernatant was removed and the pellet washed once with 1 mL70 % ethanol. The samples were air-dried for about 30 min at 60ºC, and 110 µLsterilised water was added. Samples were vortexed and left for several hours to resuspend the DNA. DNA quality was checked optically on 1 % agarose/1xTBE gels stained with ethidium bromide, and concentrations were measured in a Genequant (Pharmacia). This treatment usually resulted in final concentrations of 200 to 400 µg/mLDNA.

Mt DNA amplification and sequencing

A region of the mitochondrial cytochrom oxidase I (COI) gene was amplified using primers described by Arndt *et al.* (1996) (CO1e-F: 5'ATAATGATAGGAGGRTTTGG3', COIe-R: 5'GCTCGTGTRTCTACRTCCAT3'). These primers amplify 674 nucleotides of the echinoderm COI gene, corresponding to positions 6001 to 6674 of the mitochondrial genome of the echinoid *Strongylocentrotus purpuratus* (Jacobs *et al.* 1988).

PCR amplification is conducted using final concentrations of 1 µm of each primer, 2.5 µM $MgCl_2$, 1 x PCR Buffer, 200 µM of each dNTP and 2.5 units Taq-polymerase (Quiagen) and 40-80 ng DNA is added. PCR reactions were conducted on Perkin-Elmer (GeneAmp 9700) thermocyclers, loading the samples at 95°C. This was followed by 40 cycles of 30 sec of denaturation at 95°C, 30 sec of annealing at a temperature of 50°C, and 80 sec

extension at 72°C. The 40 cycles were followed by a final extension of 10 min, and cooling to 4°C until PCR products were removed from the thermocycler.

All PCR products were checked for the presence of bands on 1% agarose gels (1 x TBE). Most samples showed bands of the expected size (~ 640 bp), some samples which did not amplify at the first attempt did amplify in a second PCR run. For sequencing, the PCR products were cleaned using QIAaquick PCR purification kit (Quiagen), and their concentrations measured on a Genequant (Pharmacia) spectrophotometer. All PCR products were diluted to a final concentration between 10 and 25 µg/mL. For the sequencing reaction, Big Dye (ABI) sequencing reagents were used, following the instructions of the supplier. However, to achieve higher cost efficiency we scaled down the reactions to ¾ of the original instructions. DNA from each sample was sequenced in both directions, using 5 pm of either the forward or reverse primer per reaction. 5 µL of the diluted DNA were added to each reaction, and PCR amplification conducted as required by the supplier of the sequencing kit. Sequencing products were precipitated by adding 1.5 µL sodium acetate (3 M, pH 5.2) and 37.5 µL100% ethanol, centrifuging the sample and washing the pellet once with 190 µL 70% ethanol. Samples were sequenced on an ABI 377 sequencer.

PERIOD OF REPRODUCTION OF THE BLACK TEATFISH ON THE GBR

The Gonado-Somatic Index (short: gonad index, GI) was used to describe the period of reproduction in *H. nobilis*. It was initially planned to obtain 30 samples from reefs in the central section in the GBR every six weeks over 18 months. However, low densities of this species meant only 15 to 20 animals of this species were collected from two reefs, but only from one reef per sample time. Adverse weather conditions prevented samples being taken as regularly as planned.

All animals were weighed prior to dissections, which took place through an approximately five cm incision in the trivum ("ventral surface"). Gonads were removed and frozen in individual bags and the remaining body wall re-weighed. This second weight is termed "gutted" weight and is considered more accurate because variable amounts of water can be retained in the whole animal. In the laboratory, gonads were sexed macroscopically, or in some cases with the aid of microscopes, and weighed. Gonad indices presented

here are the weight of the gonad as a percentage of the weight of the body wall (total weight or gutted weight).

GROWTH RATES FOR BLACK TEATFISH

Experiments for this study were conducted on Davies Reef (18°50'S, 147°38'E) and in two locations of Lizard Island (14°40'S, 145°28'E). A total of 91 individuals of *Holothuria nobilis* were collected in May 2000 on the reef flats of Davies Reef and Bowl Reef (18°30'S, 147°33'E), in the central section of the GBR. Animals were brought on board a research vessel and kept in flowing seawater in 60 L containers. On Lizard Island, first samples were taken in June 2000 from two sample locations: 1) near Bird Islet (56 animals) and 2) near Palfrey Island (54 animals). Individuals from Davies Reefs were weighed using a spring balance (Salter Super Samson, ± 25g) and animals on Lizard Island were weighed with an electronic balance (± 1 g) after a minimum time of five minutes outside the water (this minimised the amount of water retained in the animals respiratory trees). Repeated measurements of 20 individuals showed that measurements have an average coefficient of variation of 5.8% of the mean. This variation is assumed to have resulted mainly from variation in the amount of seawater retained in the body cavity.

A small tissue biopsy (approximately 1 g) was taken from the ventral epidermis of each individual and preserved in 100% ethanol for later DNA fingerprint analysis. Tissue samples from the first sampling time on Davies Reef were snap frozen in liquid nitrogen and subsequently stored at –70°C. However, later tests showed that amplification success was higher with ethanol preserved samples. Animals were carefully returned to a distinct area at the back reef of Davies Reef that had been previously cleared of all *H. nobilis* detected in a two-hour search. On Lizard Island, animals were returned to the area of their origin, and the GPS position marked. Animals from Palfrey Island were released at two locations 90 m apart, to obtain some additional information on migration rates.

In November 2000, approximately six months later, 89 individuals from an area of up to 150 m from the release position on Davies Reef were collected during a six-hour search by manta tow. Similarly, 52 animals were collected from Bird Island and 57 from the two locations near Palfrey Island in December 2000. Animals were weighed, a tissue sample taken and individuals were released as described above. In May and June 2001, one year after the initial samples had been obtained, the procedure was repeated for a third time,

collecting 81 individuals in about four hours at Davies Reef, 59, and 55 at Bird and Palfrey Island, respectively.

Individual animals were identified by their DNA fingerprint patterns allowing growth to be assessed in specific individuals weighed over time.

DNA fingerprinting (AFLP)

DNA extractions from epidermal holothurian tissues were performed using Quiagen DNeasy extraction kits following instructions of the manufacturer. However, repeatability of the amplifications was greatly improved by reducing the initial amount of tissue to 10-20 mg, using twice the recommended amount of proteinase and performing an extra cleaning step using Sephadex CL6B spincolumns.

DNA fingerprints from each individual were obtained using the amplified fragment length polymorphism (AFLP) technique following general procedures described by Vos *et al.* (1995), with some variations as described by Wilson *et al.* (2002). In short, 100 ng of genomic DNA were digested with *Eco*RI and *MSE*I, followed by ligation of respective adapters in a 50 µL reaction volume. 1 µL of these ligations were used directly in 20 µL pre-amplifications using primers with a single selective base. *Eco*RI-AAG and *Mse*I-CGT were used as selective primers in the second amplification step after initial tests indicated that the combination of two times three selective bases yielded an appropriate amount of bands with variation. The *Eco*RI-AAG primer was end-labeled with ^{33}P and amplification products subjected to electrophoresis for 2.5 h on a 5% denaturing acrylamide gel, and later visualised through exposure to film (Kodak, BioMax). The primers resulted in approximately 80 bands in the well-resolved range between 100 and 480 nucleotides. Out of these bands, 31 that were polymorphic and could be reliably scored (as tested in repeated extractions and subsequent amplifications of 20 individuals), were chosen for assay. The presence or absence of each of these bands was scored for each individual to form a DNA fingerprint. Every individual was scored twice in two independent selective amplifications. Several individuals that had distinctly fewer bands than all other individuals were deemed as not having successfully amplified and were discarded from further analyses.

STATISTICAL ANALYSES

Survey data

Statistical analyses for the field surveys comparing number of individuals, biomass or gonad indices were conducted using a variety of ANOVA models or t-tests as performed by Statistica 4.5 (Statsoft 1994). Prior to ANOVAs, data were tested for homogeneity of variances with Cochran's C test, and transformed if necessary as indicated in the results.

Allozymes

Allele frequencies, basic statistics of genetic variability, cluster analysis, F-statistics and tests of conformation to Hardy-Weinberg expectations were performed using the TFPGA package (Miller 1997). Weir and Cockerham's (1984) methods for calculating Wright's F-statistics with corrections for unequal sample size were used with algorithms of TFPGA. Genetic variation was partitioned into that occurring within populations (F_{IS}) and that occurring between populations (F_{ST}). The significance of F_{IS} and F_{ST} values was tested using the χ^2 statistic. For tests of F_{IS}, $\chi^2 = N(F_{IS})^2$ (k-1) with degrees of freedom (d.f.) = [k(k-1)]/2 where N is the total number of individuals sampled and k the number of alleles at the locus. For tests of F_{ST} $\chi^2 = 2N(F_{ST})$ (k-1) with d.f. = (k-1)(s-1), where N and k are defined as above and s is the number of populations sampled (Waples 1987). In addition, the 95% confidence intervals for the average F_{ST} and F_{IS} values were calculated by bootstrapping across loci.

Deviations from Hardy-Weinberg equilibrium for each locus at each reef were tested by an Exact-Test choosing the conventional Monte Carlo method with the default settings in TFPGA. Significance values were appropriately corrected for multiple simultaneous tests (Miller 1966).

To test whether the allozyme data provide evidence for asexual reproduction in *Holothuria nobilis*, two parameters which proved to be reliable indicators in previous studies were calculated (Uthicke *et al.* 1998; 1999). The ratio of the number of genotypes observed in the population (N_{go}) over the sample size (N_i) provides a measure for the minimum input of sexual reproduction. The maximum input through sexual reproduction in each population can be estimated by calculating the maximum number of sexually

produced individuals (N^*) and dividing this over the sample size. The calculations of these two parameters are described in detail in Uthicke *et al.* (1998).

mtDNA

All sequences were aligned using Assemblylign. The Kimura 2-parameter substitution model was used in the following calculations. Haplotype and nucleotide diversity were calculated using Arlequin 2.001 (Schneider *et al.* 2000). This program was also used to calculate the genetic distances between populations and to perform the following computations on Analysis of Molecular Variation (AMOVA) and F_{ST} statistics. AMOVA (Excoffier *et al.* 1992) was used to partition the total molecular variance (σ^2) into covariance components due to differences within populations (σ^2_c), among populations within groups (σ^2_b) and among groups (σ^2_a). The significance of the respective fixation indices (F_{ST}, F_{SC} and F_{CT}) was tested using permutation procedures described in Excoffier *et al.* (1992) with 1000 permutations. F_{ST} values between all pair of populations were calculated and their significance tested using the permutation procedure.

RESULTS

DEVELOPMENT OF SURVEY METHODOLOGY

Estimates for *H. atra* obtained from 10 transects for each method were in the same order of magnitude, whereas densities for *S. chloronotus* were somewhat lower using manta tows (Table 3). This may reflect the fact that these animals are sometimes semi-cryptic and thus harder to detect during manta tows. No *H. nobilis* was discovered during the $40m^2$ and $100m^2$ transects. This reflects the low densities of these animals when compared to the more common species, and the much-increased area using the manta tow technique. Although the smaller transects may be sufficient for the more abundant species, it was concluded that manta tows were the best method to use during the surveys as *H. nobilis* was the main target species.

Table 3. Comparison of methods for estimating holothurian densities in reef flat areas.

Method	10 transects		Densities (ind. x $ha.^{-1}$, +- SD)		
	Total Time (min)	Total area (m^2)	*H. nobilis*	*H. atra*	*S. chloronotus*
100 m^2Transect	60	1000	0 (0)	370 (231)	110 (159)
40 m^2Transect	40	400	0 (0)	475 (299)	125 (176)
500 m^2Manta	40	5000	12.0 (19.3)	532 (283)	66 (36)

SPATIAL SURVEYS AND STOCK SIZE OF BÊCHE-DE-MER OVER THE GBR

Black Teatfish

Holothuria nobilis occurs on outer and midshelf reefs and appears to achieve highest abundances on the Ribbon Reefs (Fig. 2). In spot checks and search tows on locations closer to the shore (as indicated in Fig. 1) *H. nobilis* was never detected. Significant differences were observed between reefs closed to fishing (green reefs) and those reefs open to fishing (blue reefs) (factor Zone - Blue vs. Green reefs), and between different sectors of the GBR (factor Sector), but the interaction between those factors was also significant (Table 4). The explanation for this interaction is shown in Fig. 3. In general, densities on the green (un-fished reefs) decrease from north to south, indicating that this species is naturally more abundant in the northern two sectors, i.e. the area North of Townsville.

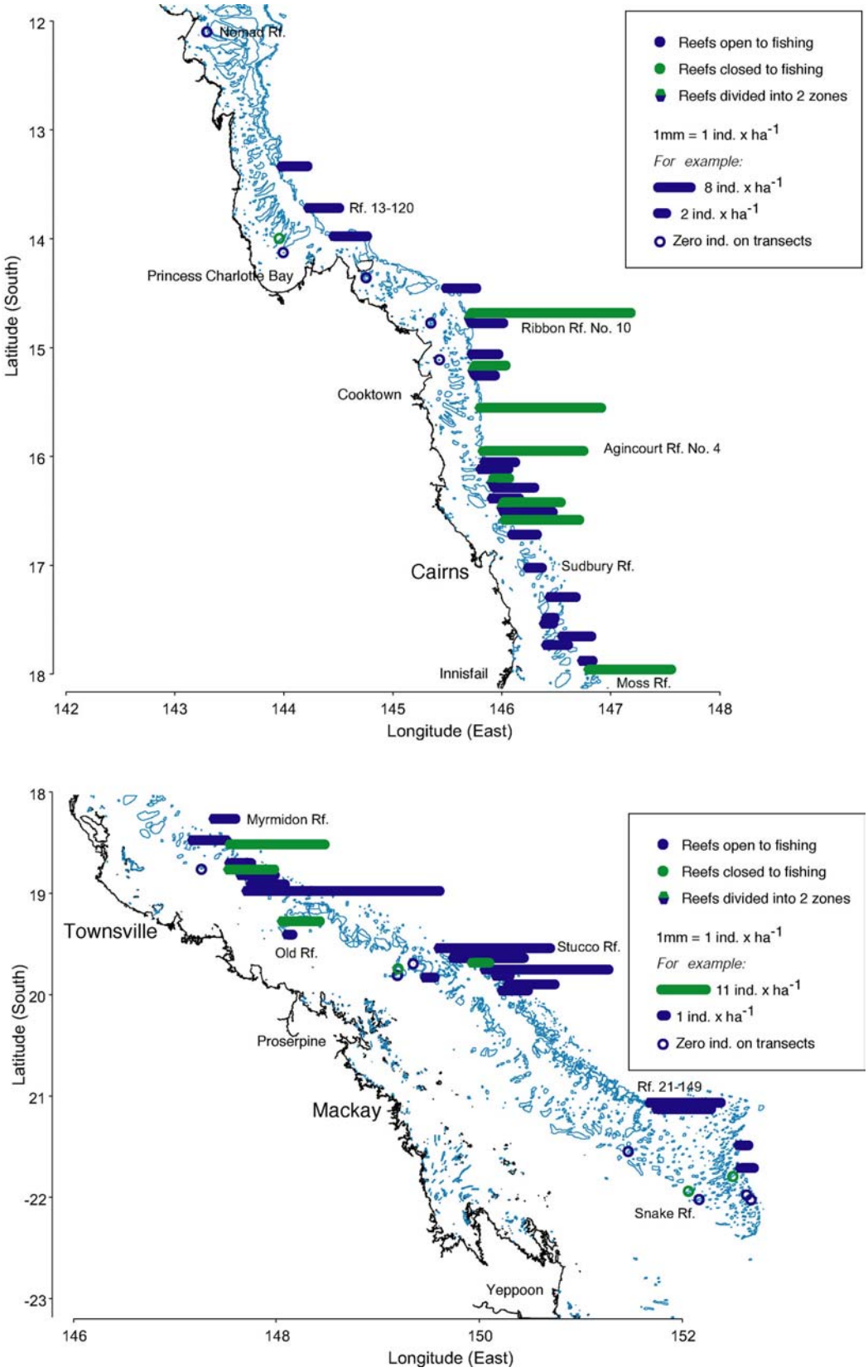

FIGURE 2. Distribution map of *Holothuria nobilis* (black teatfish) on northern (top map) and southern (bottom map) reefs of the Great Barrier Reef.

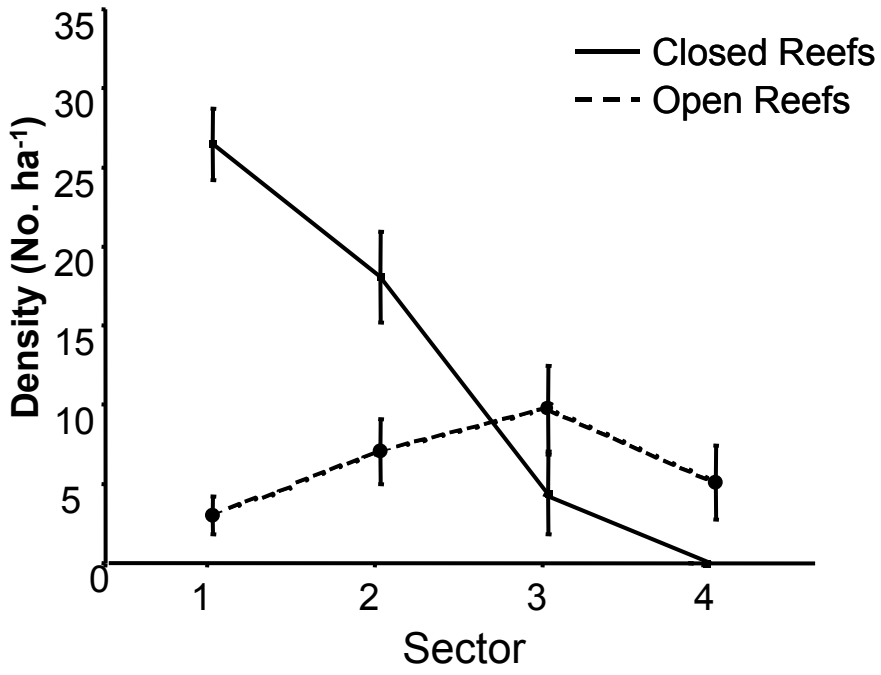

FIGURE 3. Average densities of *Holothuria nobilis* in four arbitrary sectors, and in green (Closed Reef) and blue (Open Reef) reefs of the Great Barrier Reef. Error bars indicate 1 SE.

TABLE 4. Results of nested ANOVA comparing third root transformed densities of *Holothuria nobilis* in four Sectors and differently protected Zones (green and blue) in the GBR.

Source of Variation	Df	Mean Square	F	P
Zone	1	6.928	7.67	0.008
Sector	3	6.588	7.23	<0.001
Z x **R**	3	7.925	8.77	<0.001
Reef (**Z, S**)	49	0.903	2.48	<0.001
Residual	858	0.367		

However, no *H. nobilis* were detected on the green reef sampled in the Swains region. Although not all green reefs were investigated, those most likely to have H. nobilis, based on prior experience, were chosen for sampling. The main conclusion of the interaction term depicted in Fig. 3 is that an effect of fishing (as a difference between blue and green reefs) can only be detected in the area north of Townsville, the area potentially fished. Although several reefs to the south of that (e.g. in the Whitsunday area, or in the hardline of the Pompeys), these reefs are sparsely scattered or hard to access due to oceanographic conditions, and therefore fishing in those areas is likely to be un-economical.

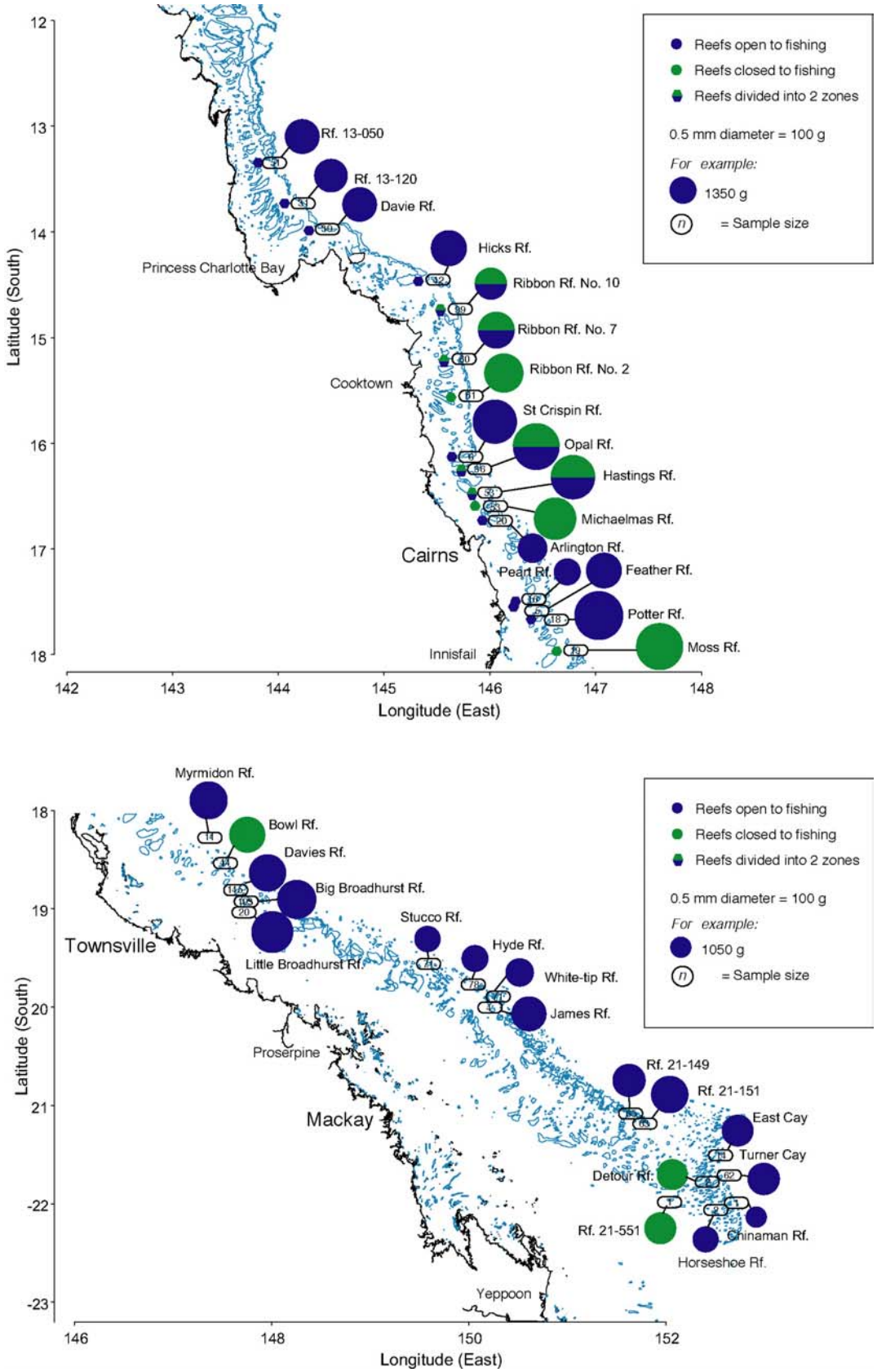

FIGURE 4. Average weights (total weight) of *Holothuria nobilis* on northern (top map) and southern (bottom map) reefs of the Great Barrier Reef.

The average total weight of *H. nobilis* for given reef populations ranged between 1300 and 2450 g (Fig. 4). ANOVA revealed significant differences in average weights of the four sectors (Table 5). However, a post hoc (Tukey) test showed that only means in Sector 3 (Whitsunday area, average 1361 g, SE = 23 g) were significantly different from those in Sector 2 (1981 g, SE = 19 g). Mean weights in Sector 1 (1737 g, SE = 24 g) and Sector 4 (1704 g, SE = 26 g) were intermediate and are not statistically significantly different from the other samples. Apart from the small weights in the Whitsunday area, no other geographic patterns in the weights are apparent. Although many average weights for fished reefs were smaller than those on green reefs, this difference was not statistically significant (Table 6).

TABLE 5. Results of nested ANOVA comparing square root transformed weights of *Holothuria nobilis* in four Sectors of the GBR.

Source of Variation	Df	Mean Square	F	P
Sector	3	902.74	2.62	**0.023**
Reef (**S**)	24	236.40	9.88	**< 0.001**
Residual	1411	23.92		

TABLE 6. Results of nested ANOVA comparing square root transformed weights of *Holothuria nobilis* in fished and unfished Zones, only for the fished Sectors 1 and 2.

Source of Variation	Df	Mean Square	F	P
Zone	1	871.12	2.62	**0.124**
Reef (**Z**)	17	332.98	13.21	**< 0.001**
Residual	932	25.20		

Because the analyses described above indicated significant differences between blue and green reefs for *H. nobilis* in the fished area of the reef north of Townsville, more detailed analyses of this area were undertaken. The densities on each of the five reefs closed to fishing were distinctly higher than on all reefs open to fishing (see Fig. 2). The average density on the five green reefs (22.84 ind. ha^{-1}) was more than four times higher than on the 16 blue reefs (5.01 ind. ha^{-1}). Although there were significant differences among locations within reefs, there were no differences among reefs within zones (Table 7). The difference between fished and un-fished reefs was highly significant.

TABLE 7. Results of nested ANOVA on square root-transformed densities of *Holothuria nobilis* comparing 16 fished and five reefs totally closed to fishing.

Source of Variation	Df	Mean Square	F	P
Zone	1	71.11	33.02	<0.001
Reef (**Z**)	18	2.15	0.314	0.995
Location (**R**)	39	6.85	1.66	0.010
Residual	292	3.931		

The two-factor ANOVA comparing densities in open and closed zones within reefs showed a significant interaction between the factor *Zone* and *Reef* (Table 8). This reflected the different result for Ribbon Reef No. 10 compared with the other reefs. Densities in the closed zone of that reef where about five times that in the open zone (Fig. 5).

TABLE 8. Results of a two-factor ANOVA on square root-transformed densities of *Holothuria nobilis* on reefs that are divided into zones open to fishing and those closed to fishing.

Source of Variation	df	Mean Square	F	P
Zone	1	23.39	4.49	0.037
Reef	3	21.36	4.10	0.009
Z x **R**	3	20.94	4.02	0.010
Residual	92	5.22		

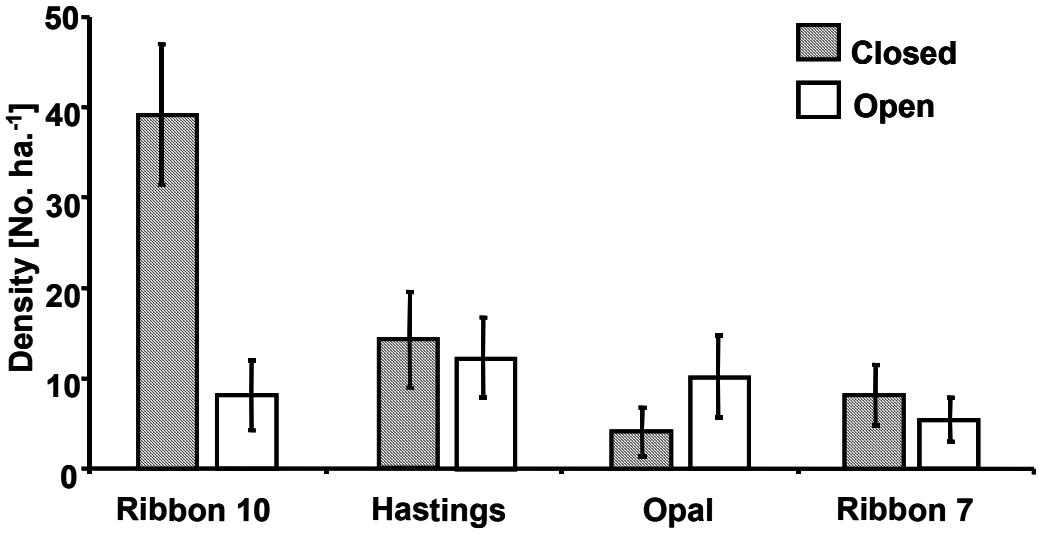

FIGURE 5. Average densities of *Holothuria nobilis* on reefs that are divided into blue (Open to fishing) and green (Closed to fishing) zones. Error bars indicate 1 SE.

A subsequent one factor ANOVA revealed that this difference was significant ($F = 4.53$, $p = 0.001$, comparison of means: $p = 0.001$). In contrast, densities between the open and closed zones of Hastings, Opal and Ribbon Reef No. 7 were not significantly different from each other (comparison of means: $p > 0.05$ in each case). The densities on these reefs were on a level comparable to reefs completely open to fishing, with the exception of Hastings Reef where density estimates were slightly higher.

Stock Size

Data from model calculations on virgin stock size and biomass, and the remaining numbers after fishing are summarized in Table 9. Estimates indicate that about 4.7 million individuals of *Holothuria nobilis* were available in the fished area of the GBR prior to fishing. This number translated to a total biomass of 5,600 tonnes. These values were reduced to about 45% during fishing. Although only about 25 % of all stock was initially protected on closed reefs, more than 50% of the remaining numbers and biomass are now on these reefs.

TABLE 9. Estimates of numbers and biomass of *Holothuria nobilis* in the fished area of the GBR (12-19° S), before fishing commenced in the mid 1980's, and after closure of the fishery in 1999. Density estimates for green reefs are 20.88 ind. x $ha.^{-1}$ (N = 6, 95% CI = 16.3 - 25.73) and 5.52 ind. x $ha.^{-1}$ (N = 29, 95% CI = 2.84 - 8.20) for blue reefs. The average gutted weight was 1,193 kg (N = 1319).

	Fished	Protected	Total
Area (ha)	167,431	56,810	224,241
Pre fishing			
Number ($*10^6$)	-	-	4.68 (3.59-5.77)
Biomass (t)	-	-	5,585 (4,288-6,882)
Post fishing			
Number ($*10^6$)	0.92 (0.48-1.37)	1.19 (0.91-1.46)	2.11 (1.39-2.83)
Biomass (t)	1,103 (568-1,639)	1,415 (1,086-1,743)	2,518 (1,654-3,382)
Total Reduction			
Number ($*10^6$)	-	-	**2.57**
Biomass (t)	-	-	**3,067**

Other Species

Several species other than *H. nobilis* were frequently observed on the transects. The most common were *S. chloronotus* (greenfish) and *H. atra* (lollyfish). The distribution map of *H. atra* (Fig. 6) indicates that this species is more abundant on midshelf reefs than on outer shelf reefs. A statistical analysis indicated that there are also significant differences between the sectors (Table 10). A post hoc analysis of means (Tukey test) showed that densities of *H. atra* in Sector 2 are significantly higher than in Sectors 3 and 1 (Table 11).

TABLE 10. Results of nested ANOVA comparing third root transformed densities of *Holothuria atra, Stichopus chloronotus, Stichopus variegatus* and *Bohadschia argus* in four Sectors and green and blue (Factor Zone) reefs of the of the GBR.

		H. atra			*S. chloronotus*			*S. variegatus*			*B. argus*		
Source of Variation	df	MS	F	P	MS	F	P	MS	F	P	MS	F	P
Zone	1	40.47	2.01	0.162	5.71	0.47	0.496	1.86	3.87	0.055	1.72	1.72	0.194
Sector	3	97.87	4.87	**0.005**	83.38	6.87	**0.001**	2.28	4.75	**0.005**	2.12	2.14	0.107
Z x **R**	3	5.75	0.29	0.835	1.72	0.14	0.934	0.58	1.22	0.313	0.21	0.21	0.887
Reef (**Z, S**)	49	20.09			12.13			0.48			0.99		
Residual	858	1.30			0.99			0.11			0.28		

Table 11. Average abundance (ind ha^{-1}) of four holothurian species in each of four sectors.

Sector	*H. atra*	*S. chloronotus*	*S. variegatus*	*B. argus*
1	50.57	50.46	0.00	2.80
2	356.21	142.25	0.44	8.61
3	60.50	48.71	5.89	4.65
4	200.84	7.94	4.73	0.84

The distribution of *Stichopus chloronotus* is similar to that of *H. atra* (Fig. 7). The factor Sector was also significant for this species (Table 10), and post hoc analysis of means indicated that average densities in Sector 2 were significantly higher than in all other Sectors (Table 11).

Stichopus variegatus (curryfish) was hardly detected in the northern two sectors (Fig. 8) but quite common in the other sectors. Because *S. variegatus* was commonly observed in deeper areas outside the transects in the north, it is likely that the apparent difference in densities may be a shift in habitat between the sectors. Differences in the means between

the sectors were only significant between Sector 3 and 1 ($p < 0.10$), 3 and 2 ($p< 0.05$), and 4 and 2 ($p< 0.1$) (Tables 10 and 11). However the analysis lacks statistical power due to the low numbers on many reefs.

Bohadschia argus (leopardfish) also appears to be most common on midshelf reefs of sectors 1 and 2 (Fig. 9), and to have very low in numbers in the south. However, statistical analysis did not detect any significant differences between sectors (Table 11). Further species counted in the transects were *Thelenota ananas* (prickly redfish, Fig. 10), *Actinopyga echnites* (blackfish, Fig. 11) and *A. miliaris* (deepwater redfish, Figure 12). However, their occurrence was so sporadic that no statistical analyses could be conducted. Several other species were observed but not counted because they could not be distinguished in manta tows, or are cryptic for a part of the day. These species include *Holothuria edulis* (pink fish), *H. leucospilota, H. couluber, Bohadschia marmorata* and *Actinopyga mauritania* (surf fish).

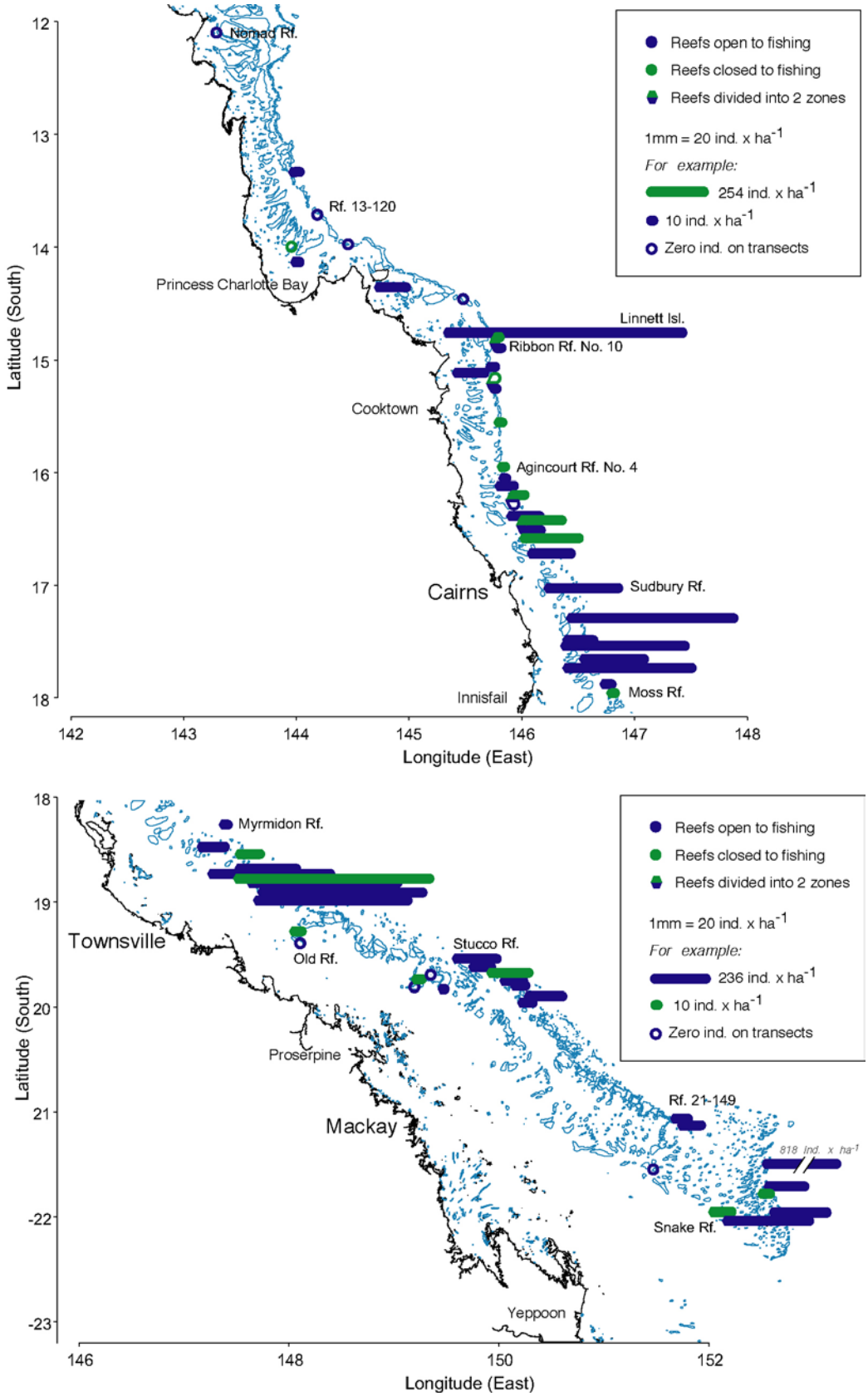

FIGURE 6. Distribution map of *Holothuria atra* (lollyfish) on northern (top map) and southern (bottom map) reefs of the Great Barrier Reef.

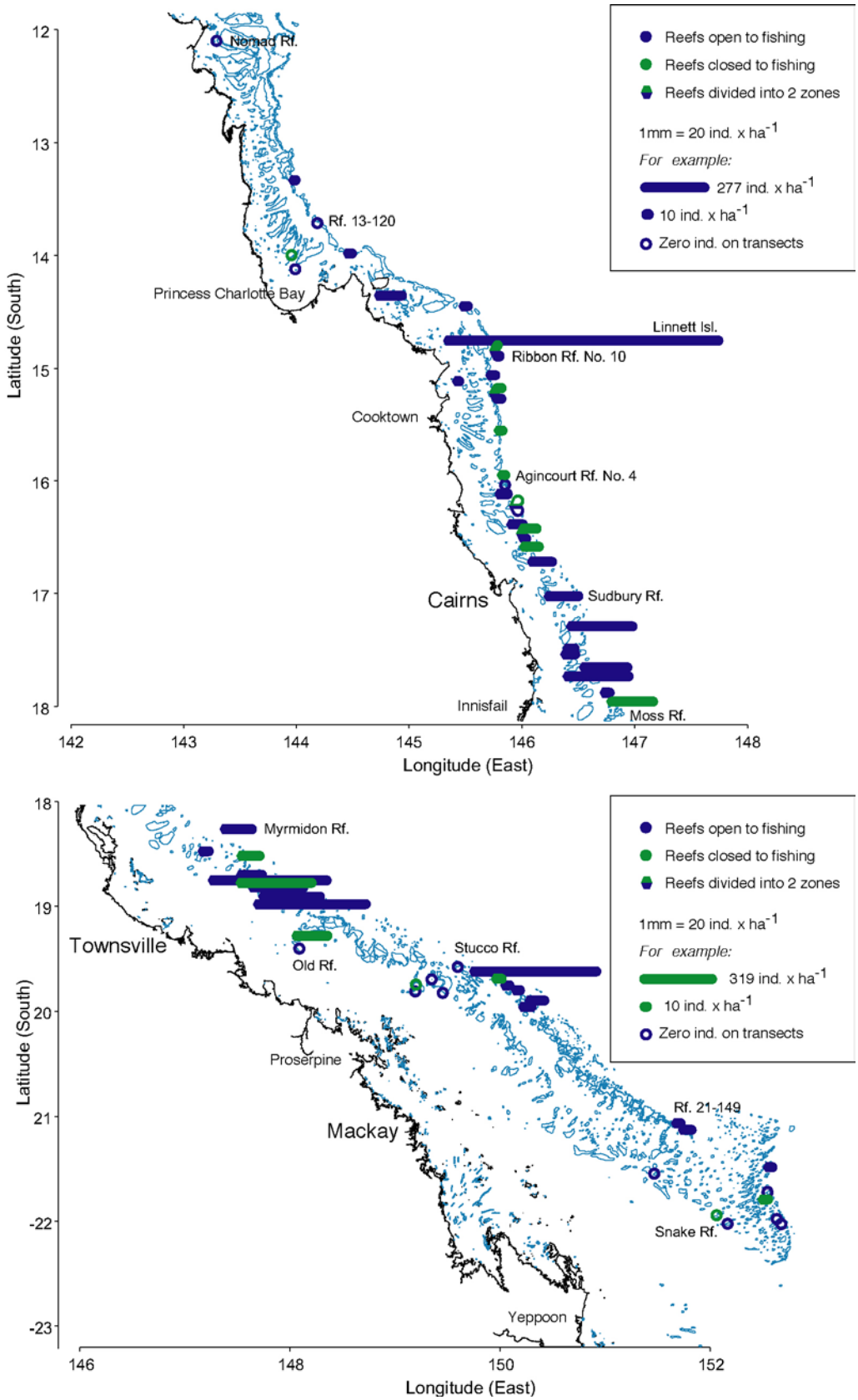

FIGURE 7. Distribution map of *Stichopus chloronotus* (greenfish) on northern (top map) and southern (bottom map) reefs of the Great Barrier Reef.

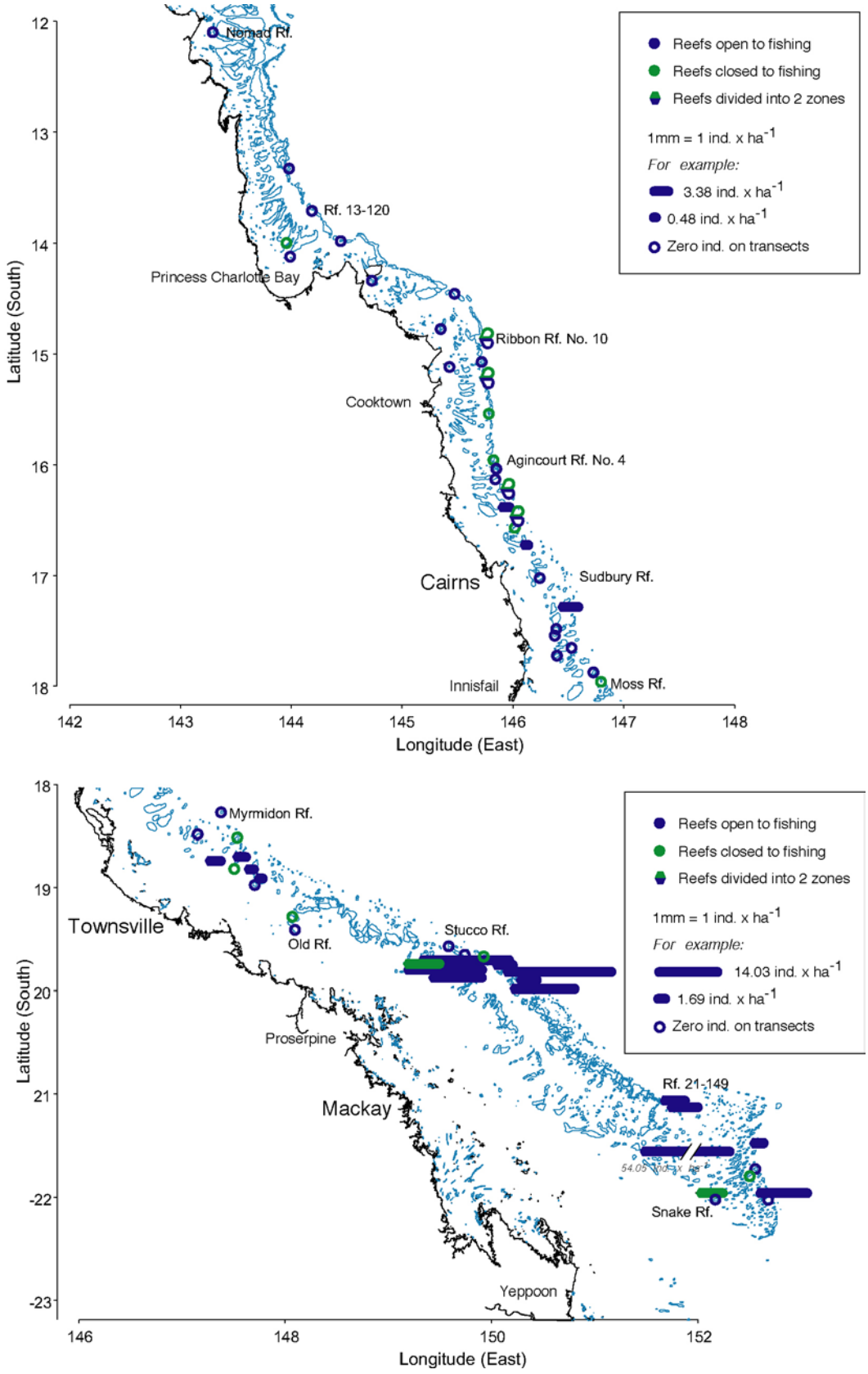

FIGURE 8. Distribution map of *Stichopus variegatus* (curryfish) on northern (top map) and southern (bottom map) reefs of the Great Barrier Reef.

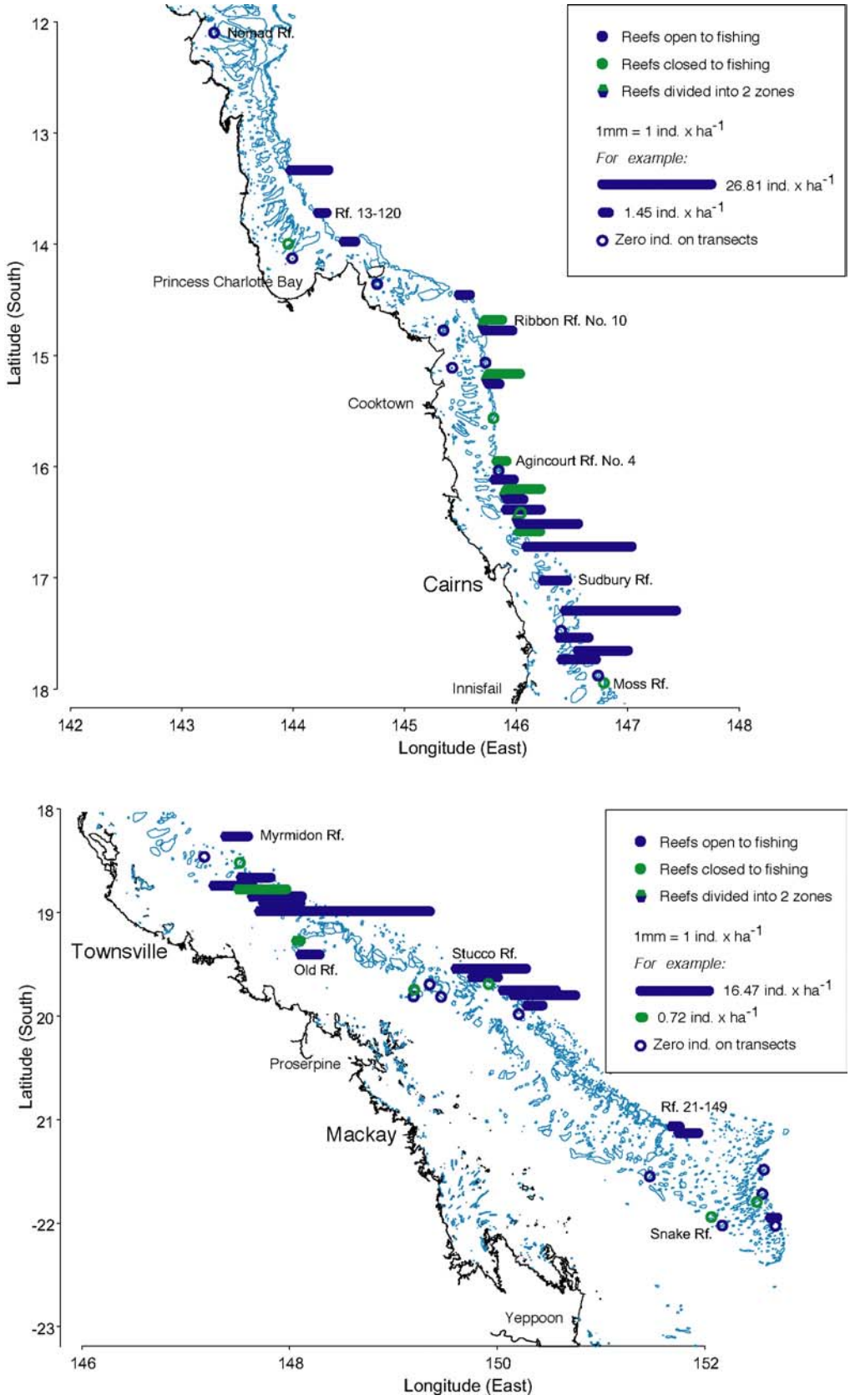

FIGURE 9. Distribution map of *Bohadschia argus* (leopard fish) on northern (top map) and southern (bottom map) reefs of the Great Barrier Reef.

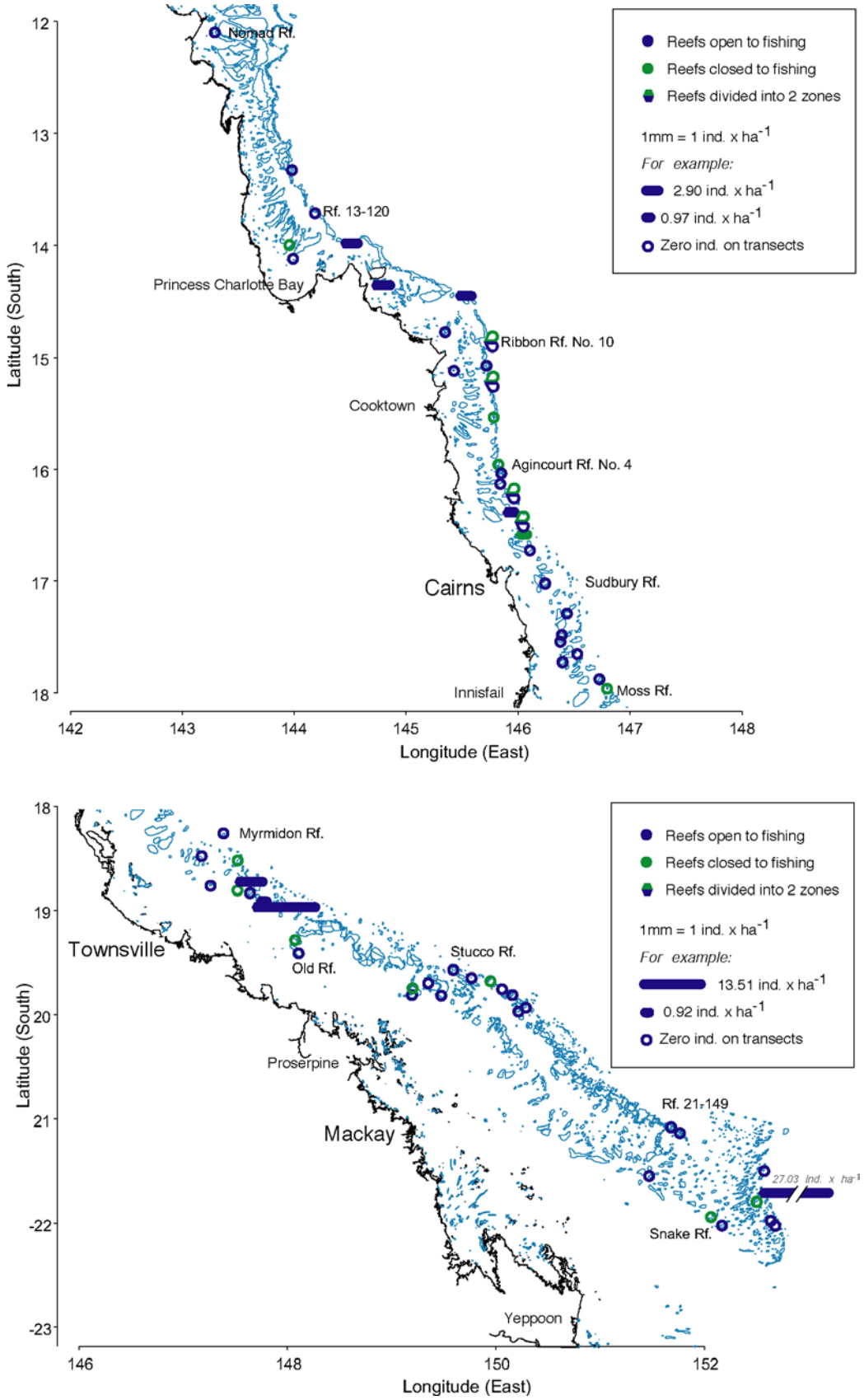

FIGURE 10. Distribution map of *Thelenota ananas* (prickly redfish) on northern (top map) and southern (bottom map) reefs of the Great Barrier Reef.

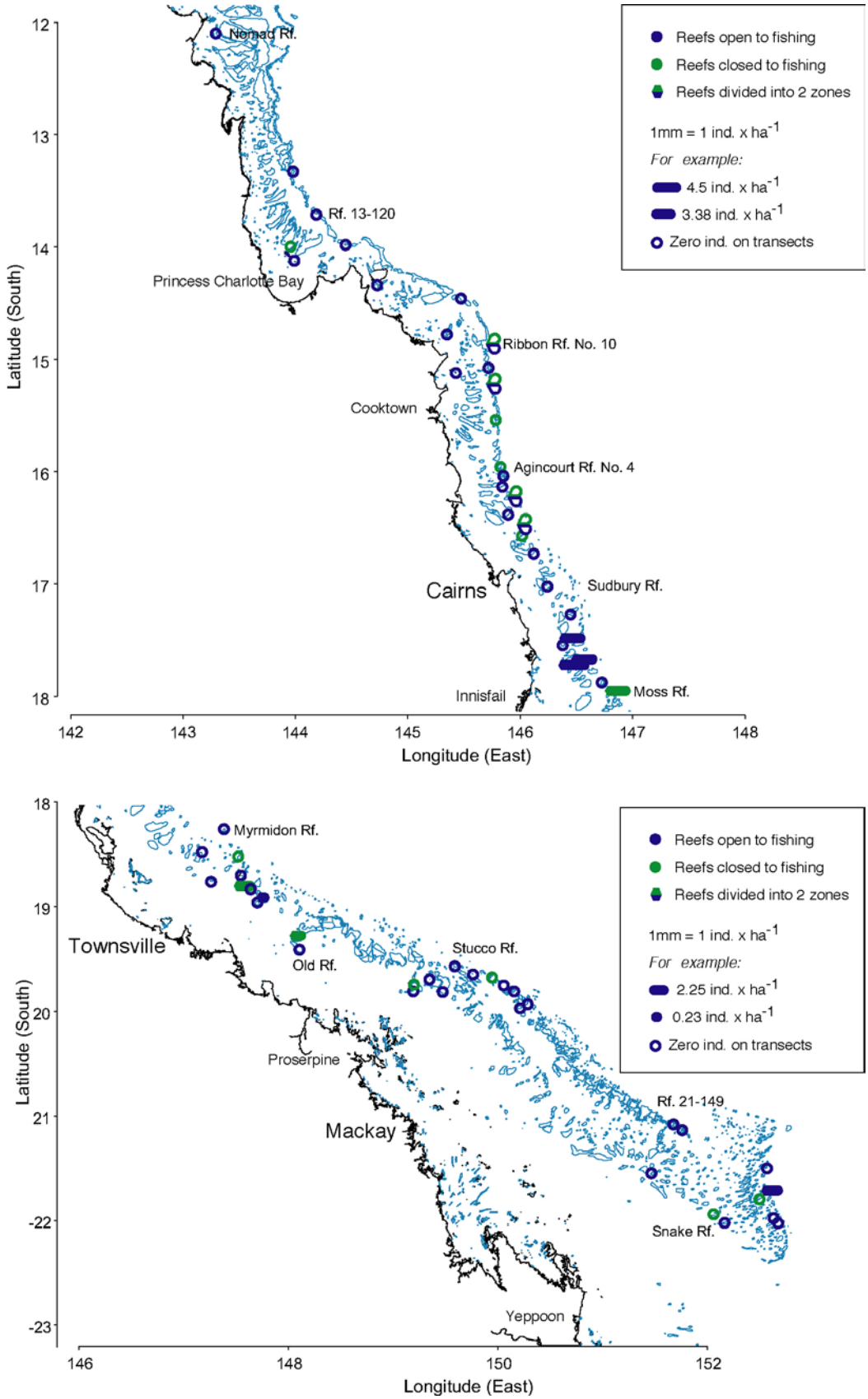

FIGURE 11. Distribution map of *Actinopyga echinites* (deepwater redfish) on northern (top map) and southern (bottom map) reefs of the Great Barrier Reef.

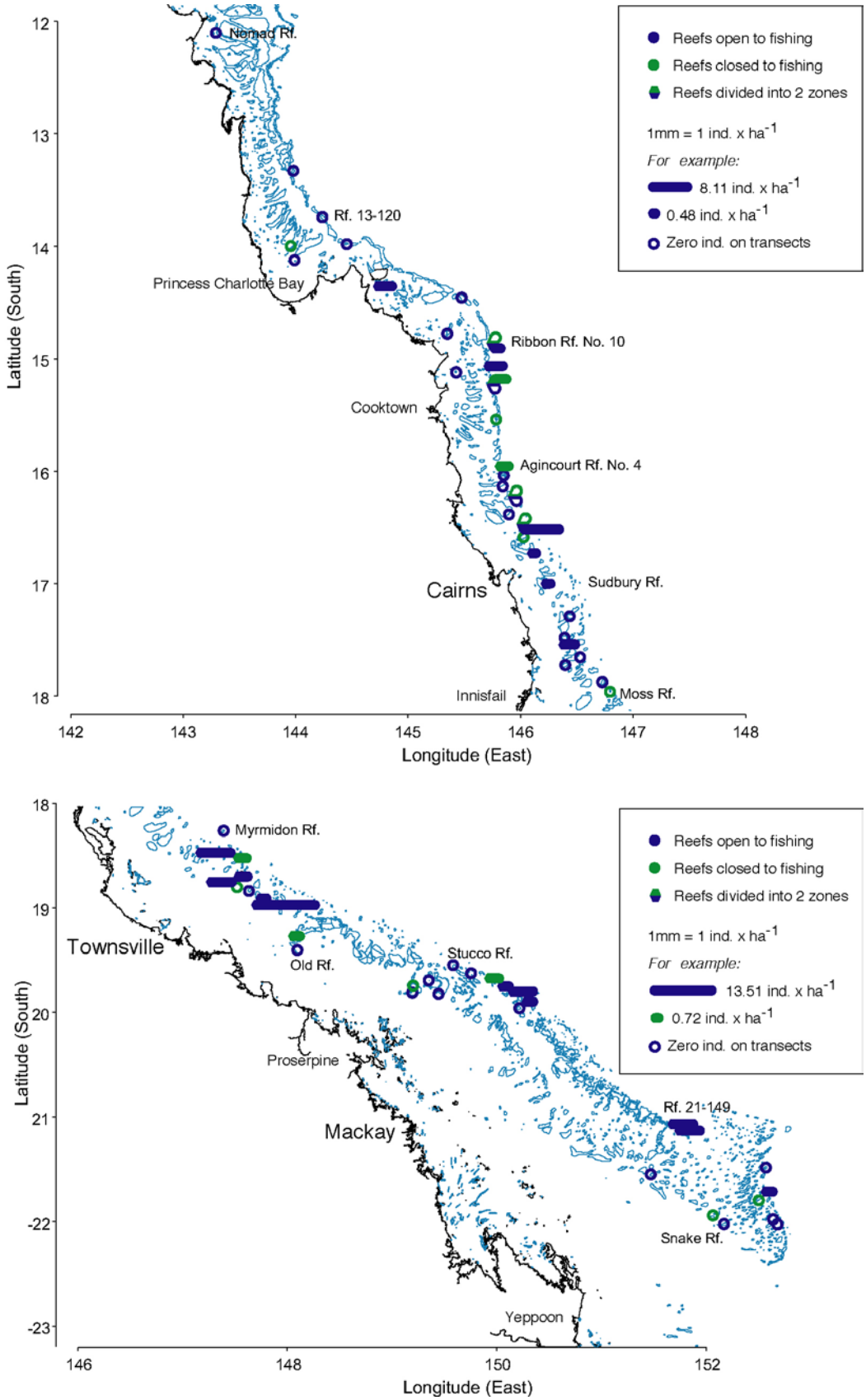

FIGURE 12. Distribution map of *Actinopyga miliaris* (black fish) on northern (top map) and southern (bottom map) reefs of the Great Barrier Reef.

TEMPORAL SURVEYS OF OVER-FISHED BLACK TEATFISH STOCKS

Twenty three reefs in the fished area of the GBR were resurveyed one year and two years after closure. ANOVA revealed that the difference between blue and green reefs remained significant for the total period surveyed (Factor Zone, Table 12). An increase of the average densities in the blue reefs (but not in the green reefs) would have been detected in a significant interaction term, but this was not the case. A subsequent ANOVA only for the blue reefs also showed no significant differences between surveys at different times ($F_{2, 39} = 0.885$, $p = 0.421$). Total densities appeared to be slightly higher on the green reefs in the second survey, but are nearly exactly at the same level two years after closure (Fig. 13).

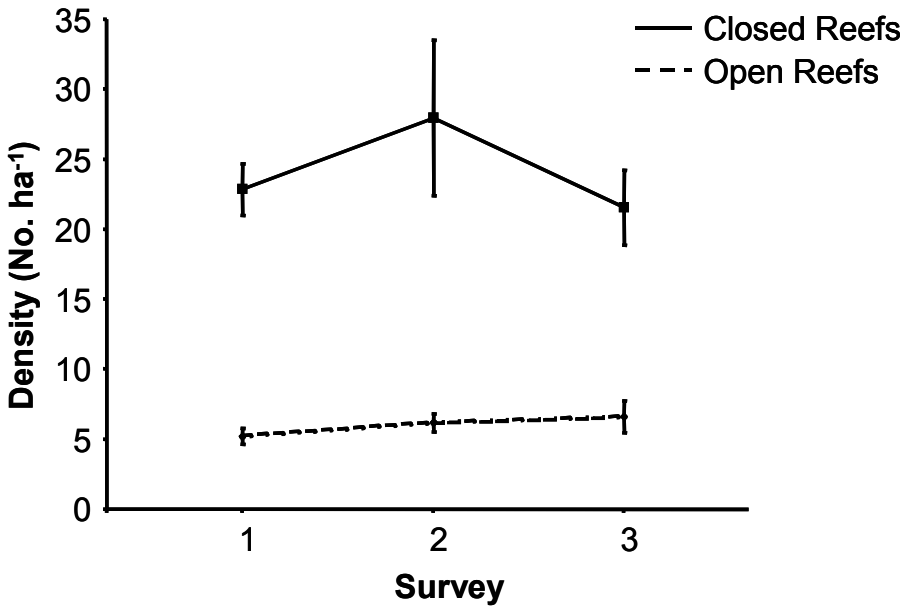

FIGURE 13. Average densities of *Holothuria nobilis* on five green (Closed) and 15 blue (Open) reefs prior to the total closure of the fishery (Survey 1) and about 1 (Survey 2) and 2 (Survey 3) years after the closure.

The total average on the blue reefs appears to increase slightly, but this increase is not statistically significant (see above). However, it is noteworthy that averages on the 8 northern most reefs of our survey area, with the exception of Ribbon Reef No. 10), are all higher in the third survey than in the first (Fig. 14).

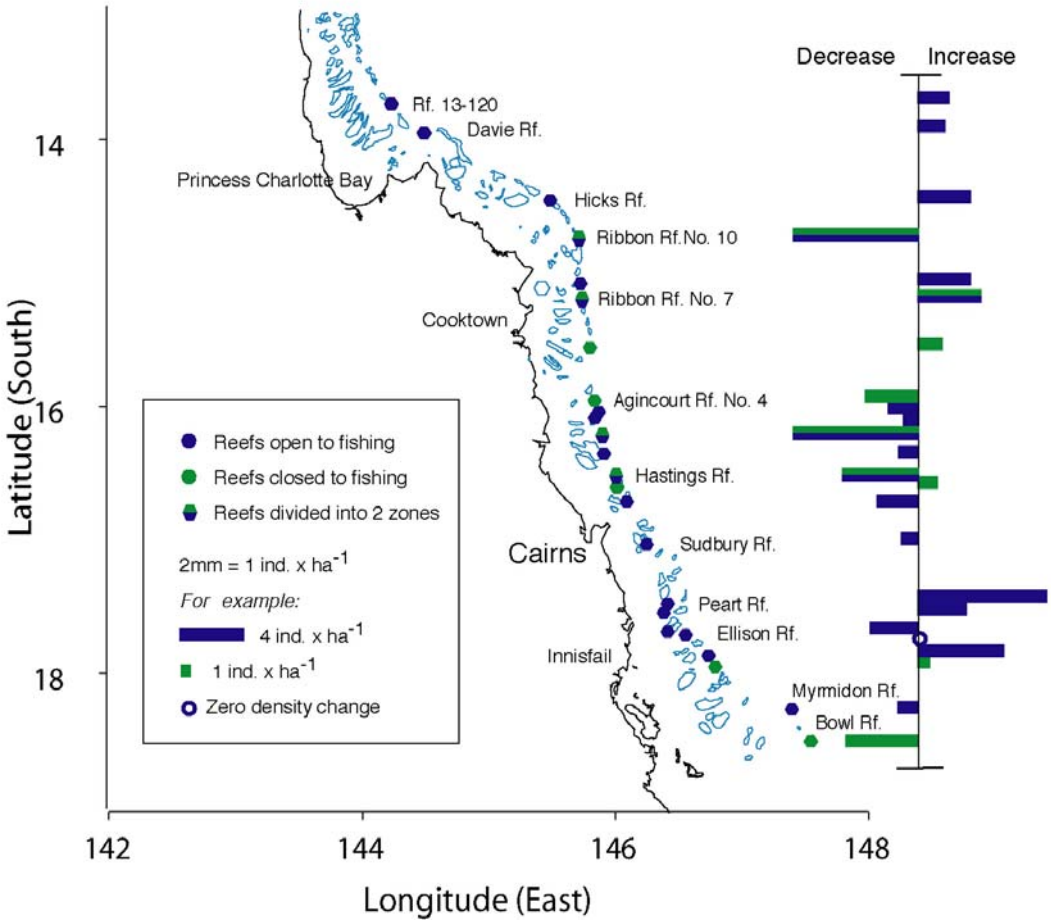

FIGURE 14. Increase and decrease of densities of *Holothuria nobilis* when compared before closure and 2 years after closure of the fishery.

TABLE 12. Results of a repeated measures ANOVA on third root-transformed ($[x+0.5]^{0.25}$) densities of *Holothuria nobilis* on reefs that were resurveyed one and two years after closure of the fishery.

Source of Variation	df	Mean Square	F	P
Between Subjects				
Zone	1	87.0497	97.50	**<0.001**
Error = Reef **(Z)**	17	0.8928		
Within Subjects				
Survey	2	0.2377	0.39	0.6788
Z x S	2	0.5364	0.88	0.4222
Error = **R**(Z) x **S**	36	0.6066		
Residual	1038	0.4233		

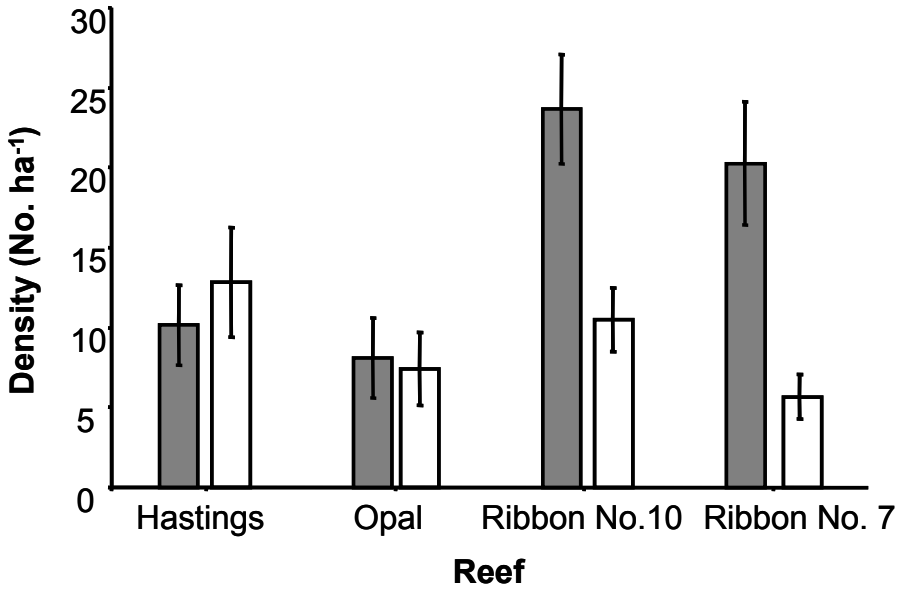

FIGURE 15. Densities of *Holothuria nobilis* on four reefs, which are divided into zones open (white bars) and closed (grey bars) to fishing prior to total closure of the fishery, averaged over three surveys.

A repeated measures ANOVA on density data from the four reefs divided into a fished and an un-fished zone reveals a significant interaction between the factors Zone and Reef (Table 13). Averaged over all surveys, densities of *H. nobilis* are distinctly higher in the green zones of the two Ribbon Reefs (No. 10 and 7), but not for Opal and Hastings Reef, confirming the trend observed on the first survey (see Fig. 5). Although the factor Survey is significant in the "Within Subjects" effect, this factor is also involved in a three-factor interaction term.

TABLE 13. Results of a repeated measures ANOVA on third root-transformed densities of *Holothuria nobilis* on reefs that were divided into one open and one closed zone, resurveyed one and two years after closure of the fishery.

Source of Variation	df	Mean Square	F	P
Between Subjects				
Zone	1	4.0577	6.79	**0.011**
Reef	3	2.4815	4.15	0.008
R x **Z**	3	1.7258	2.89	0.040
Error	90	0.5977		
Within Subjects				
Survey	2	1.6289	3.69	0.027
S x Z	2	0.2596	0.59	0.556
S x R	6	0.5929	1.34	0.240
S x R x Z	6	1.5290	3.47	0.003
Error	180	0.4409		

Animals on 11 reefs in the fished area of the GBR were weighed before closure of the fishery and during the subsequent two surveys. An analysis of variance indicated that both factors, Survey and Reef, are significant (Table 14). However, the interaction term between those was significant, and a separate one factor ANOVA for each reef was conducted. Average weights decreased on the majority of reefs after the closure, but since some reefs showed an increase no clear trend can be identified at this stage (Fig. 16).

TABLE 14. Results of a two-factor ANOVA on square root-transformed weights *Holothuria nobilis* at three different surveys and 11 reefs.

Source of Variation	df	Mean Square	F	P
Survey	2	413.85	19.09	<0.001
Reef	10	594.87	27.43	<0.001
S x **R**	20	186.62	8.61	<0.001
Residual	1123	21.68		

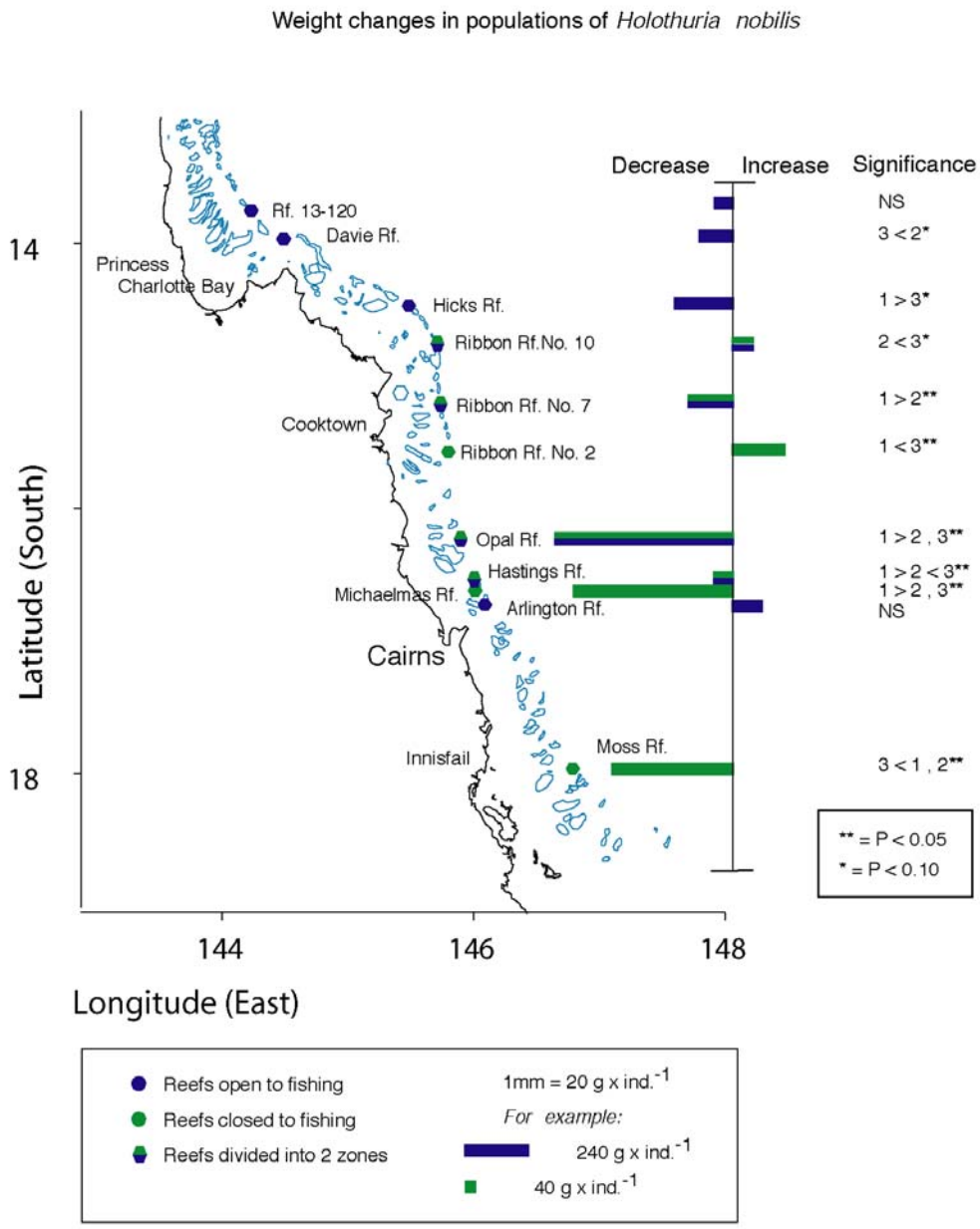

FIGURE 16. Changes in average weights in 11 populations of *Holothuria nobilis* when compared between Survey 1 and 3. Significance levels refer to separate one factor analysis for each reef, numbers indicate significance between separate surveys as determined by subsequent Tukey HSD post hoc analysis, e.g. 1 > 2,3 indicates weights at survey 1 are significantly higher than at survey 2 and three.

DISPERSAL AND RECRUITMENT IN BLACK TEATFISH

Allozymes

There were no statistical differences between sub-samples within reefs from any reef where more than one location was sampled. Samples taken at different times on Little Broadhurst Reef, Big Broadhurst Reef and Davies Reef were also not statistically different (χ^2 test, for all comparisons: df = 14, $\chi^2 < 20$, $p > 0.1$). Therefore sub-samples within reefs and over times were pooled for further analyses.

With the exception of some rare alleles (*FL-EST*90*, *HK*85*, *MPI*106*, *PGM*115* and *PGM*58*), all alleles were found in each population and there were no major differences in allele frequencies (Table 15). There were several small deviations from Hardy-Weinberg equilibrium, the majority of which were heterozygote deficits (D values, Table 16). However, statistical tests (p values, Table 16) revealed that there was no significant deviation from Hardy-Weinberg expectations in any locus, or in any population. Similarly, the observed heterozygosity was slightly below the one expected under Hardy-Weinberg equilibrium for most populations, but standard errors of these overlapped in each case (Table 17).

Other measures of genetic variability within populations (mean number of alleles, % polymorphic loci) showed no differences between populations (Table 17). One exception was the lower number of both parameters on East Cay, but this was likely to be the result of the small sample size for this reef.

The estimates for the minimum input through sexual reproduction ($N_{go} : N_i$) were between 0.74 and 1 (Table 17); and the estimates for the maximum input through sexual reproduction ($N^* : N_i$) were all one. Thus, in combination with all populations being virtually in perfect Hardy-Weinberg equilibrium, there were no indications for the occurrence of asexual reproduction in *Holothuria nobilis*.

Nei's (1978) unbiased genetic distance (D) was between 0 and 0.003 between pairs of populations and an UPGMA clustering showed no strong groups, or any associations of geographically close populations (data not shown). The relatively low cophenetic correlation (0.620) also indicated a lack of robustness in the relationships of the tree, further emphasising the lack of structure in the data. There was no significant genetic distance between any population pairs.

TABLE 17. *Holothuria nobilis*. Summary measures describing genetic variability from 15 reefs on the GBR. Standard errors are given in parentheses beneath the means where appropriate. Individual samples were only included in the number of individuals (N_i) and calculations for the number of genotypes (N_{go}) and the maximum number of sexually produced individuals (N^*) when results for all seven loci were present.

	Mean sample size per locus	Mean no. of alleles per locus	% of loci poly-morphic	Mean Heterozygosity		N_i	N_{go}	N_{go}/N_i	N^*	N^*/N_i
				Observed (H_o)	Expected (H_e)					
13-050	51.0	3.0	100	0.269 (0.072)	0.296 (0.083)	51	41	0.80	51	1
Davie	38.7	3.0	100	0.261 (0.080)	0.290 (0.091)	37	33	0.89	37	1
Hicks	41.4	3.0	100	0.283 (0.079)	0.302 (0.082)	39	35	0.90	39	1
Ribbon No. 10	98.0	3.0	100	0.275 (0.080)	0.302 (0.092)	93	73	0.78	91	1
Opal	35.6	3.0	100	0.302 (0.080)	0.317 (0.080)	34	31	0.91	34	1
Michaelmas	53.0	3.0	100	0.280 (0.088)	0.298 (0.084)	53	45	0.85	53	1
Davies	42.1	3.0	100	0.284 (0.082)	0.316 (0.087)	37	35	0.95	37	1
Big Broadhurst	123.0	3.1	100	0.274 (0.082)	0.298 (0.085)	117	87	0.74	117	1
Little Broadhurst	19.4	3.0	100	0.267 (0.066)	0.310 (0.078)	17	15	0.88	17	1
Stucco	69.9	3.0	100	0.284 (0.075)	0.312 (0.080)	69	61	0.88	69	1
White Tip	71.0	3.0	100	0.310 (0.081)	0.329 (0.089)	71	65	0.92	71	1
21-149	69.0	3.0	100	0.300 (0.083)	0.298 (0.083)	69	58	0.84	69	1
21-151	72.6	3.1	100	0.320 (0.096)	0.336 (0.101)	70	63	0.90	70	1
East Cay	14.0	2.4	86	0.286 (0.100)	0.312 (0.102)	14	14	1	14	1
Turner Cay	60.9	3.1	100	0.312 (0.085)	0.301 (0.082)	54	48	0.88	54	1

There were no significant F_{ST} values for single loci and the average value across loci (0.0024) was not significantly different from zero (Table 18). Further analyses using hierarchical F statistics to partition variation into that occurring between sectors of the GBR and between reefs within sectors revealed no significant differentiation at any level of the hierarchy (data not shown).

TABLE 18. *Holothuria nobilis*. F-statistics for 15 reefs on the GBR.
CI: 95 % Confidence interval obtained by bootstrapping across loci.

LOCUS	F	f	F_{ST}
*FL-EST**	0.1139	0.1111***	0.0031 NS
*GPI**	0.0753	0.0774*	-0.0023 NS
*HK**	-0.0264	-0.0266 NS	0.0002 NS
*MDH**	0.0491	0.0503 NS	-0.0013 NS
*MPI**	0.0698	0.0630 NS	0.0073 NS
*PGM**	0.0692	0.0640 NS	0.0055 NS
*TPI**	0.0129	0.0191 NS	-0.0063 NS
Average:	**0.0651**	**0.0628** NS	**0.0024** NS
CI: Upper	0.0780	0.0756	0.0056
Lower	0.0528	0.0512	-0.0017

As possible differences between female and male gene flow have been reported previously for two holothurian species (Uthicke *et al.* 1998; 1999), F-statistics were also performed separately for each sex, for a subset of samples (230 females, 316 males) which could be macroscopically sexed from the appearance of their gonads. There were no deviations from Hardy-Weinberg equilibrium for either sex. F_{ST} values within each sex and between the sexes showed no significant deviations from zero (data not shown).

MtDNA

Sequencing usually resulted in good quality sequences and forward and reverse sequences were easy to align. However, in several cases information from the beginning or end of the sequence could not be confirmed because one of the strands did not sequence well at the 5' end. Therefore, subsequent analysis of a slightly shortened fragment of the COI gene, representing the 559bp between 6072 and 6630 of the mitochondrial genome of *Strongylocentrotus pupuratus* were performed. Comparative searches in Genbank yielded closest matches to other echinoderm species, the closest match was to another aspidochirotide holothurian (*Parastichopus californicus*, Arndt *et al.* 1996), confirming that the sequences analysed were part of the holothurian genome and not from potential contamination by some associated fauna or flora.

The number of sequences obtained from each location is listed in Table 19. Generally, the COI sequences of *Holothuria nobilis* are characterised by high haplotype diversity, but low nucleotide diversity indicates that sequences within populations differ by less than one per cent. There does not seem to be a trend in either nucleotide or haplotype diversity with geographic location.

TABLE 19. Diversity measures for 11 GBR populations of *Holothuria nobilis*, based on the Kimura two parameter distance.

Reef	N	No. Haplotypes	Haplotype Diversity	Nucleotide Diversity
13-050	30	18	0.933	0.0060
Davie	24	16	0.957	0.0054
Hicks	33	17	0.913	0.0058
Michaelmas	29	18	0.921	0.0051
Opal	30	17	0.945	0.0058
Big Broadhurst	28	18	0.960	0.0063
Davies	22	15	0.923	0.0045
Stucco	27	12	0.926	0.0030
Whitetip	26	16	0.945	0.0054
21-151	24	18	0.971	0.0067
Turner	27	17	0.952	0.0063
Total	300	87	0.941	0.0057

An analysis of Molecular variance (AMOVA, Table 20), indicated that most of the variation in sequence divergence was within populations. The contribution of Sectors or Reefs within Sectors to the total variance was not significant, whether Kimura's two-parameter distance was used or only haplotype frequencies.

Table 20. Analyses of molecular Variance (AMOVA) with mtDNA Data collected from 300 individuals of *H. nobilis*. A) Incorporating nucleotide distances based on Kimura two-parameter distance, B) only based on Haplotype frequencies.

Source of variation	df	A) Distance		B) Haplotypes	
		Variance component	Percent of Variation	Variance component	Percent of Variation
Among Sectors	4	-0.00922	-0.58	-0.00014	-0.03
Reefs within Sectors	6	0.01689	1.06	0.00085	0.18
Within Reefs	289	1.58371	99.52	0.46993	99.85
Total	299	1.59138		0.47064	

A neighbour joining tree on corrected (for within population variation) genetic distances between populations, indicates that the differences between populations are very small

(Fig. 17). No geographical pattern was apparent in the tree. Therefore, distance methods and AMOVA were unable to detect restrictions in geneflow between populations on the GBR.

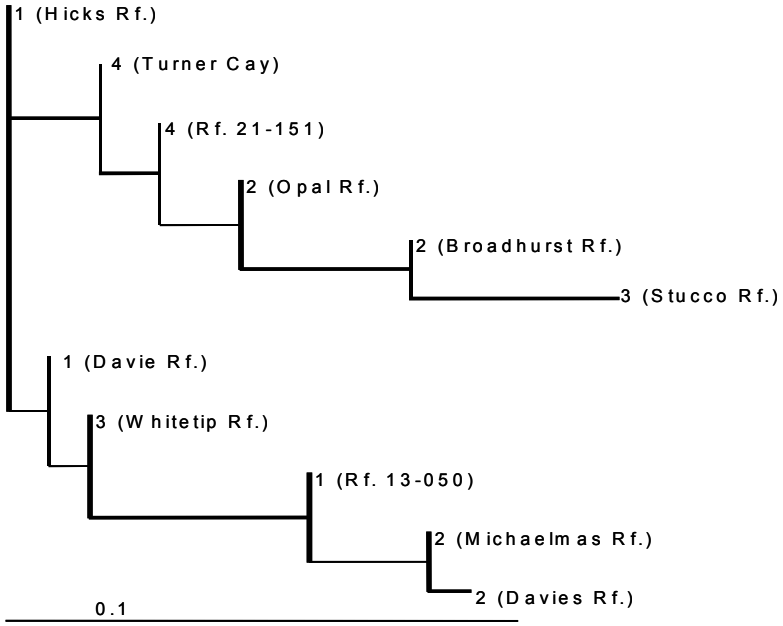

FIGURE 17. Neighbour joining tree indicating distances (Kimura 2-parameter, corrected for within population distances) between populations on *Holothuria nobilis* investigated screened for mtDNA variation. Numbers (1-4) indicate geographic location of the reefs in four arbitrary sectors.

REPRODUCTION OF THE BLACK TEATFISH ON THE GBR

The gonad index on *H. nobilis* samples from Big Broadhurst Reef and Davies Reef in the central section of the GBR was determined 13 times in the period between October 1998 and November 2000 (Fig. 18). Although several months were missed due to poor weather conditions preventing sampling, Fig. 18 clearly indicates that this species spawns in winter. The gonad indices, whether expressed as per cent of total weight or gutted weight, was consistently high between May and August in both years. Averaged over the whole period of observation, the female gonad indices were slightly higher than the ones for the males (as percent total gutted weight, female: 6.07, SD = 6.13; male: 4.58, SD = 4.07). Gonad samples were taken at several other reefs of the GBR at several occasions to test whether patterns observed in the central section are valid for larger areas of the GBR (Table 21). Although some variation exists even between close reefs sampled in the same months, the general trend with high values and presumed spawning between May and August is confirmed. However, several reefs had relatively high values even in September (Hastings Reefs) and October (Davies Reef).

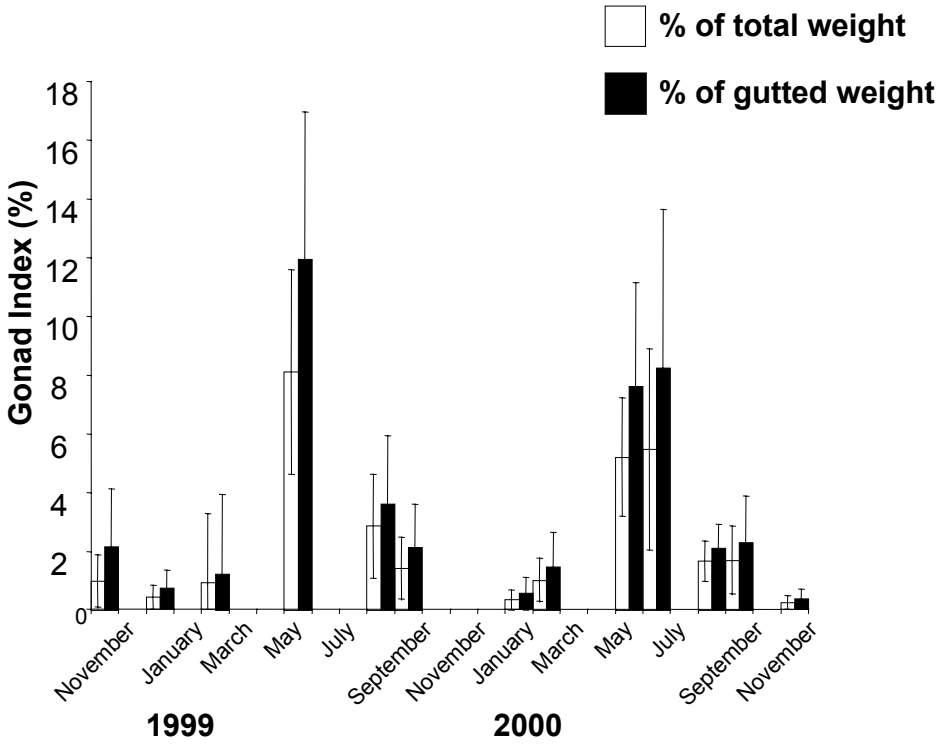

FIGURE 18. Gonad index of *Holothuria nobilis* on different sampling occasions, pooled for Big Broadhurst Reef and Davies Reef. Error bars indicate 1 SD.

Table 21. Gonad index (% of gutted weight) of *H. nobilis* samples in addition to those on Big Broadhurst and Davies Reef.

Sample Month	Reef	Gonad Index	Standard Deviation	N
May 1999	Hyde	6.02	3.55	48
	James Reef	8.96	1.32	4
	White Tip	5.04	2.70	51
August 1999	13-050	1.75	1.77	49
	13-120	1.42	1.53	31
September 1999	Ribbon Rf. No. 10	0.71	0.61	44
	Ribbon Rf. No 7	1.29	1.68	60
	Opal	3.85	2.05	4
	Hastings	4.19	3.68	31
October 1999	Arlington	2.75	2.45	19
July 2000	Potter	0.51	0.85	14
August 2000	Michaelmas	1.01	1.41	30
October 2000	Davie	3.08	2.81	24

Allometric relationships between various size and weight measures, based on all 639 samples collected for gonad analysis are shown in Figure 19. The relation between both weight measures is described well by a power function, whereas the regression line explains a smaller proportion of the relationship of these parameters and the length. This indicates that length is highly variable and probably the least reliable size measure in *H. nobilis*. As in most aspidochirotide holothurians, length-weight relationships are described by allometric constants distinctly smaller than three (Conand 1989a, Uthicke 1994).

Weight-frequency distributions for *H. nobilis* collected for gonad index analysis are bell-shaped (Fig. 20), with most animals in the weight classes between 1400 and 2200 g (total weight). The weight frequency distribution highlights once more the scarcity of juveniles or small animals in these populations. The number of animals with gonads reaches 50% in the size class between 800 and 1000g. However, this value reaches 80% in the next weight class.

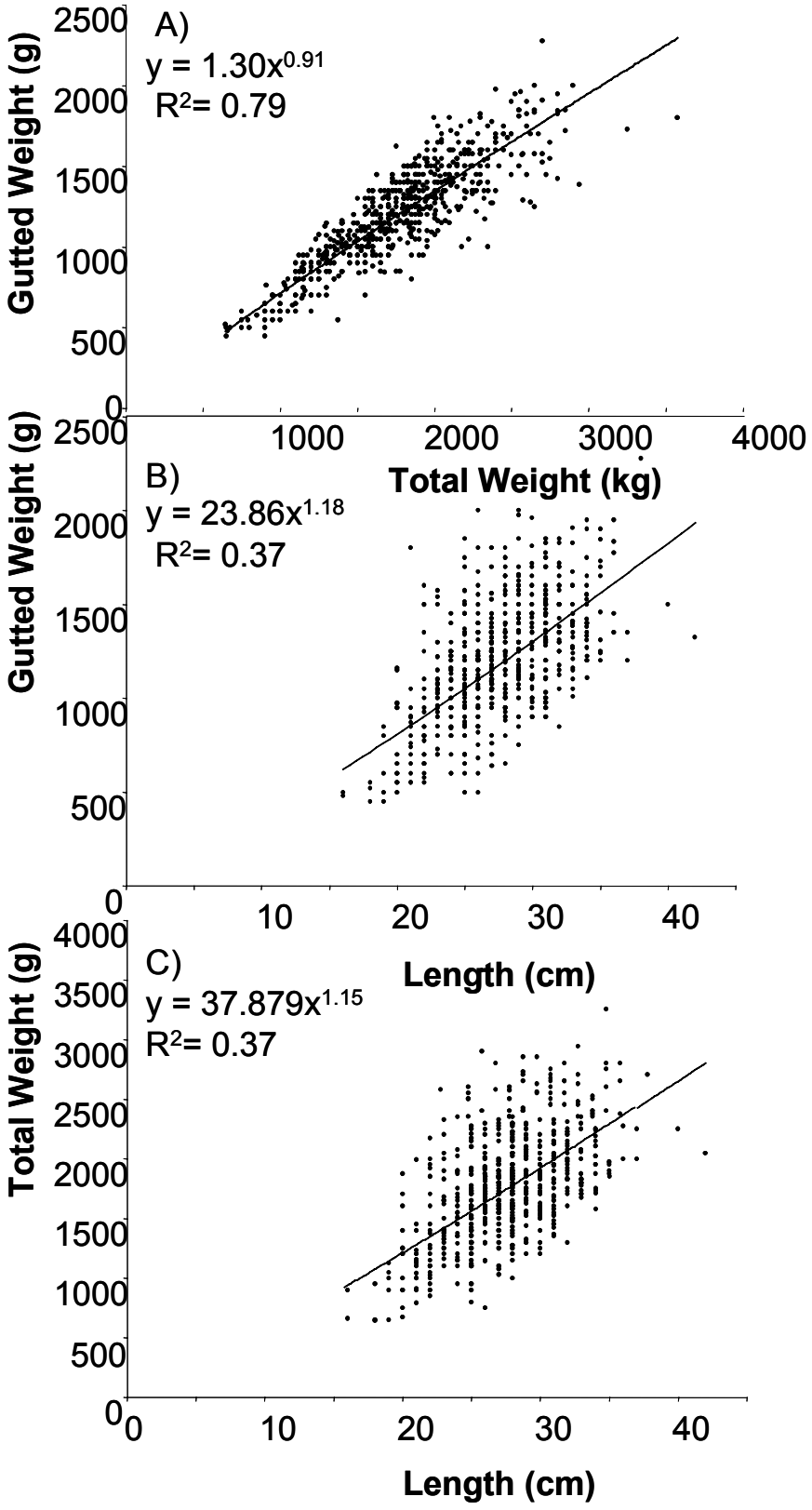

FIGURE 19. Relationship between total weight and gutted weight (A), length and gutted weight (B) and length and total weight (C) in *Holothuria nobilis* collected for gonad index analysis.

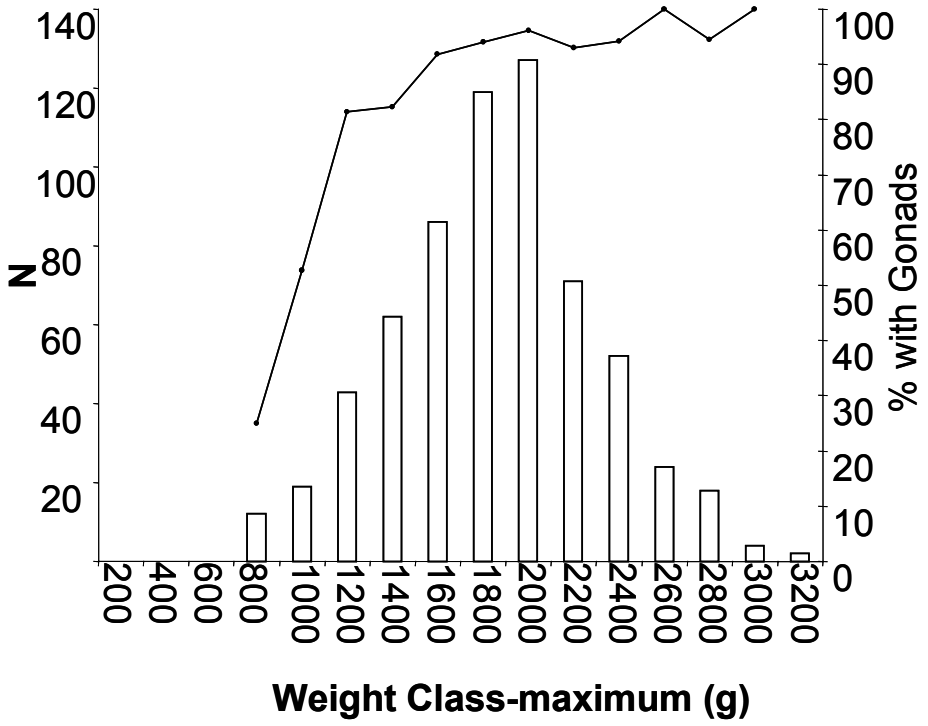

FIGURE 20. Weight frequency distributions (numbers of individuals, left hand y-axis) and percent of individuals with detectable gonads (right hand y-axis) for *Holothuria nobilis* collected for gonad index analysis.

GROWTH RATES FOR BLACK TEATFISH

AFLP analyses using one primer combination yielded individual DNA fingerprints for each *Holothuria nobilis* successfully amplified within each sampling period at both locations. The proportion of samples that were successfully amplified was smaller for samples obtained in the first time collection at Davies Reef and which had been snap frozen. Samples from later sampling periods and from Lizard Island were preserved in 100% ethanol and this appeared to improve amplification success (Table 22).

On Davies Reef, 27 of the samples collected in November 2000, had fingerprints identical to those observed in tissues collected in the previous time period (Table 22). Similarly, 37 samples collected in May 2001 matched samples from November 2000, and 25 samples were the same as one year prior to that. Similar numbers of animals could be re-identified on both locations of Lizard Island. However, due to the smaller collection size at Lizard Island, the recapture rates are actually higher at that location. Based on the frequency of each band in the sample from the first time period at Davies Reef, the probability of identity (P_{ID}, Waits *et al.* 2001) is less than 0.01 (i.e. 0.007), even if the conservative

formula for high occurrence of siblings and inbreeding is used. Therefore, all individuals in the second and third sample with the same fingerprints as individuals from the first or second sample were accepted as being the same individuals.

Although variation is considerable, a large proportion (53-68%) of the weight in re-captured individuals can be predicted by the weight of the initial sample using Francis' growth model (Table 23). Smaller (1.0 kg) individuals were predicted (Parameter g_1, Table 23) to grow between 35 and 533 g annually, with somewhat higher rates when modelled from data obtained between December and June. Animals of 2.5 kg, however, were consistently predicted (Parameter g_2, Table 23) to shrink more with the exception of those at Bird Island between June and December. The weight of zero growth was around 1.4 to 2.8 kg ($c^{1/b}$, Table 23). Due to the largest sample size we consider the pooled data for all reefs as the most reliable, the results for all locations pooled are shown in Figure 21. These data also suggest seasonal differences in growth, with growth rates for 1 kg individuals estimated to be 170g per year when estimated from the period from June to December, and 80g per year when estimated from December to June. Large individuals in the pooled data set where predicted to shrink for both periods, but more so between December and June.

Data obtained here can also be used to assess the mobility of individuals. As expected, no animals from Davies reef matched any on Lizard Island or vice versa, because these locations are hundreds of km apart. On Lizard Island, no individuals migrated between Bird and Palfrey Island (Distance: 2.1km). Animals on the Palfrey Island reef flat were released in two locations only 80-90m apart. During the whole study period, only three individuals (out of 62 matches) were found to have migrated between those two locations.

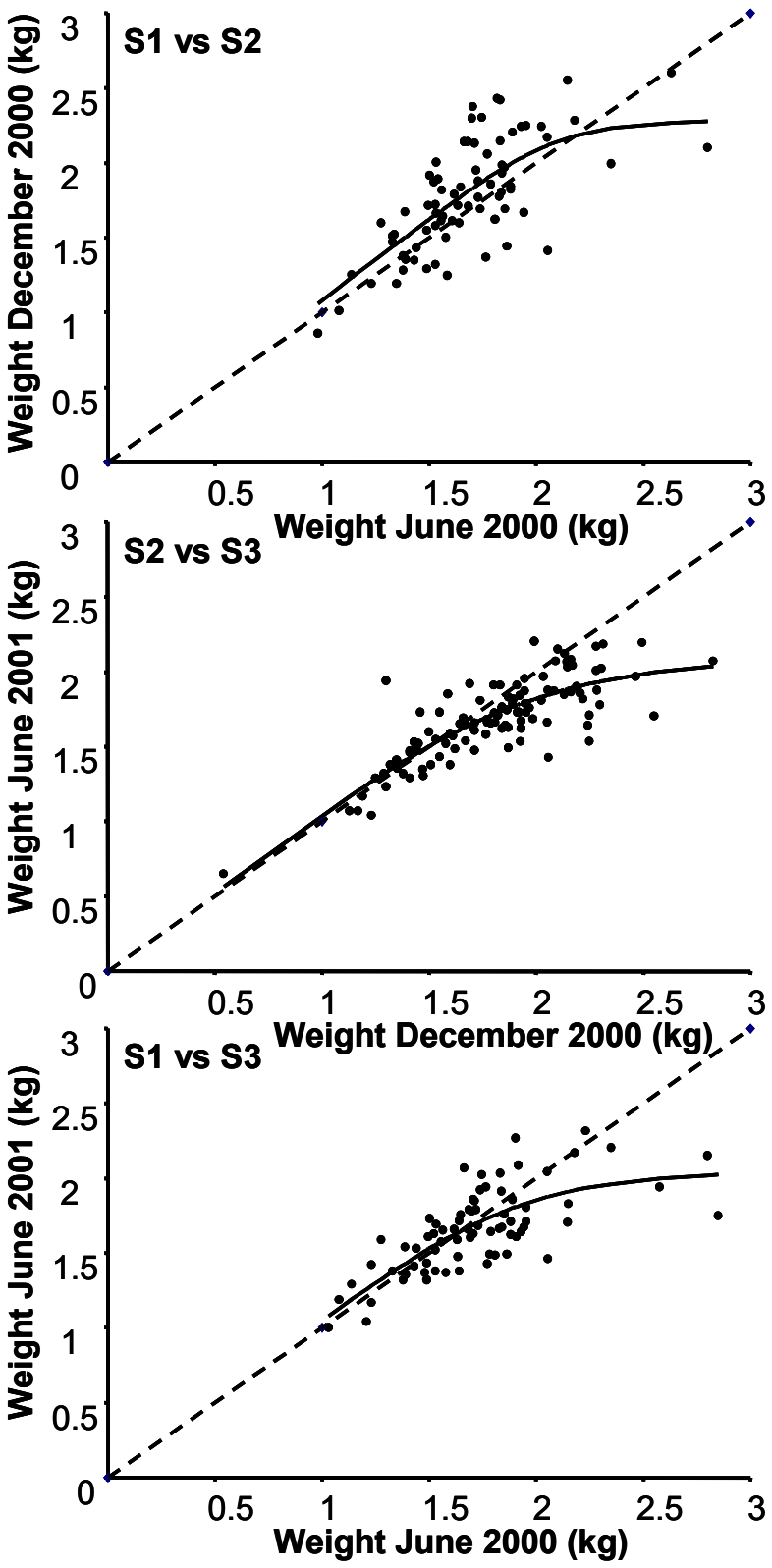

FIGURE 21. Initial versus final weight of re-captured individuals between June 2000 and December 2000 (S1-S2), December 2000 and June 2001 (S2-S3), and June 2000 and 2001 (S1-S3). The broken line indicates a hypothetical line of zero growth, the solid line is fitted with Francis' (1995) analogue of Schnute's (1981) growth model. Dots represent original data points.

Table 22. Number of *H. nobilis* samples collected, DNA fingerprinted and re-identified at three sampling locations on the Great Barrier Reef.

	Sample 1 May/June 2000		Sample 2 December 2000			Sample 3 May/June 2001				
	Animals collected	Successfully Amplified	Animals collected	Successfull y Amplified	Matches with S1	Animals collected	Successfully Amplified	Matches with S1	Matches with S2	Matches with S1 and S2
Lizard Island										
Bird Isl.	59	55 (95%)	56	52 (93%)	27	59	59 (100%)	29	33	19
Palfrey Isl.	54	54 (100%)	57	54 (95%)	21	55	54 (98%)	20	37	16
Central GBR										
Davies Reef	91	72 (79%)	89	77 (87%)	27	81	81 (100%)	25	37	18

Table 23. Parameters of Francis' (1995) growth function for populations of *H. nobilis*.

	Bird Isl.	Palfrey Isl.	Pooled LI	Davies Reef	All Pooled
June to December 2000	R^2 =0.41 (N=27)	R^2 =0.56 (N=21)	R^2 = 0.44 (N=48)	R^2 =0.53 (N=27)	R^2 = 0.53 (N=75)
g1 kg yr^{-1}	0.2162	0.5336	0.3099	0.1481	0.1733
g2 kg yr^{-1}	0.2518	-0.1898	-0.1223	-0.7941	-0.2914
b	-10.2589	-2.7654	-6.8830	-3.4517	-11.22
$C^{1/b}$	2.83	2.26	2.36	1.47	2.18
December to June 2001	R^2 =0.48 (N=33)	R^2 =0.65 (N=37)	R^2 =0.48 (N=70)	R^2 =0.68 (N=37)	R^2 = 0.68 (N=107)
g1 kg yr^{-1}	0.288	-0.0687	-0.0476	0.0641	0.0814
g2 kg yr^{-1}	-0.8059	-0.5517	-0.6847	-0.7032	-0.6828
b	-0.5516	-14.3023	-8.5563	-8.5082	-4.7933
$C^{1/b}$	1.46	undefined	undefined	1.63	1.52
June 2000 to June 2001	R^2 =0.52 (N=29)	R^2 = 0.58 (N=20)	R^2 = 0.46 (N=49)	R^2 = 0.65 (N=25)	R^2 = 0.55 (N=74)
g1 kg yr^{-1}	0.0352	0.3398	0.1577	0.0805	0.04758
g2 kg yr^{-1}	-0.3846	-0.1527	-0.3140	-0.6290	-0.5127
b	-2.4634	1.5461	-0.7388	-2.6685	-6.0992
$C^{1/b}$	1.37	1.98	1.70	1.40	1.64

DISCUSSION

SPATIAL SURVEYS AND STOCK SIZE OF BÊCHE-DE-MER OVER THE GBR

General distributions of bêche-de-mer

The surveys conducted were mainly designed for black teatfish, *H. nobilis*, in that they concentrated on the reef flat area. *H. nobilis* was shown to be a mid to outer shelf species with it's main abundance at the northern half of the GBR. However, some reefs in the southern areas had high abundances (Whitsunday area and Pompey region). This species was the only high value bêche-de-mer species common in shallow water habitats in the GBR. *H. atra* and *S. chloronotus* were more abundant in the Central section of the GBR and were less abundant on the outer shelf than inshore. These species are also abundant on some near shore reefs where extremely high densities are maintained by asexual reproduction (Uthicke 2001b). The fact that these two species are also abundant on the Capricorn Bunker section of the GBR (Harriott 1980, own observation) shows that these species also occur in the south and that temperature thus does not seem to be the factor regulating their densities on the GBR. It is not know why *Stichopus variegatus* was more common in the southern transects.

Most other bêche-de-mer species were rare on the transects. As mentioned above, surveys may not have been in the main distribution area for some of the species such as the prickly redfish. However, since large areas on the reef flat and sometimes also parts towards the lagoonal portions of reefs were towed it was concluded that the main habitat of any other reef species would be considerably smaller than the area occupied by *H. nobilis*. Therefore, even if some other species occur in high densities in local areas it is considered unlikely that their overall population size approaches that of *H. nobilis*. The few possible exceptions to this are the low value but common species *H. atra*, *S. chloronotus* and *H. leucospilota*.

Black teatfish

There are very few published data on densities of *Holothuria nobilis* populations, and none of these provide any specific comparison of fished and un-fished sites. For some reefs on the GBR Hammond *et al.* (1985) reported values up 17.5 ind. ha^{-1} and Harriott (1984, 1985) reported values lower than in our survey on some fished and one un-fished reef. Both of the latter surveys were done before onset of the recent fishery, and the lower values reported by Harriott (1984, 1985) were therefore unexpected. However,

this author found that her survey methods generally underestimated population densities by a factor of two to four. In addition, the present survey excluded some areas of the reef flat, which are unlikely habitat for *H. nobilis*, i.e. sandy areas towards the leeward end of the reef flat. If these were included in Harriott's surveys, her density estimates will have been reduced correspondingly. The range of densities observed in the present study fit well within the values reported by previous authors. Average density estimates for the Torres Straits (Long *et al.* 1996), Papua New Guinea (Massin and Doumen 1986, Lokani 1990), New Caledonia (Conand 1989a), the Solomon Islands (Lincoln-Smith *et al.* 2000) and Tonga (Preston and Lokani 1990, cited from Preston 1993) range between 9.4 and 18.4 ind. ha^{-1}, but maximum densities of 100 ind. ha^{-1} (Conand 1989b) or 275 ind. ha^{-1} (Lokani 1990) have been reported. Some of this variation might reflect differences in the survey technique or reporting method in different studies.

These data indicate considerable variation in density at different sites and underscore the difficulty in determining natural densities or virgin biomass, which is an important pre-requisite for sustainable fisheries management. In addition, because the fishing on this species in a boom-and-bust fashion has occurred for several hundred years on the GBR, it is difficult to assess how much of the current distribution patterns observed is natural and how much human induced. However, the detailed information gleaned in this survey, particularly the opportunity to compare data from reefs protected throughout the last fishing phase, has added significantly to our ability to interpret the results, and identify the implications for fishery management.

Given the significantly lower densities of black teatfish on open compared to closed reefs it appears that bêche-de-mer fishing on the GBR has led to a reduction in *Holothuria nobilis* densities of at least 75 % on recently fished reefs. The densities of the fished reefs were all similar (around 5 ind. ha^{-1}). Our own experience during sampling of *H. nobilis* suggests that this density corresponds to a catch rate of 2-3 animals per hour by a snorkler and conversions given in Conand (1989b) yield a similar estimate. If this catch rate is close to the limit at which it is economic to fish, then once populations are fished to this level they are unlikely to be reduced further at current market prices.

Our estimates of the reduction of holothurian stocks due to fishing are likely to be minimum estimates because they are based on the assumption that densities found on closed reefs represent natural densities. However, it is possible that fishing may have reduced the recruitment to protected sites through reducing the regional pool of larval recruits and illegal fishing may directly reduce densities on protected reefs. Evidence of

illegal fishing in protected areas comes from the fact that protected zones on two reefs which are frequented by tourists have higher densities of *Holothuria nobilis* than other "protected" areas, e.g. densities in the closed area of Ribbon Reef No. 10 were distinctly higher than on the totally closed reefs. The difference may be due to natural variation, but this reef is also visited by tourists regularly, which may deter illegal fishing. Another area with very high densities (91.2 ind. ha^{-1}, determined from 8 transects, SD = 46.0) is the backreef area of Michaelmas Cay, which is just several hundred meters from a major tourist site.

The similarity of densities between open and closed zones on the other three reefs may result from deliberate or accidental incursions by fishermen into protected areas because of the small size (11km) or by migration of adult holothurians from the protected region into the fished zone. In the case of the large protected reef (Ribbon Reef No. 10) adult holothurian migration may not be able to even out densities in fished and un-fished areas, illegal fishing may be less because of the larger distances fishermen would need to cover, or because tourist operations deter illegal fishing. However, the limited data collected in the present study of growth of black teatfish has also shown very little movement of adults in the Lizard Island region, suggesting that migration between protected and non-protected areas on these small reefs does not explain the loss of animals from the protected zones.

Considerable variation in average weights of *Holothuria nobilis* between reefs was observed in the present survey. High variation in average weight was also found between *H. nobilis* populations in New Caledonia (Conand 1989a). Variation in average weights between habitats appears to be a feature in many holothurian species (e.g. Conand 1993, Uthicke 1994). Although average weights on many fished reefs are smaller than on reefs closed to fishing, this difference was not statistically significant. This result is in contrast to a previous analysis (Uthicke and Benzie 2000), but which was based on fewer reefs. Fishing may reduce weights on many reefs, but natural variability among reefs was too high to detect statistically significant differences overall.

The time span since a reef was last fished may also influence the population structure and increase variability. It may be that some reefs with low densities but high average weights, such as Potter Reef or the closed zones of Hastings and Opal Reef, were last fished several years ago. The small animals remaining would have grown in the period between the last fishery event and the present survey. However, if this is so, the interpretation implies low recruitment rates onto these reefs because densities remained low at those

sites. Although there is no specific legal size minimum for *H. nobilis* in the Queensland fishery, the general size minimum for bêche-de-mer (15 cm, this is equivalent to approximately 850 g total weight in *H. nobilis*, Uthicke, unpublished data) would have protected some small individuals which, in any case, are generally more difficult to find and less commercially valuable.

The reduced densities combined with lower average weights resulted in a reduction in biomass greater than 75% on fished reefs. A four-fold reduction in densities leads to a doubling of the average distance between individuals, and this can influence fertilisation success in broadcast spawners (Levitan and Young 1995). The combination of lower densities, reduced likelihood of fertilisation, lower average weight and smaller number of mature individuals may reduce the output of larvae from the fished populations. Although reefs completely protected from fishing may act as refugia for *H. nobilis* populations, it is not known whether dispersal of larvae from the protected reefs to fished reefs is effective in the short-term. The number of the protected reefs with suitable habitats is also small. In addition to direct effects on the fished stocks it is not known how reduced holothurian densities affect the general ecology of coral reefs, since holothurians have important ecological functions such as bioturbation and nutrient recycling (Massin 1982, Birkeland 1988, Uthicke and Klumpp 1998, Uthicke 1999, 2001a).

The surveys conducted were mainly designed for *H. nobilis* in that they concentrated on the reef flat area, which is the main habitat of this species. However, high densities of this species can also be observed on reef slopes and deeper backreef areas on reefs protected from fishing. It is assumed that these areas (reef slopes and deeper backreef areas) are first over fished because they are easier to access during low tides, and also smaller and less complex. The ease of collection is a threat for other species in these areas, such as the prickly red fish. None of the other species statistically analysed showed the distinct difference between green and blue reefs in the fished area. Because these species were not fished at that time, this is additional evidence that the patterns found in *H. nobilis* are caused by fishing, and not some unknown habitat factor.

Stock size

Model calculations on black teatfish stocks indicated that the virgin biomass was in the order of 5,500 t and about 5 million individuals. Some of the simplistic fishery models assume a MSY of virgin biomass multiplied by 0.5 times the natural mortality rates (Gulland 1983). However, estimates of mortality rates of holothurians hardly exist, and the few data available (summarised in Conand 1989c) are rough estimates based on other

species, several of which have additional asexual reproduction. Using these data to calculate maximum sustainable yields for holothurian populations can be very erroneous. For example, using a mortality rate of 1.0 as a 'reasonable-estimate' for holothurians in general (Long *et al.* 1996) would suggest that 50% of the virgin biomass of *H. nobilis* could be caught annually on the GBR and provide a sustainable yield. Based on our lower confidence limit estimates for the biomass this would correspond to 2,000 t. However, the maximum annual catch for *H. nobilis* achieved in the GBR was about 370 t (catch data provided by QFS). This represents much less than 10% of the biomass of *H. nobilis* in the target region. In fact, calculations based on the lower confidence limit show catch rates in most years were below 5%. Thus, with hindsight, it can now be concluded that an annual catch rate of less than 10% of the virgin stock was sufficient to induce severe over fishing. This does not necessarily suggest that fishery models are not adequate for holothurians, but does indicate that mortality rates are much lower than have been commonly assumed. Indeed, the slow growth rates and lack of recovery of the stocks observed during the present study provide evidence that productivity in these populations is very low, and that both recruitment and natural mortality are low.

Another interesting aspect of the model calculations is the total reduction in biomass, suggested to be around 3000 t. This estimate is very close to the total catches of 2500 t (QFS) reported from 1987 to 1999. There were no indications that stocks had rebuilt between the fishing years, and that only an 'excess production' had been caught. On the contrary, these data suggest that during the period from when the fishery started in the mid 1980s until its closure, the fishery has simply reduced total biomass without substantial recovery between the years, providing additional evidence that recruitment rates in this species are low over periods of several years.

TEMPORAL SURVEYS OF BÊCHE-DE-MER AND RECOVERY AFTER CLOSURE TO FISHING

This study is among the first to attempt to estimate recovery rates of holothurian stocks. There were no indications of a recovery two years after closure of the fishery for *H. nobilis*. Densities on both green reefs (protected from fishing even before the closure of the fishery) and blue reefs since closed to fishing remained relatively stable, with green reefs continuing to have some four to five times the average densities of black teatfish as the reefs recently closed to fishing. Hardly any new recruits were observed in any of the populations. This may be taken as further evidence that animals may be relatively long-lived, because densities would reduce if mortality was high and recruitment low.

Densities of black teatfish on reefs divided into green and blue zones did not change significantly over time either. On two of the Ribbon Reefs, densities were higher in the green zone, indicating that fishing has previously occurred in the blue zone. Because the density differences remain constant, it appears that there is no major migration between the two zones. On two smaller reefs, the difference between the blue and green zone was not distinct, and although densities on the blue reefs showed some fluctuation there was no clear sign of a recovery in population size.

DISPERSAL AND RECRUITMENT IN BLACK TEATFISH

Genetic analysis was used to infer geneflow and hence dispersal among black teatfish populations because it is not possible to tag adults long-term (the animals shed tags) and the planktonic larvae are too small to physically tag even if they could be captured and identified reliably. Allozyme analyses of 866 specimens of *Holothuria nobilis* obtained on 15 reefs from the GBR revealed no genetic differentiation, and high levels of geneflow were inferred among all populations even though they were separated by as much as 1300 km. Mitochondrial DNA markers were then surveyed in a subset of populations because these markers are deemed to provide higher resolution because of their higher mutation rates and smaller effective population size. However, no population genetic structure was also detected using this marker.

The results for *H. nobilis* are similar to those reported previously for the asteroid *Linckia laevigata* over a similar range of the GBR (Williams and Benzie 1993): both studies detected no deviations from Hardy Weinberg equilibrium, non-significant F_{ST} values around 0.002 and genetic distances between populations below 0.003. On the GBR, both species occur in similar habitats although *L. laevigata* is found on a larger number of reefs and can also be found on reefs closer to the shore. Results obtained by Martinez and Richmond (1998) suggested a larval life for *H. nobilis* slightly longer than 28 days, similar to that of *L. laevigata*. High levels of dispersal are usually associated with a long larval life (e.g. Hedgecock 1986) and genetic analyses of populations of fish (Doherty *et al.* 1995), giant clams (Benzie and Williams 1992; Macaranas *et al.* 1992) and seastars (Benzie and Stoddart 1992; Williams and Benzie 1993) with larval lives of 10 days or more indicate little or no genetic structure over the length of the GBR. The close connectivity of the many reefs in the GBR separated by short distances and the movement of larvae on the longshore currents during the spring and summer when most of these species spawn is thought to play a role in their dispersal through the GBR (Williams *et al.* 1984).

In contrast to most other species studied on the GBR, *H. nobilis* spawns in winter in New Caledonia (Conand 1993) and, as we have shown in the present study, on the GBR. Longshore drift on the GBR in winter is significantly more restricted compared to summer (Williams *et al.* 1984) and therefore dispersal of winter spawners might be expected to be more restricted compared to summer spawners. However, in the case of *H. nobilis*, this did not result in measurable population differentiation.

Length of larval life is not the only influence on dispersal and the genetic structure of populations. F_{ST} values up to an order of magnitude higher than those were found for GBR populations of species with the potential for asexual reproduction such as the zoanthid *Zoanthus coppingeri* (Burnett *et al.* 1995), or species that are viviparous such as the scleractinian *Seriatopora hystrix* (Ayre and Dufty 1994). Data on the population genetic structure of *Holothuria nobilis* did not provide any indication of the occurrence of asexual reproduction in this species. Asexual reproduction usually results in distinct deviations from Hardy-Weinberg equilibrium (e.g. Johnson and Threlfall 1987; Ayre and Dufty 1994; Uthicke *et al.* 1998; 1999). No such deviations were observed in any locus on any population of the present study. In addition, the two parameters N_{go}:N_i and N^*:N_i, previously shown to be reliable estimators for the occurrence and magnitude of asexual reproduction in holothurians (Uthicke *et al.* 1998; 1999), indicated no asexual reproduction in *H. nobilis*.

Given the lack of asexual reproduction in natural *Holothuria nobilis* populations it is interesting that this species can be artificially induced to split and that animals can fully regenerate after they have split into two halves (Reichenbach *et al.* 1996). Strong regenerative potential appears to be a feature of many holothurian species and several species undergo seasonal evisceration and regeneration of their guts, or eviscerate when handled (summarised in Emson and Wilkie 1980). Many *H. nobilis* populations occur on high flow environments and are often exposed to high wave energy and low water levels (Uthicke, unpublished data). The potential of *H. nobilis* to regenerate may be a mechanism to repair damage sustained in these hostile environments. The finding that fission in this species does not occur on the GBR does not exclude the possibility that it exists in other regions, but *H. nobilis* does not appear to be a typical asexual species. It is relatively large, has a thick body wall and does not occur in the very high densities typical for many asexual species (Uthicke 1997).

In summary, it appears that sexually produced larvae are the only source of recruits to *Holothuria nobilis* populations. The allozyme and mitochondrial DNA data indicate panmixis in the GBR, suggesting the entire GBR population can be managed as one stock.

PERIOD OF REPRODUCTION OF THE BLACK TEATFISH ON THE GBR

Most holothurian species investigated in the western Pacific spawn in the summer months (e.g. Conand 1993). The only exception to this was *H. nobilis* in New Caledonia, and this species was shown in the present study to spawn in winter on the GBR. The number of animals with gonads reaches 50% in the size class between 800 and 1000g, and 80% for 1000 to 1200g animals. The current commercial size limit for bêche-de-mer fisheries is 15 cm for all holothurians and therefore potentially does not protect any individuals of *H. nobilis* up to the point where they would have spawned at least once. A minimum size limit of 1200 g total weight (equivalent to 820 g gutted weight or 20 cm using conversion factors given in Fig. 19), together with closures during the spawning season should allow about 80% of all animals to reproduce at least once.

GROWTH RATES FOR BLACK TEATFISH

The development of a novel DNA fingerprinting method allowed growth in *H. nobilis* in the wild to be measured for the first time and has revealed several interesting an unexpected features of the species' biology. Growth appears to be slow and animals in the wild have the capacity to shrink. The lack of small individuals in the data set means it is impossible to predict the age of holothurians in the size range observed. However, even if small individuals grow three times as fast as predicted for 1kg individuals recaptured after one year in this study (50 g x 3 = 150 g for the whole dataset), animals of 1 kg weight would be older than four years. Large individuals of other large holothurian species (with weights higher than 3 kg) have been estimated to be older than 10 years (Conand 1988).

The growth estimates obtained here for 1.0 kg holothurians are similar to those obtained in studies on smaller species whose growth rates ranged between 100 and 200 g per year (Franklin 1980, Uthicke 1994, Chao *et al.* 1994). However, growth rates obtained here are low when compared to other large holothurian species, which were observed to grow up to 450 g per year (Conand 1988, 1989a,b, c, Shelley 1985).

The finding that the larger individual *H. nobilis* consistently shrunk was unexpected. It is known that holothurians shrink in aquaria (Conand 1983, Shelley 1985, Wiedemeyer

1992), but this has never been reported from wild populations. Negative growth has also been inferred for other echinoderms such as echinoids and it was suggested that size in these animals could be controlled by environmental conditions (Ebert 1967). It may be that the environmental conditions were not favourable in the years over which this study was conducted. Alternatively, the number of animals transplanted to the study side may have been in excess of the carrying capacity of the habitat. Although densities were not measured, they were thought to be within (albeit on the higher end of) the range naturally occurring on the GBR (Uthicke unpublished data, Uthicke and Benzie 2000). In either case, the shrinkage of large individuals strongly suggests that average weight of holothurians in the wild is highly plastic and that density, size and biomass of holothurians may be limited and regulated by food supply, as previously suggested by Uthicke (1997, 2001a).

It was not possible here to present complete growth curves for *H. nobilis* because small individuals or new recruits were observed rarely and none collected. Complete growth curves will need to be developed over time and once data from several study areas become available. However, the ability to recapture and identify a sufficient number of animals to obtain growth rates from marine animals otherwise difficult to tag has been demonstrated. Apart from growth studies, it will be possible, using slightly different experimental designs to estimate migration rates, population size and recruitment to local populations (Mueller and Wolfenbarger 1999). It is pertinent that only a small number of adult individuals moved the short distance between the two Lizard Island sites (a distance of 90 metres) suggesting that the adults may not disperse far, at least for periods of several months to years.

MANAGEMENT IMPLICATIONS

Holothurian fisheries are currently good examples of boom and bust fisheries. Recent drastic declines in catches and shifts towards less valuable species (Conand 2001) indicate that the current global boom period has reached its end. However, the lack of basic biological information has prevented a better understanding of the reasons for these collapses, or more important, an ability to manage these fisheries better.

The present study has allowed the collection of some of the best information to date on the biology of bêche-de-mer, particularly black teatfish. Despite the considerable variation between reefs in population densities, the ability to obtain samples from reefs open to the recent fishing and those known to have been protected, has allowed clear interpretation

of the likely effects of fishing, and inferences concerning mortality and recruitment at population scales.

Genetic markers demonstrated conclusively that sexually produced larvae are the only source of recruits to *Holothuria nobilis* populations and suggested the entire GBR population can be managed as one stock. The high degree of connectivity among *H. nobilis* populations could be used to argue that dispersal among populations is high and that recruits might be derived from a large number of sources. This circumstance might be expected to lead to a rapid recovery of over-fished reefs. However, genetic data measure the integrated effects of recruitments over many generations (hundreds of years or more), and it is possible that major recruitment to particular reefs may be low for several years.

Several other biological observations obtained in this survey provided evidence that dispersal among populations in ecological time scales is more restricted because of a lack of successful recruits, and that recovery of over-fished populations may not be rapid. The first was the lack of juveniles observed throughout the three years of surveys on any reefs. The second was the lack of increase in population size in reefs recently closed to fishing up to two years after closure of the fishery. The third was the increase in size but not density in reefs that were presumed to have been last fished several years ago (Potter Reef), suggesting growth of individuals, no additional mortality in the populations but also no recruitment. The fourth was the evidence for the limited mobility of adults, both from the DNA fingerprinted populations, and by inference from the difference in population densities in zones within individual reefs that were open and closed to fishing and where adherence to regulations was likely as a result of the close proximity of those reefs to tourist operations. These data indicate that recruitment of juveniles is sporadic. The lack of juveniles in the reefs protected from fishing for some time (green reefs) suggests this effect is broader than simply a reduction on the density of animals of fished reefs that might lead to a reduction in spawning success on those reefs. The scarcity of juveniles is a common phenomenon in studies on holothurians (e.g. Uthicke 2001b) and suggests these interpretations are not specific to the GBR but that low and or sporadic recruitment may be a feature of bêche-de-mer populations generally and explain in part why these species are vulnerable to overfishing.

A surprising finding from the use of a novel fingerprinting technique that allowed growth of individual black teatfish to be followed in the wild was the slow growth of black teatfish and the likely age of average sized animals in the population to be estimated as possibly

more than 10 years. This finding also implies longer times to first reproduction than has been assumed, and slower recovery of populations that had been over-fished. Calculations of changes in stock size have indicated a take from the fishery very similar to that recorded by the industry catch statistics which, given the large errors involved is remarkable. These data show that a take of round 5% of the standing stock was more than the populations could sustain, and is consistent with the increased vulnerability of the population inferred from slow growth rates, inferred low natural mortality and low recruitment in these populations.

The closure of the black teatfish harvest fishery in 1999 was justified, albeit at a late point in time. Our data could confirm that reefs at that time were heavily over fished, potentially to a point where no successful reproduction can take place on these reefs. The low recovery rates, together with apparently slow growth rates and the absence of recruits indicate that the fishery on black teatfish will have to remain closed, potentially for several years. A target value of about 20 individuals per ha. appears a minimum estimate of "natural" densities and should be reached before re-opening the fishery. Extreme caution should be taken in applying mortality rates from other holothurian species to calculate MSYs. Data presented here for the black teatfish clearly suggest that even catch rates of less than 5-10% of the virgin biomass per year were sufficient to reduce stock size in the long term.

If seasonal fishery closures are considered, these should be at least in the months between April and August to allow black teatfish reproduction. However, a protection of all spawners would only be warranted in a seven month closure between April and October. The reproductive cycle of most other bêche-de-mer species on the GBR remains to be investigated, but is likely to be in the summer months. Therefore, closures for all other species should be in the summer months. A minimum size limit of 1,200 g total weight (equivalent to 820 g gutted weight or 20cm using conversion factors given in Fig. 19) is recommended. Together with closures during spawning season this figure should warrant that about 80% of all animals reproduce at least once.

The high geneflow along the GBR indicates that recruits to a depleted population may come from reefs further afield. Therefore, opening of new areas, particularly relatively isolated reefs such as Ashmore Reef or the Coral Sea reefs should be considered carefully, as external sources of recruitment are fewer than those for reefs within the GBR, where recovery is slow in any case.

In summary, data obtained previously and collected here suggest that bêche-de-mer, and especially black teatfish on the GBR are a very vulnerable resource. Low recruitment rates and slow growth lead to very slow recovery of over-fished stocks. Because these studies commenced at such a late stage in the over-fishing cycle, it is not possible to predict whether recruitment rates would have been higher if stocks were not already reduced. Although this project concentrated on black teatfish, it appeared that no other medium or high value reef species are available for sustainable fishing on shallow reef flats of the GBR.

BENEFITS

Direct information on the abundance, biomass and levels and patterns of genetic variation in allozyme and mtDNA markers have provided basic information that will assist the bêche-de-mer industry, particularly that of black teatfish in the Great Barrier Reef, although the information will provide generic assistance in the management of other populations. The surveys have shown that bêche-de-mer species are more frequent on midshelf and offshore reefs, and that black teatfish is the most common high-value species found in shallow waters. The surveys have also shown that the densities of black teatfish on reefs protected from fishing are significantly higher than that in reefs open to fishing, particularly where entire reefs are protected. These data provide clear information concerning the utility of protected areas in maintaining bêche-de-mer abundance and biomass. Reproduction has been demonstrated to occur in winter with some spawning as late as October, providing direct information on the times the fishery should be closed if a strategy using fishery closures at the time of breeding is used as a management tool.

The data show that the GBR populations of black teatfish can be considered as one genetic stock, where there is sufficient gene flow between all member populations to prevent significant genetic differentiation of any one population, at least on the time scales addressed by genetic data (hundreds to thousands of years). However, the temporal surveys of reefs after they were closed to fishing found no recruits after two years, suggesting that individual reefs may take several years to recover. Recruitment does vary on shorter, ecological, time scales (annual or decadal) and information on these dynamics is best gained from surveys of juvenile settlement. It has not proved possible to distinguish between different local sources of juveniles because no recruits were found.

The slow growth of black teatfish, and the possibility that many of the average sized individuals on the GBR may be in the order of ten years old also has important implications for fisheries management in that the resilience of populations to fishing may be limited and their sensitivity to over-fishing may be greater than has been assumed in the past.

Direct benefits of the project therefore include the provision of basic information of direct relevance to fisheries management such as stock size and delineation, reproductive period, growth rate and likely effects of protected areas, and the assessment of survey and

genetic techniques applicable to bêche-de-mer. and, therefore, more cost-effective management of a productive fishery.

FURTHER DEVELOPMENT

The surveys have provided new and unexpected information on the biology of black teatfish that are pertinent to the management of that fishery on the GBR, but which also have general implications for bêche-de-mer fisheries in general. Although genetic connectivity of populations is high, recruitment levels are low, growth rates are low and the age of most individuals of the population is 10 years or more. It was not possible to measure natural mortality rates directly, but the comparison of reef populations both closed to, and open to, fishing suggest that natural recruitment and mortality rates (at least after settlement) are low. A possibility is that rare recruitment events provide the bulk of the productivity of the fishery for many years. Knowledge of these factors has clearly highlighted the areas where information is still lacking.

A clear priority would be to better estimate the parameters of recruitment, age and mortality directly. Nothing is known of the relationship between these and climatic or other environmental variables. These relationships need to be established. The fingerprinting technique developed in this study provides additional avenues to assess survival and growth in wild bêche-de-mer populations.

Continued survey of the reefs recently closed to fishing, together with a subset of green reefs to act as controls would provide invaluable data on recovery of these populations, and potentially identify a good recruitment year if and when such an event occurred. Further monitoring of the recovery should be conducted in the third year after closure, and at least every second year after that. This will provide the first such data set anywhere in the world.

Further modeling of the fishery using reduced mortality rates would also provide greater insight in to the role of various factors in influencing the population dynamics of the fishery.

Legislation requires all Australian fisheries to be ecologically sustainable. It is not known what impact removal of holothurians has on the ecology of reefs and seagrass beds. Work

on low value commercial species (Uthicke 2001a) suggested that these animals have an important function in the nutrient recycling and productivity on coral reefs. Therefore, removal of bêche-de-mer may reduce overall productivity in these ecosystems. Whether this is true for high value species and in seagrass systems must be investigated in manipulative ecological experiments.

PLANNED OUTCOMES

A basis for improved management of a productive and valuable fishery in the Great Barrier Reef (GBR) has been developed through the provision of key biological information on several species of bêche-de-mer in the GBR, and the black teatfish in particular. Successful survey of several bêche-de-mer species, and of black teatfish (*Holothuria nobilis*) in particular, and the description of the population genetic structure of black teatfish has provided important information on stock definition, assessment of stock size and determination of levels of dispersal and recruitment among populations. Surveys over time have shown low recruitment rates and potentially long times required for the recovery of over-fished black teatfish stocks. The low growth rates measured also indicate the potentially longer times that might be required for recovery after populations are depleted. The determination of the reproductive period for black teatfish in the GBR will potentially assist the planning of closed fishing periods. The results will help develop bêche-de-mer management in regions outside the GBR, but the outcomes planned for the NT and WA sandfish fisheries in this study (concerning dispersal and gene flow) could not be met because insufficient samples were obtained from these regions. Interpretations of the biological data with respect specifically to fisheries management, and the potential role of protected reefs in the fishery management, were also achieved.

CONCLUSIONS

Nine of the eleven main objectives of the project were achieved:

1) To develop a survey methodology applicable for all shallow water bêche-de-mer species.

A methodology based on manta tows, applicable to all shallow water bêche-de-mer species was developed.

2) To adapt established techniques for enzyme electrophoretic analyses of holothurians to several bêche-de-mer species.

Established techniques for enzyme electrophoresis were adapted for use in several species of bêche-de-mer, including *Actinopyga echinites*, *A. miliaris*, *Holothuria nobilis, H. fuscogilva*, *H. fuscopunctata*, *Thelenota ananas*, *Stichopus variegatus*.

3) To determine the stock size of bêche-de-mer over a large geographic area in the GBR.

Densities of several species of bêche-de-mer were obtained from manta tows for the main area of the GBR fished for bêche-de-mer, and the spatial pattern of their abundance mapped.

4) To determine the stock size and biomass of the black teatfish over a large geographic area in the GBR.

Densities of black teatfish were obtained from manta tows for the main area of the GBR fished for bêche-de-mer, and the spatial pattern of their abundance mapped. Model calculations of black teatfish stocks indicated that the virgin biomass in the fished area of the GBR was in the order of 5,500 tonnes and about 5 million individuals, now reduced to 1,103 t and 920,000 individuals in the areas open to fishing.

5) To establish the period of reproduction of the black teatfish on the GBR.

The main reproductive period on black teatfish on the GBR was established to be winter, between April and August, although gonad indices on several reefs were high into October.

6) To measure dispersal and recruitment in black teatfish using genetic markers.

Allozyme electrophoretic markers, and mitochondrial DNA markers, indicated no restrictions to geneflow, and hence to dispersal and recruitment, in *H. nobilis* populations throughout the entire length on the GBR suggesting that the GBR bêche-de-mer fishery is one stock.

7) To identify and report the implications of these findings for management of bêche-de-mer fisheries.

The implications of the high genetic connectedness and several aspects of biology of black teatfish, such as low recruitment, slow growth, apparent low mortality and seasonal reproduction to fishery management was discussed, together with evidence of the effectiveness of whole reefs and portions of reefs closed to fishing.

8) To measure the recovery time for over-fished black teatfish stocks, (numbers and biomass).

Surveys covering two years after reefs were closed to fishing showed no evidence of population increase or of any recruitment to those populations.

9) To assess the likely source of recruits to recovering populations, including the role of protected reefs.

The likely source of recruits to recovering reefs could not be assessed as no recruits were observed.

10) To estimate growth rates for black teatfish.

A novel technique to measure growth in bêche-de-mer using genetic fingerprinting revealed that medium sized black teatfish grew slowly and some large individuals actually shrunk. Average sized individuals on the GBR reefs may be 10 years old or more.

11) To describe large-scale geneflow and dispersal of sandfish among fished populations in NT and WA.

Geneflow and dispersal of sandfish in NT and WA was not estimated because sufficient samples could not be obtained from these regions.

This work also met the performance indicators for the project by developing survey methods and techniques for the genetic estimation of gene flow and recruitment in bêche-de-mer, databases on bêche-de-mer stocks on the GBR and on fished reefs recently closed to fishing (recovering reef populations), databases on the genetic constitution of these populations, particularly black teatfish, and reporting specifically interpretations of the biological and genetic data from fishery management. The ongoing support of the Bêche-de-mer industry and Management agencies was demonstrated by their interest and participation in collection of materials and in the preliminary results presented at HarvestMAC meetings in Queensland.

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APPENDIX I: INTELLECTUAL PROPERTY

No patentable inventions or processes have been developed as part of this project. All results will be published in relevant scientific articles and other public domain literature.

APPENDIX II: STAFF

Dr John Benzie	Principal Investigator (AIMS and UNSW)
Dr Sven Uthicke	Co Investigator, Associate Research Scientist (AIMS)
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APPENDIX III: DATA FILES

TABLE A1. Densities and standard deviations for common holothurian species on the GBR.

	H. nobilis		*A. miliaris*		*H. atra*		*S. chloronotus*		*S. variegatus*		*B. argus*		*T. ananas*	
Reef	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
Agincourt Rf. No. 1	7.24	10.08	0.00	0.00	1.45	5.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Agincourt Rf. No. 4	23.65	33.29	1.69	5.85	1.69	5.85	1.69	5.85	0.00	0.00	1.69	5.85	0.00	0.00
Arlington Rf.	5.79	12.08	0.48	3.13	185.33	290.40	90.25	172.71	0.48	3.13	25.10	30.63	0.00	0.00
Big Broadhurst Rf.	8.06	14.91	0.92	4.25	749.30	723.33	263.51	382.74	0.46	3.04	8.52	16.47	0.92	4.25
Black Rf.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.27	45.33	0.00	0.00	0.00	0.00
Bowl Rf.	22.52	22.94	1.13	4.78	82.21	59.59	65.31	88.37	0.00	0.00	0.00	0.00	0.00	0.00
Cenetepede Rf.	5.07	9.17	1.69	5.85	255.06	370.59	89.53	77.04	1.69	5.85	6.76	15.78	5.07	12.60
Davie Rf.	7.60	15.08	0.00	0.00	0.00	0.00	7.60	10.49	0.00	0.00	2.53	7.17	2.53	7.17
Davies Rf.	8.45	15.72	0.00	0.00	684.11	852.88	233.11	211.33	0.84	4.14	10.98	23.89	0.00	0.00
East Cay	1.69	5.85	0.00	0.00	817.56	476.26	11.82	20.19	1.69	5.85	0.00	0.00	0.00	0.00
East-Black Rf.	2.25	6.76	0.00	0.00	2.25	6.76	0.00	0.00	11.26	10.68	0.00	0.00	0.00	0.00
Ellison Rf.	4.50	8.67	0.00	0.00	583.33	491.16	275.90	170.61	0.00	0.00	7.88	12.32	0.00	0.00
Feather Rf.	2.25	6.55	2.25	6.55	556.30	610.37	41.67	63.52	0.00	0.00	6.76	12.04	0.00	0.00
Grub Rf.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Half Tide Rf.	0.00	0.00	0.00	0.00	18.82	28.07	11.58	28.35	0.00	0.00	0.00	0.00	0.00	0.00
Hardy Rf.	0.00	0.00	0.00	0.00	10.62	39.83	0.00	0.00	6.76	16.13	0.00	0.00	0.00	0.00
Hastings Rf.	13.18	15.10	4.05	14.10	130.74	101.56	33.45	45.69	0.00	0.00	7.09	15.10	0.00	0.00
Hicks Rf.	6.76	10.14	0.00	0.00	0.00	0.00	6.76	20.27	0.00	0.00	2.25	6.76	2.25	6.76
Hook Rf.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.37	21.67	0.00	0.00	0.00	0.00
Hyde Rf.	30.41	32.80	1.13	4.78	83.33	100.97	6.76	24.08	3.38	14.33	12.39	24.22	0.00	0.00
James Rf.	6.24	17.33	0.00	0.00	38.98	49.97	26.51	46.36	14.03	29.12	0.00	0.00	0.00	0.00
Keeper Rf.	0.00	0.00	4.50	13.51	551.79	633.40	527.02	482.03	2.25	6.76	9.01	27.03	0.00	0.00
Linnett Isl.	0.00	0.00	0.00	0.00	1097.96	381.90	1270.25	788.78	0.00	0.00	0.00	0.00	0.00	0.00
Little Broadhurst Rf.	40.54	35.11	13.51	23.41	702.69	572.01	499.99	137.98	0.00	0.00	40.54	40.54	13.51	23.41
McCulloch Rf.	5.91	9.41	0.00	0.00	760.13	512.45	277.02	219.59	3.38	9.76	26.18	32.45	0.00	0.00
Michaelmas Rf.	18.48	21.96	0.00	0.00	253.97	226.50	59.62	72.13	0.00	0.00	4.77	10.05	1.19	4.84
Moss Rf.	20.27	18.39	0.00	0.00	4.50	11.11	188.06	338.38	0.00	0.00	0.00	0.00	0.00	0.00
Myrmidon Rf.	5.07	9.17	0.00	0.00	6.76	13.20	121.62	347.77	0.00	0.00	5.07	9.17	0.00	0.00
Nomad Rf.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

	H. nobilis		*A. miliaris*		*H. atra*		*S. chloronotus*		*S. variegatus*		*B. argus*		*T. ananas*	
Reef	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
Old Rf.	0.84	4.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.22	10.32	0.00	0.00
Opal Rf.	7.09	11.90	0.00	0.00	26.35	43.67	0.00	0.00	0.00	0.00	6.08	11.58	0.00	0.00
Peart Rf.	1.69	5.85	0.00	0.00	119.93	74.08	30.41	26.64	0.00	0.00	0.00	0.00	0.00	0.00
Potter Rf.	6.76	13.20	0.00	0.00	283.78	179.64	202.70	200.10	0.00	0.00	11.82	20.19	0.00	0.00
Rebe Rf.	2.90	7.36	4.34	8.63	46.33	100.39	14.48	24.41	24.61	34.82	14.48	18.52	0.00	0.00
Rf. 13-050	5.79	9.50	0.00	0.00	18.82	14.80	1.45	5.42	0.00	0.00	8.69	10.41	0.00	0.00
Rf. 13-120	7.24	17.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.45	5.42	0.00	0.00
Rf. 17-065	2.25	6.55	0.00	0.00	25.90	44.41	11.26	19.94	0.00	0.00	0.00	0.00	0.00	0.00
Rf. 19-156	17.37	18.24	0.00	0.00	83.98	64.56	567.56	469.14	0.00	0.00	5.79	15.32	0.00	0.00
Rf. 19-159	4.34	8.63	1.45	5.42	154.92	280.83	10.14	22.13	0.00	0.00	0.00	0.00	0.00	0.00
Rf. 21-149	17.37	20.82	4.34	11.73	55.02	51.17	13.03	27.09	4.34	11.73	1.45	5.42	0.00	0.00
Rf. 21-151	13.03	28.23	4.34	11.73	76.74	61.16	33.30	37.80	5.79	12.39	4.34	8.63	0.00	0.00
Rf. 21-433	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	54.05	46.81	0.00	0.00	0.00	0.00
Rf. 21-551	0.00	0.00	0.00	0.00	69.82	147.03	0.00	0.00	4.50	8.67	0.00	0.00	0.00	0.00
Rf. 22-101	0.00	0.00	0.00	0.00	236.48	356.20	0.00	0.00	10.81	24.07	1.35	5.23	0.00	0.00
Ribbon Rf. No. 2	29.28	21.11	0.00	0.00	1.13	4.78	2.25	6.55	0.00	0.00	0.00	0.00	0.00	0.00
Ribbon Rf. No. 8	5.79	9.38	2.90	9.69	12.55	21.71	4.83	8.85	0.00	0.00	0.00	0.00	0.00	0.00
Ribbon Rf. No. 7	6.76	11.08	1.35	5.14	2.70	11.58	21.62	47.89	0.00	0.00	5.41	10.56	0.00	0.00
Ribbon Rf. No. 10	23.65	28.21	0.68	3.70	15.54	21.74	6.76	19.44	0.00	0.00	5.41	10.56	0.00	0.00
Slasher Rf. No. 1	8.45	18.25	6.76	23.41	106.42	105.19	18.58	30.51	0.00	0.00	0.00	0.00	0.00	0.00
Snake Rf.	0.00	0.00	0.00	0.00	378.37	629.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
St Crispin Rf.	5.79	11.36	0.00	0.00	48.26	48.17	18.34	30.00	0.00	0.00	3.86	13.78	0.00	0.00
Stanley Rf.	8.69	19.45	0.72	3.83	22.44	74.09	130.31	426.45	0.00	0.00	0.72	3.83	0.00	0.00
Stucco Rf.	26.60	27.41	0.00	0.00	176.10	236.12	0.00	0.00	0.00	0.00	16.47	55.50	0.00	0.00
Sudburry Rf.	3.38	9.35	0.68	3.70	318.24	242.55	127.03	155.10	0.00	0.00	5.41	10.56	0.00	0.00
Swizer Rf.	0.00	0.00	2.90	7.66	153.47	209.95	98.45	128.43	0.00	0.00	0.00	0.00	2.90	7.66
Three Islands	0.00	0.00	0.00	0.00	118.24	135.92	3.38	8.28	0.00	0.00	0.00	0.00	0.00	0.00
Tongue Rf.	5.79	11.36	0.00	0.00	126.45	142.89	37.64	60.57	0.97	4.42	7.72	18.66	0.97	4.42
Turner Cay	3.38	10.43	1.13	4.78	157.66	140.19	0.00	0.00	0.00	0.00	0.00	0.00	27.03	72.25
Wharton Rf.	0.00	0.00	0.00	0.00	10.14	14.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Wheeler Rf.	11.06	13.94	0.00	0.00	886.35	1288.26	318.79	598.78	0.00	0.00	11.06	16.63	0.00	0.00
White-tip Rf.	11.26	19.94	1.13	4.78	153.15	232.70	61.94	180.70	3.38	10.43	3.38	7.77	0.00	0.00

Table A2. Densities (No per ha) of *H. nobilis* on reefs resurveyed after closure of the fishery.

		Survey Year		
		1999	2000	2001
Agincourt Rf. No. 1	Average	7.24	7.24	4.34
	SD	10.08	12.84	8.63
Agincourt Rf. No. 4	Average	23.65	24.95	18.71
	SD	33.29	34.27	25.45
Arlington Rf.	Average	5.79	6.76	1.93
	SD	12.08	14.62	6.02
Bowl Rf.	Average	22.52	42.79	15.77
	SD	22.94	24.96	20.34
Davies Rf.	Average	7.60	6.76	10.14
	SD	15.08	9.98	13.67
Ellison Rf.	Average	4.50	6.76	0.00
	SD	8.67	12.04	0.00
Feather Rf.	Average	2.25	2.25	6.76
	SD	6.55	6.55	12.04
Hastings Rf.	Average	13.18	15.20	6.08
	SD	15.10	20.67	11.58
Hicks Rf.	Average	6.76	3.86	11.58
	SD	10.14	10.37	12.11
Michaelmas Rf.	Average	18.48	23.17	20.27
	SD	21.96	26.54	25.55
Moss Rf.	Average	20.27	11.26	21.40
	SD	18.39	15.89	14.70
Opal Rf.	Average	7.09	10.14	6.08
	SD	11.90	12.30	14.85
Peart Rf.	Average	1.69	10.14	13.51
	SD	5.85	16.17	15.78
Potter Rf.	Average	6.76	7.88	6.76
	SD	13.20	15.76	15.55
Rf. 13-120	Average	7.24	5.79	10.14
	SD	17.07	12.39	15.40
Rf. 17-065	Average	2.25	8.45	10.14
	SD	6.55	16.07	18.33
Ribbon Rf. No. 2	Average	29.28	37.50	31.53
	SD	21.11	35.58	27.16
Ribbon Rf. No. 8	Average	5.79	5.79	10.62
	SD	9.38	9.38	13.78
Ribbon Rf. No. 10	Average	23.65	17.45	12.07
	SD	28.21	17.57	17.94
Ribbon Rf. No. 7	Average	6.76	18.10	14.48
	SD	11.08	27.77	18.18
St Crispin Rf.	Average	5.79	7.24	4.34
	SD	11.36	12.84	8.63
Sudburry Rf.	Average	3.38	5.07	1.69
	SD	9.35	13.70	5.72
Tongue Rf.	Average	5.79	3.86	3.86
	SD	11.36	10.37	8.16