Abstract—We investigated the use of otolith morphology to indicate the stock structure of an exploited serranid coral reef fish, *Plectropomus leopardus*, on the Great Barrier Reef (GBR), Australia. Otoliths were measured by traditional one- and two-dimensional measures (otolith length, width, area, perimeter, circularity, and rectangularity), as well as by Fourier analysis to capture the finer details of otolith shape. Variables were compared among four regions of the GBR separated by hundreds of kilometers, as well as among three reefs within each region, hundreds of meters to tens of kilometers apart. The temporal stability in otolith structure was examined by comparing two cohorts of fully recruited four-year-old *P. leopardus* collected two years before and two years after a significant disturbance in the southern parts of the GBR caused by a large tropical cyclone in March 1997. Results indicated the presence of at least two stocks of *P. leopardus*, although the structure of each stock varied depending on the cohort considered. The results highlight the importance of incorporating data from several years in studies using otolith morphology to discriminate temporary and possibly misleading signals from those that indicate persistent spatial structure in stocks. We conclude that otolith morphology can be used as an initial step to direct further research on groups of *P. leopardus* that have lived at least a part of their life in different environments.

The use of otolith morphology to indicate the stock structure of common coral trout (*Plectropomus leopardus*) on the Great Barrier Reef, Australia

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Marine fish populations are generally distributed over large geographical ranges in a heterogeneous environment. Variable physical and biological processes may restrict the exchange of dispersive larvae and adults between areas within a population's species range, resulting in groups of individuals that are phenotypically or genetically distinguishable. Genetic and environmental processes may also have variable effects on the productive capacity (e.g., growth and reproduction) of individuals in different areas. Such variations may be directly measurable with variable life history characteristics, but also indirectly measurable with phenotypic characteristics, such as meristic and morphological characteristics.

Groups of individuals with different genetic or phenotypic characteristics can be defined as separate stocks. Although the precise definition of a stock has been widely debated (see reviews by MacLean and Evans, 1981; Begg, 1998; Booke, 1999), its ultimate meaning should depend on the management objective related to its use. If the management objective is to protect the genetic diversity of a species, for example, genetic information should be sought. If the purpose is

to prevent over-fishing and localized depletion, information about life history characteristics is required. This information is needed because groups with different life history characteristics may respond differently to fishing pressure and therefore have different vulnerabilities to over-fishing (Cole, 1954; Adams, 1980; Jennings et al., 1998).

Variations in morphological characteristics of otoliths have proved useful for identifying stocks for a range of temperate marine fishes (e.g., Bird et al., 1986; Castonguay et al., 1991; Smith, 1992; Campana and Casselman, 1993; Friedland and Reddin, 1994; Begg et al., 2001; Smith et al., 2002), but have not been examined for tropical reef fishes. Differences in morphological characteristics between putative stocks indicate that the stocks have spent some periods of their lives in different environments (Begg et al., 1999; Cadrin, 2000) and therefore have the potential to develop different life history characteristics. Otolith morphological characteristics used as indicators of stock separation generally fall within one of three categories. The first category includes the traditional one-dimensional linear measurements of size-related attri-

butes, such as otolith length and width (e.g., Begg and Brown, 2000; Bolles and Begg, 2000) and distances between specific features on the otolith (e.g., Turan, 2000). Internal otolith measurements, such as nucleus length (e.g., Messeih, 1972; Neilson et al., 1985) and width of hyaline bands or increments (e.g., Begg et al., 2001) also fall within this category. The second category comprises two-dimensional size measurements, such as area, perimeter (e.g., Campana and Casselman, 1993; Begg and Brown, 2000; Bolles and Begg, 2000) and different shape indices, including circularity and rectangularity (e.g., Friedland and Reddin, 1994; Begg and Brown, 2000; Bolles and Begg 2000, Tuset et al., 2003). A third, more recent morphological technique examines the two-dimensional outline of otolith shape using Fourier analysis (e.g., Bird et al., 1986; Smith, 1992; Campana and Casselman, 1993; Begg and Brown, 2000; Smith et al., 2002). Fourier analysis produces a series of cosine and sine curves from the coordinates of a traced outline which, when added together, describe the outline of the traced form. The cosine and sine curves can be defined mathematically in a series of Fourier descriptors and used as variables to compare otolith shapes among individuals or potential stocks (Christopher and Waters, 1974; Younker and Ehrlich, 1977).

Plectropomus leopardus (common coral trout) (also known as leopard coralgrouper, FishBase[1]) is the most important commercially and recreationally harvested reef fish on the Great Barrier Reef (GBR), Australia (Mapstone et al.[2]; Williams[3]). *Plectropomus leopardus* comprises between 35% and 50% of the commercial reef line catch annually (Mapstone et al.[2]) and in 2004 a total allowable commercial catch (TACC) of 1300 t was implemented. Regional (hundreds of km) or inter-reef (hundreds to thousands of m) variations have been demonstrated in some life history characteristics of *P. leopardus* on the GBR (Begg et al., 2005), such as differences in density (Ayling et al.[4]), reproductive strategies (Adams, 2002), size and age (Russ et al., 1995; Lou et al., 2005), and mortality (Russ et al., 1995, 1998; Map-

stone et al., 2004). Current management arrangements (such as TACC, fish size limits, gear restrictions, recreational bag limits, and spatial and temporal closures), however, do not incorporate the localized or regional spatial structure in the life history characteristics of *P. leopardus* or any other exploited species on the GBR.

The overall aim of this study therefore was to examine the use of otolith morphology for determining the stock structure of *P. leopardus* on the GBR. We investigated the broad spatial scale of *P. leopardus* by comparing aspects of otolith morphology among fish collected from four regions of the GBR, separated by hundreds of kilometers (north to south). Otolith structure was also assessed at finer spatial scales, among *P. leopardus* collected from neighboring reefs separated by hundreds of meters to tens of kilometers, within each of the four regions. In addition, because temporal variation in otolith shape could confound the spatial information if samples were taken from only one time, we also compared otolith morphological characteristics from two cohorts of *P. leopardus* with non-overlapping life histories either side of a significant environmental disturbance that affected the southern half of the GBR (the large and persistent Cyclone Justin in March 1997). Spatially variable effects of the Cyclone, such as a significant drop in temperature and salinity in large parts of the GBR (AIMS[5]), provided us with a unique opportunity to test the temporal stability of spatial patterns in otolith morphology.

Methods and data analysis

Background

Common coral trout (*Plectropomus leopardus*) were collected as part of the Cooperative Research Centre for the Great Barrier Reef World Heritage Area (CRC Reef) Effects of Line Fishing (ELF) experiment (Campbell et al., 2001; Mapstone et al., 2004). The ELF experiment, which began in 1995 and concluded in 2006, monitored line-caught fish populations from a group of six neighboring reefs in each of four regions extending over 7° of latitude along the GBR (Fig. 1; Mapstone et al.[6], 1996, 1997, 2004; Davies et al.[7]). At the start of the experi-

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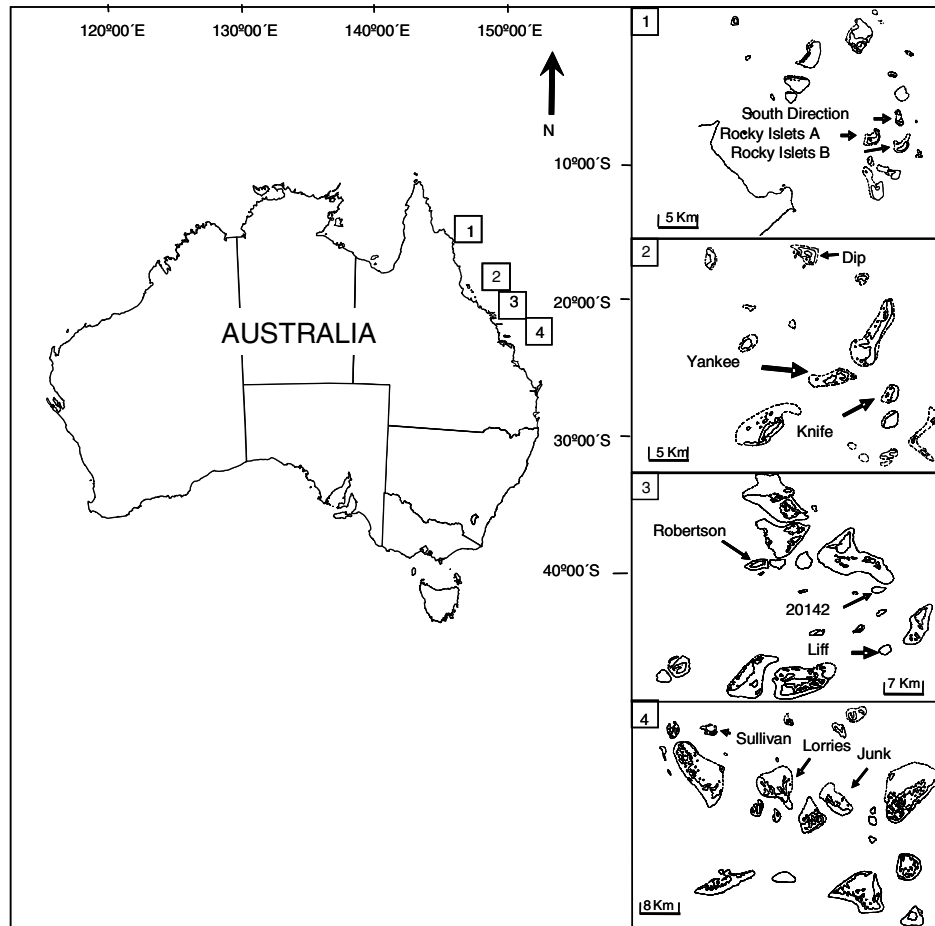
Figure 1

Great Barrier Reef, Australia. Study regions and reefs (1–4) from which common coral trout (*Plectropomus leopardus*) were collected are shown. 1 = Lizard Island; 2 = Townsville; 3 = Mackay; 4 = Storm Cay.
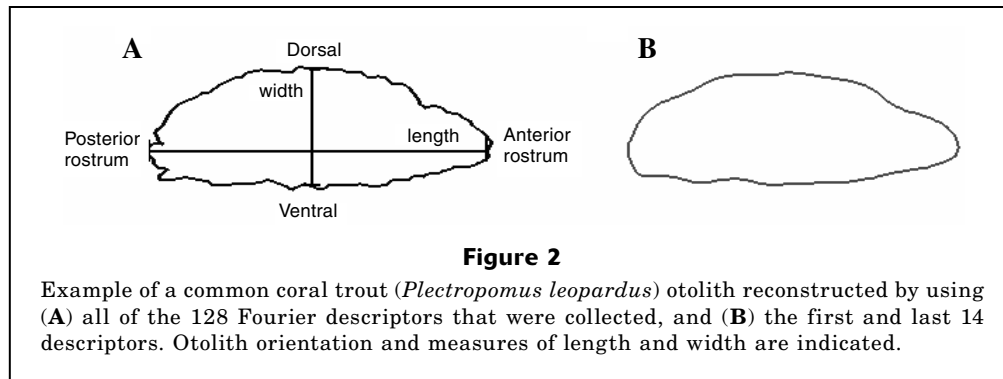
ment, four reefs in each region had been closed to fishing for 10–12 years under GBR Marine Park Zoning Plans (zoned Marine National Park B, MNP-B) and two reefs in each region had been open to fishing (zoned as General Use, GU). Two of the reefs closed to fishing remained closed during the experiment, other than to the annual research line fishing surveys. The other two closed reefs were each subjected to one year of fishing, in 1997 and 1999, after which they were closed again. The two reefs in each region that had historically been open to fishing were subjected to increased fishing pressure for one year (i.e., were temporarily opened). These reefs were then closed for five years before reverting to their original zoning status (GU).

All reefs were sampled each year in the austral spring (October–December) to coincide with the peak spawning period of the main target species, *P. leopardus*. Each reef was divided into six approximately equal-size, contiguous blocks, and sampled on a single day on each sampling occasion. Standardized commercial reef line fishing effort was distributed uniformly across two depth strata within each block. All fish caught were

measured, tagged for later identification, and kept for weighing and for extraction of gonads and otoliths. For further sampling details of the ELF experiment see Davies et al.[7] and Mapstone et al. (2004). Samples of *P. leopardus* were aged by CRC Reef staff using standardized methods developed by Ferreira and Russ (1994).

Sample collection

Otolith morphological variables were analyzed from four-year-old *P. leopardus* collected in 1995 and 1999 from three reefs within each of the four regions (Fig. 1; Table 1). Four years is the youngest age at which *P. leopardus* in all regions are fully recruited to the fishing gear used in the ELF Experiment. Although the zoning status of a reef and the level of fishing pressure on it were unlikely to have affected otolith structure, one reef from each treatment regime in the ELF experiment was included from each region to avoid potential biases in spatial variation related to particular fishing histories. Thus, within each region, samples were analyzed from one MNP-B reef that was closed to fishing, one MNP-

Figure 2

Example of a common coral trout (*Plectropomus leopardus*) otolith reconstructed by using (**A**) all of the 128 Fourier descriptors that were collected, and (**B**) the first and last 14 descriptors. Otolith orientation and measures of length and width are indicated.

Table 1

Study regions, reefs, and years for which the common coral trout (*Plectropomus leopardus*) was sampled over the Great Barrier Reef and examined for otolith morphology.

Region	Reef (GBRMPA number)	1995, 1999 replicates	Total length range (mm)
Lizard Island	Rocky Islet Reef A (14132a)	20, 6	304–495
	Rocky Islet Reef B (14132b)	20, 6	306–481
	South Direction Reef (14147)	20,12	350–468
Townsville	Knife Reef (18081)	8, 4	370–451
	Dip Reef (18039)	7,12	318–462
	Yankee Reef (18074)	20, 8	322–464
Mackay	unnamed reef (20142)	20, 18	322–456
	Liff Reef (20296)	20, 16	303–427
	Robertson Reefs (no2) (20136)	20, 11	342–514
Storm Cay	Junk reef (21131)	20, 15	345–422
	Lorries reef (21130)	20, 11	328–475
	Sullivan reef (21124)	18, 19	280–424
Total		213, 138	303–514

B reef that was open for one year of fishing and then closed, and one GU reef that was subjected to increased fishing for a year prior to closure for five years.

Because otoliths from only four-year-old fish were analyzed in 1995 and 1999, the two cohorts included individuals with nonoverlapping life histories. Consequently, individuals collected in 1995 were not exposed to the influence of the unusually large and persistent Cyclone Justin that influenced the southern half of the GBR throughout March in 1997, unlike those four-year-old fish collected in 1999. Standardizing sampling by age also minimized the potential for confounding spatial variation in otolith shape with ontogenetic changes.

Sagittal otoliths from up to 20 four-year-old *P. leopardus* were sampled from each reef each year (Table 1). Otoliths from fewer than 20 fish were analyzed only if less than this number were collected from a reef. This sampling design enabled the examination of broad (region) and fine (reef within region) spatial and temporal (1995 and 1999 cohorts) patterns in the otolith morphology of *P. leopardus* across much of the GBR, spanning an unusual environmental event (Cyclone Justin, 1997) that had the potential to significantly influence the results.

Morphological analysis

A microscope image (10× magnification) was projected onto a computer screen by using a video camera (Panasonic GP-KR222E, Panasonic, Matsushita Communication Industrial Co., Osaka, Japan. Whole otolith area, length, perimeter, and width (Fig. 2) and two shape indices (circularity and rectangularity) were recorded from each otolith by using the OPTIMAS image analysis system (OPTIMAS, vers. 6.51, Silver Spring, MD). Rectangularity was calculated as the area of the otolith divided by the area of its minimum enclosing rectangle, and circularity as the perimeter of the otolith squared divided by its area. The perimeter of the otolith was traced in a counter clockwise direction and digitized into 128 *x-y* equidistant coordinates by using the distal edge of the otolith rostrum as a common starting point

for the coordinates. A fast Fourier transform (FFT) was calculated as a Cartesian FFT. The Cartesian FFT uses the 128 *x-y* coordinates as complex numbers ($a + ib$), where a is the real component and ib the imaginary component, representing the amplitudes of the cosine and sine waves, respectively. The resultant 128 set of complex numbers or descriptors were subsequently normalized for differences in otolith position by setting the 0th descriptor to 0, and for size and rotation of the otolith by dividing all the descriptors with the first descriptor. The normalized descriptors ($a' + ib'$) were used to calculate the absolute value (harmonic) of each descriptor according to the following equation (Christopher and Waters, 1974):

$$Harmonic = \sqrt{a_n'^2 + \left(ib'\right)_n^2}.$$

The harmonics were then used in combination with the other morphological variables and shape indices to compare otoliths between cohorts and among regions and reefs within regions.

The higher the number of equidistant points and subsequent complex numbers included in the model, the closer is the fit to the original shape. The main features of the otolith shape, however, are generally captured by the first 10–20 harmonics (e.g., Campana and Casselman, 1993; Friedland and Reddin, 1994). The minimum number of Fourier descriptors required to explain at least 90% of the recorded shape of the otoliths in our study was calculated similarly to the range-finding procedure of Smith et al. (2002). A total of 128 descriptors were collected from two randomly selected otoliths from each reef and cohort (24 in total) and normalized for position, size and rotation as described above. The shape of each otolith was reconstructed (by computing the inverse FFT) by using all the descriptors and then reconstructed by using only the first and last descriptors. The Euclidian distance between the inverse FFT using all the descriptors and the inverse FFT using only the first and last descriptors was defined as the maximum percent error of reconstruction, i.e. 100% reconstruction error (Smith et al., 2002). Because the Cartesian descriptors are asymmetrical around the middle frequency, both ends of the array are required in the reconstruction. Otolith shape was reconstructed, therefore, by using the first two and last two descriptors, the first three and last three descriptors, and so on until the first and last 22 descriptors were used. This range-finding test allowed us to estimate the decrease in mean percent reconstruction error by using more and more descriptors and it was estimated that 14 of the first and last descriptors were required for the reconstruction error to be less than 10%. See Figure 2 for a comparison of the otolith shape reconstructed from the first and last 14 descriptors and all 128 descriptors. These descriptors, therefore, were used in the statistical analyses to compare the spatial and temporal patterns in otolith shape of *P. leopardus*.

Statistical methods

The assumption of normality and homogeneity of variance for each morphological variable was examined by using Shapiro-Wilk's and Levene's tests, respectively, and homogeneity of the group covariance matrix by Box's *M* test. Variables of circularity, breadth and area were log_{10}-transformed and Fourier harmonics 2, 4, 9, 11–14, 120–121, 114–118, and 123–127 square-root–transformed to conform to the assumption of normality and homogeneity of variances.

A relationship between otolith shape and otolith growth rate (assumed to be correlated to fish length) may confound spatial or temporal differences in otolith shape (Campana and Casselman, 1993). We minimized the potential for such effects by 1) including only fish with a fork length (FL) between 280 and 514 mm (overall FL for four-year-olds sampled during the ELF experiment in 1995 and 1999 ranged from 250 to 551 mm), and 2) standardizing morphological variables by fish FL where a significant relationship existed between the variable and FL before further analyses. The effect of FL on each morphological variable was examined by analysis of covariance (ANCOVA; Winer et al., 1991). Our primary interests in these analyses were 1) to test whether morphological variables differed with FL for any group of samples; and 2) if so, to test whether the slopes of regressions of morphological variable on FL were homogeneous among groups. If a significant regression was detected and homogeneous among groups, the effect of FL was removed from each measurement by using the relationship

$$O_{ij,adj} = O_{ij} + b.(FL_{ij} - MFL_{\cdot j}).$$

where $O_{ij, adj}$ = otolith morphological measurement of fish i adjusted to mean fork length of group j;

O_{ij} = original otolith morphological measurement for fish i from group j;

$b.$ = slope of the relationship O_{ij}:FL_{ij} common to **all** groups;

FL_{ij} = fork length of fish i in group j; and

$MFL_{\cdot j}$ = average fork length within group j.

If significant slopes of the relationship differed among groups, the correction for FL was made separately for each group by using the equation above, but by replacing the common slope (*b.*) with the group-specific slope (*b_j*). These corrections had the effect of scaling all morphological variables from all otoliths to their predicted group mean FL.

Multivariate analysis of variance (MANOVA; Tabachnick and Fidell, 1983) was used to investigate the effects of sex (females, males, and individuals in the process of changing sex; i.e., transitional fish) on otolith shape (MANOVA). A total of 302 of the 351 individuals had been examined for sex, of which 166 were females, 123 males and 13 transitional fish. Separate MANOVAs were computed for the one- and two-dimensional shape

variables and the Fourier harmonics by using a three-way crossed model (outlined below) with fixed factors: cohort, region, and sex. Data were pooled across reefs within regions owing to insufficient sample numbers to test for reef-specific effects.

A MANOVA was used to test for spatial and temporal differences in otolith shape. A principal component (PC; Tabachnick and Fidell, 1983) analysis was done first on the combined data set of both the shape variables and Fourier harmonics to reduce the number of variables to be incorporated in the MANOVA. The number of PCs to extract and subsequently include in the MANOVA was determined by examining the size of the eigenvalues (representing the variance explained by each PC), as well as their relative contribution to the percent variance explained compared to the other eigenvalues (i.e., scree test; Tabachnick and Fidell, 1983). The latter determines the number of PCs beyond which the addition of more PCs would contribute little to the variance explained by the solution (Tabachnick and Fidell, 1983). Wilk's lambda criterion was used to test for group differences in the MANOVAs. Sums of squares and degrees of freedom of interactions were pooled when the *F*-ratios of interaction effects were ≤1. Pooling increases the degrees of freedom for the denominator and consequently the power of the test of remaining (unpooled) effects in the analyses.

A posteriori univariate analysis of variance (ANOVA) was used to explore patterns for each of the PCs separately when significant effects were indicated in the MANOVA. The univariate linear model for the analysis of each PC was

$$x_{ijkl} = \mu_{....} + C_{i...} + R_{.j..} + r(R)_{.k(j)} + C_{ij..} + Cr(R)_{ik(j).} + e_{l(ikj)},$$

where x_{ijkl} = the PC score for otolith l from cohort i, region j and reef k;

$\mu_{....}$ = the estimate of the population mean PC score over all cohorts, regions, reefs, and otoliths;

$C_{i...}$ = the fixed effect of cohort i averaged over regions and reefs;

$R_{.j..}$ = the fixed effect of region j averaged over cohorts and reefs;

$r(R)_{.k(j)}$ = the random variation attributable to reef k within region j averaged over cohorts; and

$e_{l(ikj)}$ = unexplained random variation associated with otolith l within cohort i, region j and reef k.

Tukey's honestly significant difference (HSD) test was used to determine which means differed following significant effects detected in the ANOVAs. The communalities (representing the proportion of the total variance of a variable accounted for by the PC) and variable loadings of the PCs that were significant in the ANOVAs were subsequently examined. A loading below 0.45 indicated that the variable explained less than 20% of the PC and therefore was not interpreted further.

Finally, two forward stepwise canonical discriminant analyses (CDAs; Tabachnick and Fidell, 1983) were computed by using the shape variables and Fourier harmonics to examine the otolith shape of *P. leopardus* in multivariate space and to investigate whether otolith shape could be used to classify samples to spatial scale and cohort of origin. The factor used as a separating variable in the CDA depended on the significant effects determined in the MANOVA (i.e., cohort, region, or reef [region], or any interactions between these factors). The CDA was used in this way as a confirmatory technique. Wilk's lambda criterion was used to test for significant differences between the discriminant functions. Jack-knife classification was used to minimize potential bias in the reclassification of individuals.

Results

Slopes of the relationship between FL and several otolith morphological variables for *P. leopardus* differed among a range of spatial scales and between cohorts (Table 2). The within-group slope, therefore, was calculated for each group according to the level at which the slopes of the relationship differed and was used to correct for the influence of FL (Table 2). For some variables, the common between-group slope was used to correct for the influence of FL because there was a significant overall relationship between the variable and FL, which was homogeneous among groups (ANCOVA homogeneity of slopes test, *P*>0.05; Table 2). No morphological variable was significantly correlated with FL after standardization. Furthermore, shape variables and Fourier harmonics were not significantly different between otoliths of females, males or transitional fish (MANOVA, *P*>0.05). The morphological data, therefore, were pooled across sex for the remainder of the analyses.

Principal component analysis (PCA)

Four PCs were extracted from the analysis of the combined data set of shape variables and Fourier harmonics and included in the MANOVA (Table 3). The communalities ranged between 0.11 and 0.88, and some morphological variables were better defined by the PC solution than others (Table 3). About 44% of the total variance in the morphological data was explained by the four extracted PCs (17.9%, 10.2%, 9.9% and 5.9% by PC I, II, III, IV, respectively). A combination of higher order harmonics describing the finer details of the otolith outline, and lower order harmonics, perimeter, length, and circularity representing the broad shape of otoliths explained most of the variation in PC I and III (Table 3). Variation in the broader details of otolith shape also accounted for most of the variation in PC II and PC IV; otolith area, breadth, perimeter, length, and harmonic eight explained most of the variation in PC II and breadth and harmonic 127 explained most of the variation in PC IV (Table 3).

Table 2

Results of the homogeneity of slopes test for the influence of fish fork length (FL) on otolith morphological variables of four-year-old *Plectropomus leopardus*. The level of correction represents the level at which the slopes were heterogenous. For example, the level of correction "reef(region)" means that the slopes of the relationship between FL and a morphological variable differed among reefs nested within regions and that the slope for each reef was used to correct for the influence of FL. Level of correction "FL" means that there was a significant overall relationship between FL and a morphological variable, but that the slopes of this relationship were homogenous among reefs(region), regions and cohorts. Only variables with a statistically significant relationship with FL are shown.

Variable	df	F	P	Level of correction
Log_{10} breadth	8, 306	1.99	0.0467	reef(region)
Log_{10} circularity	8, 306	1.99	0.0479	reef(region)
Length	3, 315	4.05	0.0075	region
Log_{10} area	8, 306	2.01	0.0452	reef(region)
Perimeter	8, 306	2.34	0.0187	reef(region)
Rectangularity	1, 341	14.43	0.0002	FL
Sqrt harmonic 2	1, 349	11.15	0.0009	FL
Harmonic 3	1, 349	10.32	0.0014	FL
Sqrt harmonic 4	1, 349	6.90	0.0090	FL
Harmonic 5	3, 314	3.44	0.0171	cohort×region
Harmonic 6	7, 314	2.12	0.0410	reef(region)
Harmonic 7	1, 349	13.56	0.0003	FL
Sqrt harmonic 9	7, 307	2.30	0.0270	cohort×reef(region)
Sqrt harmonic 13	1, 349	8.05	0.0048	FL
Sqrt harmonic 14	7, 317	3.31	0.0021	reef(region)
Sqrt harmonic 114	1, 327	8.69	0.0034	vohort
Sqrt harmonic 115	7, 307	2.48	0.0170	cohort×reef(region)
Sqrt harmonic 116	7, 317	3.95	0.0004	reef(region)
Sqrt harmonic 117	7, 317	2.89	0.0061	reef(region)
Sqrt harmonic 118	3, 314	2.90	0.0354	cohort×region
Harmonic 119	1, 349	6.79	0.0096	FL
Sqrt harmonic 120	3, 325	2.91	0.0346	region
Sqrt harmonic 121	7, 307	2.10	0.0435	cohort×reef(region)
Harmonic 124	3, 314	4.01	0.0080	cohort×region
Harmonic 125	1, 349	10.79	0.0011	FL
Harmonic 126	1, 349	31.76	<0.0001	FL
Harmonic 127	1, 327	4.37	0.0373	cohort

The MANOVA of the four PCs identified significant differences in the PC scores of otolith shape among regions, but were not consistent between cohorts (1995 and 1999; cohort×region interaction, Table 4). Univariate ANOVAs indicated that these differences were due to variation in PC II among regions and in PC IV among reefs within regions—both spatial patterns varying depending on the cohort in consideration (Table 5). Differences in PC II were due to differences between Lizard Island and Mackay regions in 1995 and between cohorts in Mackay (Fig. 3A; HSD, $P<0.05$). The apparent inconsistency between the significant reef within region effect in the ANOVA (PC IV) and no such reef effect detected in the MANOVA is most likely explained by the difference between two reefs in the Lizard Island region, for which there was a significantly lower mean PC IV score for Reef 14132a than for Reef 14147 in 1995, whereas the reverse was observed in 1999 (Fig. 3B; HSD, $P<0.05$). There was also a significant difference among cohorts on Reef 14132a (Lizard Island) and Reef 20296 (Mackay Region; Fig. 3B; HSD, $P<0.05$).

Although only explaining 18% (PC II and PC IV combined) of the variation in our morphological data, the PC analysis indicated that the presence of at least two stocks of *P. leopardus* on the GBR in 1995, one in the northern part of the GBR (Lizard Island) and one in the southern part (Mackay), whereas one homogeneous stock was indicated in 1999.

Table 3

Variable communalities and loadings on the four significant principal components (PCs) explaining 44% of the total variance in the otolith morphological data of four-year-old *Plectropomus leopardus* collected from three reefs in each of four regions in 1995 and 1999 on the Great Barrier Reef.

Variable	Communalities	Loadings PC I	PC II	PC III	PC IV
Perimeter	0.88	0.39	0.69	0.51	0.01
Length	0.85	0.07	0.57	0.71	0.14
Log_{10} area	0.81	−0.05	0.80	0.32	−0.27
Log_{10} breadth	0.80	−0.03	0.70	−0.10	−0.55
Harmonic 127	0.78	0.18	−0.16	0.65	0.55
Log_{10} circularity	0.76	0.67	0.13	0.42	0.33
Sqrt harmonic 4	0.70	0.80	0.04	−0.21	−0.09
Harmonic 6	0.64	0.72	0.18	−0.27	−0.11
Harmonic 5	0.58	0.63	−0.29	0.27	−0.15
Harmonic 8	0.51	0.17	0.57	−0.38	0.09
Harmonic 10	0.49	−0.32	0.44	−0.29	0.34
Sqrt harmonic 2	0.49	0.70	0.00	−0.04	0.03
Harmonic 3	0.49	−0.40	−0.05	0.57	0.01
Harmonic 122	0.45	0.43	0.40	−0.25	0.21
Sqrt harmonic 13	0.44	0.58	−0.08	−0.07	−0.30
Sqrt harmonic 117	0.41	0.42	−0.21	0.11	−0.42
Harmonic 7	0.41	0.54	−0.23	0.06	0.25
Sqrt harmonic 120	0.40	0.37	0.26	−0.32	0.30
Sqrt harmonic 11	0.37	0.54	−0.12	0.03	−0.25
Harmonic 119	0.37	0.45	−0.30	0.22	−0.14
Harmonic 125	0.35	−0.22	0.02	0.55	0.03
Sqrt harmonic 115	0.35	0.33	−0.12	0.22	−0.42
Sqrt harmonic 121	0.31	0.39	−0.37	0.16	−0.01
Harmonic 124	0.30	0.48	0.11	0.03	0.24
Sqrt harmonic 116	0.26	0.30	0.14	−0.35	0.18
Sqrt harmonic 118	0.26	0.49	0.02	−0.11	0.06
Sqrt harmonic 114	0.18	0.19	0.00	−0.37	0.10
Sqrt harmonic 12	0.18	0.22	−0.11	0.33	−0.05
Harmonic 126	0.16	0.32	0.18	0.04	0.13
Sqrt harmonic 9	0.14	0.09	−0.20	−0.05	0.30
Sqrt harmonic 123	0.14	0.27	−0.03	−0.17	0.18
Rectangularity	0.13	−0.25	−0.17	−0.18	0.08
Sqrt harmonic 14	0.11	0.30	−0.09	−0.02	0.10

Discriminant analyses

Two stepwise canonical discriminant analyses (CDAs) were computed for the morphological variables (Fourier harmonics and shape variables combined) in response to the significant cohort × cegion effect in the MANOVA of the PC scores. First, separate CDAs were done for each region using cohort as the separating variable and second, separate CDAs were done for each cohort by using region as the separating variable. The region-specific CDA, where cohort was used as a separating variable, showed a significant first discriminant func-

tion (DF) for all four regions, accounting for 100% of the variance in the data (Table 6). Some separation was observed in the distribution of the DF I scores between the two cohorts within each region, although the temporal pattern was not consistent across regions (Fig. 4). Likewise, the cohort specific CDA using region as a separating variable showed some separation between the four regions in the first two significant DFs of cohort 1995 (explaining 59.9% and 30.7% of the variance, respectively) and one significant function of cohort 1999 (explaining 100% of the variance, Table 6). The means of the DF I scores in the Lizard Island and

Townsville regions were distinctly greater than those of the Mackay and Storm Cay regions in 1995 (HSD, *P*<0.05; Fig. 5A) and in Townsville were greater than the Mackay and Storm Cay regions in 1999 (HSD, *P*<0.05; Fig. 5B).

Different combinations of harmonics 9, 114, 118 describing the finer details of the otolith shape, and harmonic 124 and shape variables area and circularity representing the broad details of otolith shape, were selected into the DFs to maximize the separation of cohorts within regions (Table 7). The primary predictor variables selected for separating regions in each of the cohorts were similar to those selected in the region-specific CDA, although the variables were not the same in each cohort (Table 7). Between 66.7% and 76.3% of *P. leopardus* within a region could be correctly classified to their cohort of origin (Table 8). Fewer cohort specific individuals could be classified to a region however;

Table 4

Results of MANOVA comparing principle component (PC) I–IV scores of otolith shape from two cohorts of four-year-old *Plectropomus leopardus* collected from three reefs in each of four regions in 1995 and 1999 on the Great Barrier Reef. *P*-values in bold indicate significant differences (*P*<0.05).

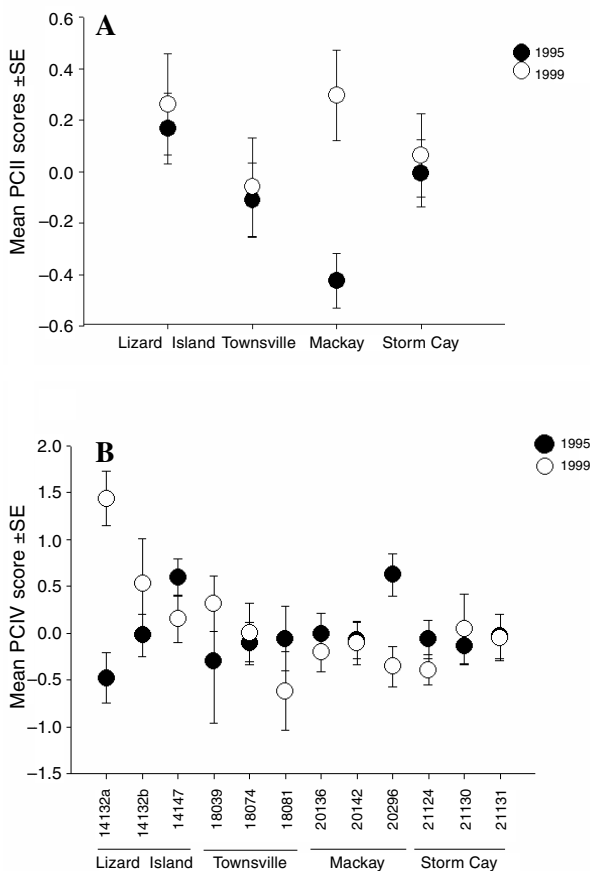| Factor	Wilks' lambda	*F*	df	*P*
Cohort	0.3800	2.04	4, 5	0.2269
Region	0.0513	2.34	12, 14	0.0687
Cohort×region	**0.0315**	**3.04**	**12, 14**	**0.0269**
Reef(region)	0.8880	1.19	32, 1167	0.2126
Cohort×reef(region)	0.8900	1.18	32, 1167	0.2318

Figure 3

Mean (**A**) principal component (PC) II scores per region and cohort, and (**B**) PC IV scores per reef within region and cohort of four-year-old common coral trout (*Plectropomus leopardus*), collected from three reefs in each of four regions in 1995 and 1999 on the Great Barrier Reef.
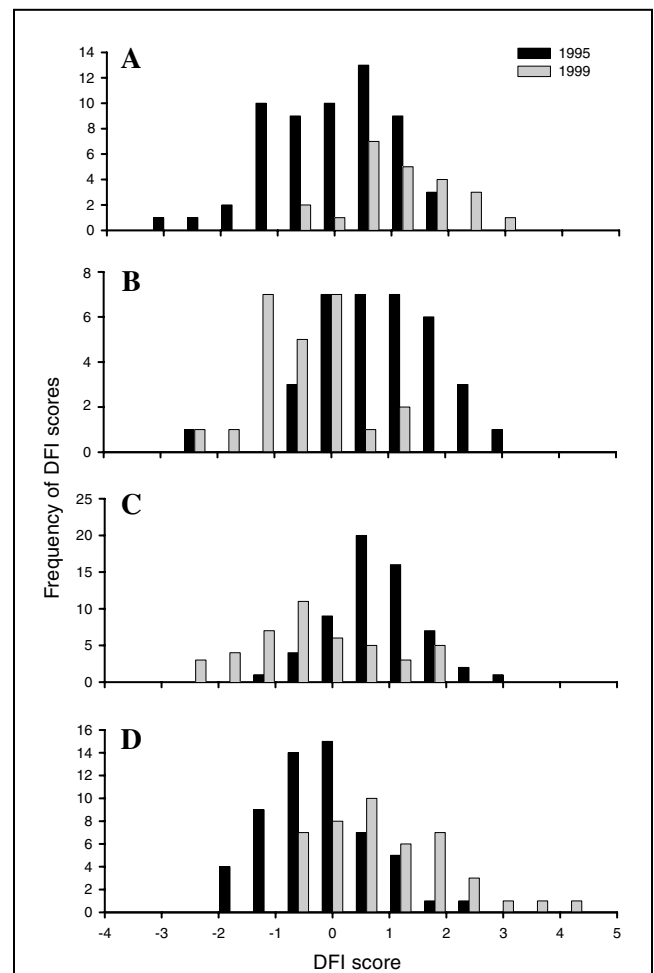

Figure 4

Frequencies of discriminant function (DF) I scores from otolith morphological variables (Fourier harmonics and shape variables combined) per cohort (sampled in 1995 and 1999) for regions (**A**) Lizard Island, (**B**) Townsville, (**C**) Mackay, and (**D**) Storm Cay for four-year-old common coral trout (*Plectropomus leopardus*), from the Great Barrier Reef. (The data were pooled across reefs within regions.)

34.3% and 39.7% were correctly classified for cohorts sampled in 1995 and 1999, respectively (Table 9).

Discussion

Spatial and temporal variation in otolith structure

This study detected differences in the otolith shape of *P. leopardus* at different spatial and temporal scales across the Great Barrier Reef (GBR), Australia. Although the spatial patterns in otolith shape of *P. leopardus* were not always consistent among different types of shape variables, two main inferences can be made from our results. Firstly, our results indicate that otolith morphology can be useful for identifying groups of individuals of this species that are likely to have spent a significant part of their lives in different environments and therefore may indicate potential stock separation. Overall otolith shape suggested the presence of at least one southern and one northern stock along the GBR. The Storm Cay and Mackay regions consistently belonged to the southern stock and the Lizard Island region to the northern stock, and it was inconclusive as to which stock individuals in the Townsville region belonged. It is possible that the main morphological features of otoliths are established in the larval stage and that large amounts of larval dispersal and mixing between areas results in overlap of signatures between stocks. Finer details of otolith shape, however, are most likely influenced by environmental processes during the post larval phase and may therefore provide insights to the separation of stocks during postlarval life.

Although otolith shape also suggested variation at a smaller, among reef, spatial scale (hundreds to thousands of m) the significant differences in the multivariate morphological measurement (PC IV) occurred only between two reefs in the Lizard Island region, and therefore it seems unlikely that such small-scale separation is a common phenomenon on the GBR. Nevertheless, the presence of some difference among reefs emphasizes the importance of careful and sufficient sampling in order to capture the range of values manifest in small-scale, presumably random, variation within regions and to avoid erroneously ascribing to regional structure what are really a reflection of local-scale variation. Given the hydrodynamic mixing over scales of kilometers, we consider it unlikely that reef-scale variations in morphological variables represent persistent environmentally induced patterns in stock structure.

Table 5

Results of ANOVAs comparing otolith principle component (PC) scores of otolith structure from two cohorts of four-year-old *Plectropomus leopardus* collected from three reefs in each of four regions in 1995 and 1999 on the Great Barrier Reef. Only final analyses resulting from pooled terms with $F \leq 1$ are shown. *P*-values in bold indicate significant differences ($P<0.05$). Only PCs with significant differences are shown. MS=mean square.

Variable	Factor	df	MS	F	P
PC II	Cohort	1	1.5165	1.60	0.2067
	Region	3	0.8057	0.47	0.7126
	Reef(region)	8	1.7209	1.82	0.0732
	Cohort×region	**3**	**3.0900**	**3.26**	**0.0217**
PC IV					
	Cohort	1	0.3246	0.11	0.7473
	Region	**3**	**3.2901**	**8.11**	**0.0083**
	Cohort×region	3	3.5574	1.21	0.3675
	Reef(region)	8	0.4059	0.43	0.9011
	Cohort×reef(region)	**8**	**2.9452**	**3.14**	**0.0020**

Table 6

Significance test of cohort-specific and region-specific canonical discriminant analysis (CDA) of shape variables and Fourier harmonics combined from otoliths of four-year-old *Plectropomus leopardus* collected from three reefs in each of four regions in two cohorts, 1995 and 1999 on the Great Barrier Reef. Only significant discriminant functions (DF) are shown. DF=discriminant function; df=degrees of freedom.

Scale	Factor	DF	Wilks' lambda	χ^2	df	P
Spatial scale						
Lizard Island	Cohort	I	0.7818	19.21	2	<0.0001
Townsville	Cohort	I	0.6863	20.89	3	0.0001
Mackay	Cohort	I	0.7685	26.34	4	<0.0001
Storm Cay	Cohort	I	0.7683	25.43	3	<0.0001
Temporal scale						
1995	Region	I to III	0.7573	56.72	12	<0.0001
1995	Region	II to III	0.8918	23.36	6	0.0006
1999	Region	I	0.91	11.95	3	0.0075

The second inference from our results is that regional patterns in otolith shape are not always consistent among cohorts and may be subject to temporally dynamic events such as large-scale environmental perturbations. Ignoring such temporal signals could give misleading information about stock structure. A single homogenous stock of *P. leopardus* may have been suggested if otolith structure based on the 1999 PCA results alone were considered, whereas two potential stocks would be inferred from both the PCA and CDA analyses of the 1995 data (Figs. 2A; 5A). In addition, cohorts were distinguishable within all regions based on the frequency distributions of DF scores. Other investigations of the interannual stability in otolith morphology of marine fishes have found differences between years, as well as age groups (Castonguay et al., 1991; Campana and Casselman, 1993; Begg and Brown, 2000; Begg et al., 2001). Consequently, when using otolith morphology to investigate stock structure, it is preferable to compare multiple cohorts with individuals of the same age, collected over several years. Such a sampling design would minimize confounding spatial variation in otolith shape with particular times of sampling and derive a time-averaged assessment of the spatial structure of a stock.

The spatial and temporal patterns in otolith shape are in part consistent with the assessment (based on otolith microchemistry) of stock structure of *P. leopardus* on the Great Barrier Reef (Bergenius et al., 2005). Differences in otolith chemistry of *P. leopardus* collected at the same spatial and temporal scales as those considered in this study have indicated the presence of two or three regional stocks. Thus, although the number and potential boundaries of phenotypic stocks based on otolith chemistry and otolith structure remain uncertain, the combined results of these studies emphasize the potential presence of several stocks of *P. leopardus* on the GBR and indicate a north-south demarcation. Differences in both otolith
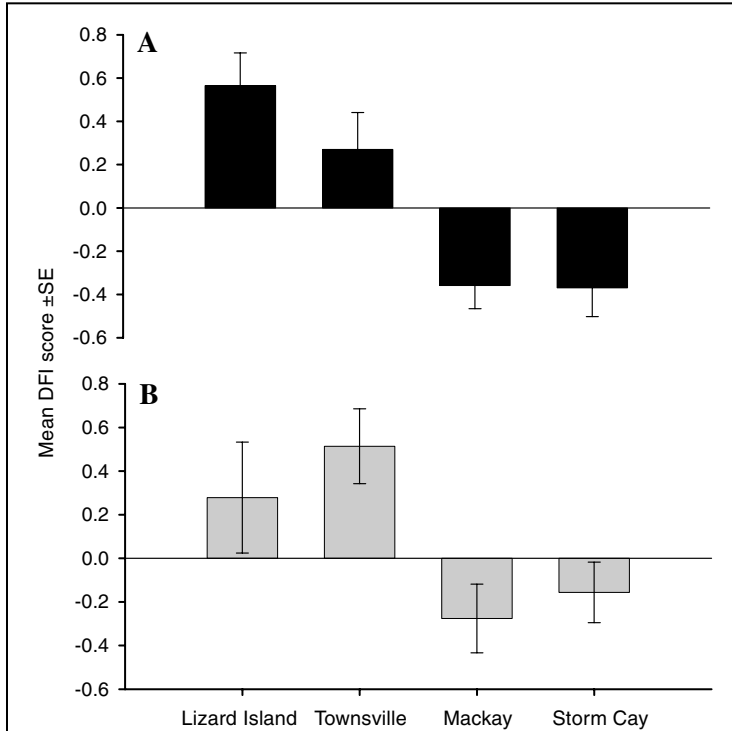

Figure 5

Mean ±SE of discriminant function (DF) I scores from otolith morphological variables (Fourier harmonics and shape variables combined) per region for (A) cohort 1995, and (B) cohort 1999 in four-year-old common coral trout (*Plectropomus leopardus*) from the Great Barrier Reef. (The data were pooled across reefs within regions.)

Table 7

Canonical coefficient function representing correlation between the morphological variables (Fourier harmonics and shape variables combined) and significant discriminant functions (DFs), separating two cohorts (1995 and 1999) of four-year-old *Plectropomus leopardus* within each of four regions of the Great Barrier Reef. All variables selected in the models are shown, but variables with loadings <0.45 are not interpreted further.

Variable	Lizard Island DF I	Townsville DF I	Mackay DF I	Storm Cay DF I	1995 DF I	1995 DF II	1999 DF I
Log_{10} area			−0.62		0.59	−0.37	
Rectangularity				0.38			
Log_{10} circularity		−0.58					1
Sqrt harmonic 9		0.49	0.57				
Sqrt harmonic 114				0.62			
Sqrt harmonic 115	−0.24						
Sqrt harmonic 118	0.84				−0.46	0.23	
Sqrt harmonic 123			0.35				
Sqrt harmonic 124		0.22		0.49	0.45	0.52	
Sqrt harmonic 125			0.41		−0.12	0.69	

Table 8

Correctly classified individuals of four-year-old *Plectropomus leopardus* per cohort (1995 and 1999) in each of four regions of the Great Barrier Reef.

Cohort	Lizard Island	Townsville	Mackay	Storm Cay
1995	63.79	71.43	78.33	76.79
1999	73.91	83.33	70.45	63.64
Total	66.70	76.30	75.00	71.00

Table 9

Correctly classified individuals of four year old *Plectropomus leopardus* per region on the GBR in each of two cohorts (1995 and 1999).

Region	1995	1999
Lizard Island	39.66	17.39
Townsville	28.57	58.33
Mackay	45.00	53.49
Storm Cay	41.07	11.36
Total	39.70	34.30

shape and chemistry indicated that the movements of adults of *P. leopardus* may be limited and are consistent with the presumed sedentary (reef-specific) habit of most serranids after settlement (e.g., Chapman and Kramer, 2000; Stewart and Jones, 2001). Our results confirm those of several tagging studies of *P. leopardus* on the GBR which showed that individuals are unlikely to move among reefs after settlement (Davies, 1995; Zeller, 1998; Zeller and Russ, 1998). Such limited inter-stock movement of adults means that the recovery rate of stocks after significant harvest is largely (if not solely) dependent on some unknown level of larval dispersal. Further investigations, therefore, are required to clarify the implications of this potential stock structure of *P. leopardus* to ensure sustainable harvests at the appropriate scale for both management and the stock structure of the fish.

Reasons for temporal and spatial variation in otolith structure

Little is known about what processes influence the expression of traits responsible for otolith shape (Ihssen et al., 1981). Differences in growth rate has been the main reason given for differences in linear morphological variables of otoliths (e.g., Reznick et al., 1989; Secor and Dean, 1989; Begg et al., 2001), as well as outline shape variables (e.g., Castonguay et al., 1991; Smith, 1992; Campana and Casselman, 1993; Begg and Brown, 2000). In our study, *P. leopardus* subsamples were restricted to a certain size range to minimize the potential of a growth rate effect that could confound spatial or cohort related differences in otolith shape. Significant differences in growth rates (indicated by mean FL of four-year-olds), however, were apparent among reefs within regions in the complete sample from the ELF experiment (Bergenius et al.[8]), whereas there was no consistency in the patterns of variation in FL and any of the otolith morphological variables. It is possible that the large variation in FL among reefs could have masked potential

differences in broad-scale regional differences in growth rates, or that some other developmental rate, such as maturation or reproductive output, were more important in influencing the otolith shape of *P. leopardus*.

Spatial and temporal variability in otolith shape would be expected in *P. leopardus* populations along the GBR given the natural variability in biological and hydrodynamic factors between reefs and regions (Wolanski, 1994), which in turn could affect various fish developmental rates. There was no monotonic latitudinal trend apparent in any of the otolith morphological variables related to a temperature gradient. The maximum difference in monthly average sea surface temperature among the latitudes examined in our study is typically less than 2°C (Lough, 1994). In contrast, there is regional (broad spatial scale) variability in the upwelling and inflow of cool nutrient rich oceanic water across the continental shelf of the GBR (e.g., Andrews and Gentien, 1982; Andrews, 1983; Wolanski, 1994; Middleton et al., 1995) which could potentially influence fish developmental rates through changes in food availability or "step-wise" patterns in water temperature.

There is also a possibility that the temporal variability in otolith shape of *P. leopardus* collected before and after Cyclone Justin in 1997 was related to associated temperature or other climatic changes. Cyclone Justin was an unusually large cyclone that remained in the Coral Sea adjacent to the GBR for over three weeks during March 1997. It caused a large and rapid drop in water temperature over the southern half of the GBR (south of ~17–18°S) during this time, and bottom water temperatures dropped in just one month (March) to below the average winter minimum not usually reached until July (AIMS[5]). Our study revealed a significant difference in otolith shape between nonoverlapping cohorts sampled in 1995 and 1999 in the Mackay region, which is one of the regions where the temperature changes would have been greatest.

Management and future directions

Although differences in otolith shape of *P. leopardus* may give an indication of stock structure, an examination of the relevance of these patterns for fisheries management should be the next step. A recent increase in the total

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[8] Bergenius M. A. J., B. D. Mapstone, G. R. Russ, and G. A. Begg. 2005. Unpubl. data from the Effects of line fishing experiment (Mapstone et al., 2004). CRC Reef Research Centre, Townsville, Australia. [Data are on files at the CRC Reef Research Centre, James Cook University, Townsville, Queensland 4810, Australia.]

area of the GBR Marine Park (designated as marine national park zones [or no-take zones]) from about 5% to 33% enhances the potential for displacement of fishing effort between regions. If the proposed stocks have different life history characteristics and, potentially, different fishery productivities, less productive stocks may be subjected to greater harvest levels and increased risk of local depletion. Information on vital life history characteristics (e.g., growth, mortality, and maturity) is therefore needed to examine the potential impacts of fishing and related harvest strategies for the postulated stocks. Moreover, until more is known about how larvae relate between stocks, prudent and precautionary management should recognize groups of fish that function as individual units or stocks in response to harvest and management (Haddon and Willis, 1995).

Otoliths are often collected during routine sampling undertaken as part of the monitoring procedures and assessments of exploited fish stocks. As a result there may be large archives of otoliths available for morphological analyses in most fisheries laboratories. We therefore suggest that patterns in otolith morphology provide a cost and time effective starting point for directing further research on groups of harvested coral reef fishes that have lived at least parts of their life in different environments.

Acknowledgments

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