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Evidence for discrete subpopulations of sea perch (*Helicolenus ercoides*) across four fjords in Fiordland, New Zealand

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In coastal populations of invertebrates and fishes, the distribution of discrete subpopulations is influenced by adult and larval dispersal, as well as by the effects of habitat heterogeneity on site fidelity or connectivity. Here, we examine evidence for spatial structure of sea perch, *Helicolenus percoides*, populations among four fjords in the Fiordland region of southwestern New Zealand. We examine patterns in adult morphology, length-at-age, $\delta^{13}C$ and $\delta^{15}N$ of muscle tissue, and trace elemental composition of whole otoliths as proxies for population isolation among the four inner fjord regions. A multivariate analysis of morphometrics reveals significant differences among populations from each of the four sites, suggesting existence of four distinct subpopulations. These patterns are consistent with observed differences in $\delta^{13}C$ and $\delta^{15}N$, and length-at-age estimates among the four subpopulations. Differences in whole otolith concentrations of Sr, Ba, Mg and Li, and high classification scores based on the whole otolith elemental fingerprint are also consistent with significant subdivision among areas. Patterns across all four markers are consistent with discrete subpopulation structure of adult sea perch among the four study sites. These data indicate that the newly implemented network of marine protected areas in Fiordland is likely to contain discrete populations of sea perch.

Keywords: morphology; growth; stable isotopes; otolith microchemistry; metapopulation

Introduction

Connectivity among discrete subpopulations through dispersal of individuals as larvae, juveniles or adults is a critical structural property of marine metapopulations (Sale et al. 2005; Kritzer & Sale 2006). Understanding dynamics of marine metapopulations, and success in application of marine protected areas, can be enhanced by an understanding of how populations are connected by dispersal across fragmented landscapes (Forgarty 1998; Jones et al. 1999; Thorrold et al. 2001), and how variations in habitat quality and nutrition affect reproductive output (Crowder et al. 2000).

The majority of research into population connectivity of marine fishes has concentrated on the dispersal of larval stages (Jones et al. 1999; Cowen et al. 2000), recognised as a primary driver of population dynamics (Hixon et al. 2002). However, recent studies have indicated that local retention of fish larvae is common (Swearer et al. 2002), dispersal distances may not be large for some species (Taylor & Hellberg 2003) and many coastal fish populations may not be as open as

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previously thought (Cowen et al. 2000). These results suggest that adult dispersal may be an important alternative mechanism for maintaining connectivity in some species.

Adult dispersal patterns are well known for several commercially exploited marine fishes, largely through mark–recapture studies and archival tag records (e.g. Block et al. 2005); however, for the majority of coastal fishes there is scant information (Sale et al. 2005). Directly studying population connectivity in the ocean is challenging, as it is difficult to predict or track migration over large areas, and mark–recapture studies can be hampered by low rates of return and spatial bias in recapture patterns (Bolle et al. 2005). Relevant studies have tended to focus on connectivity patterns through ontogeny (Gillanders & Kingsford 2000), natal homing (Thorrold et al. 2001) and stock structure (Campana et al. 2000; Silva 2003). Population connectivity can be studied indirectly using information on population structure. Observed differences between groups of fish, such as variation in morphology (Silva 2003) or growth rate (Agnew 1988), can be used as a proxy to identify discrete subpopulations within a species that has continuous distribution over a large area. Consistent differences among several such proxies imply population structuring with low levels of adult migration (Begg & Waldman 1999).

Other 'natural tags', such as stable isotopes, otolith ('earstone') microchemistry and genetic markers, can also resolve population structure of marine fishes (Begg & Waldman 1999), however genetic techniques are rarely effective in moderately mixed populations, as differences can be masked by even low rates of exchange (Thresher 1999). $\delta^{15}N$ and $\delta^{13}C$ of a consumer's tissues provides an integrated measure of the isotopic signature of their diet (DeNiro & Epstein 1978, 1981), so if food sources in particular habitats are distinct for $\delta^{15}N$ and $\delta^{13}C$, the tissues of animals feeding in these habitats over extended periods will also have distinct $\delta^{15}N$ and $\delta^{13}C$. As turnover of $\delta^{15}N$ and $\delta^{13}C$ is likely to occur over several years for slow growing fishes (Hesslein et al. 1993), the isotopic signature of their tissues can be used as a natural marker to distinguish population structure (Roelke & Cifuentes 1997) and identify migratory animals (Rodgers & Wing 2008) over timescales of 1–2 years.

The use of otolith microchemistry as a natural tag is facilitated by two key properties: (1) the trace elemental composition of otoliths reflects, at least in part, the chemical and physical characteristics of the surrounding water, and (2) otoliths are acellular and metabolically inert, so incorporated elements are permanently retained (Campana 1999). Exposure to contrasting environments can result in unique patterns, or 'fingerprints', of otolith elemental concentrations that can be used to reconstruct temperature and salinity histories, stock structure and migration pathways (Begg et al. 2005). It is difficult to determine past conditions and geographic associations robustly from such fingerprints alone, since the effects of temperature, salinity and physiology on metal incorporation into the otolith are not fully understood, and aquatic environments rarely remain stable over long periods of time (Kalish 1991, Thresher 1999). However, since the otolith grows continuously throughout the lifetime of the fish, individuals that have spent most of their lives in the same stock are expected to share similar whole otolith elemental fingerprints. Assuming that environmental conditions vary across time and space, such integrated fingerprints are likely to distinguish among isolated stocks exposed to different overall past conditions (Campana et al. 2000).

Here, we investigate the population structure of sea perch (*Helicolenus percoides*) in the Fiordland region of New Zealand, which is comprised of 15 deepwater glacially carved fjords separated from the open ocean by a shallow sill of rock moraine (Grange et al. 1981). Within each fjord, high rainfall drives estuarine circulation (Stanton & Pickard 1981). The isolated basins of the inner fjords and predominant estuarine circulation play an important role in limiting larval dispersal and

gene flow among fjords for marine organisms with a planktonic larval phase. Evidence for this comes from several sources, including genetic studies of the eleven-armed sea star, *Coscinasterias muricata* (Sköld et al. 2003; Perrin et al. 2004), the sea urchin *Evechinus chloroticus* (Perrin et al. 2003) and brachiopods, *Liothyrella neozelanica* and *Terebratella sanguinea* (Ostrow et al. 2001). Similarly, a highly divided stock structure and low rates of movement among regions has been shown for blue cod (*Parapercis colias*) (Carbines & McKenzie 2004; Rodgers & Wing 2008). These studies suggest that dispersal of adult fish may be an important determinant of connectivity in this system.

Helicolenus percoides are relatively small, long-lived (up to 59 years), benthic Scorpaenids, distributed throughout New Zealand and southern Australia's continental shelf and slope (Paul 1998). They have been caught from depths greater than 1000 m, but are particularly common at depths less than 20 m in Fiordland (Francis & Ling 1985). Information on the population biology of *H. percoides* is limited, and little is known of their movements at any life-history stage. Regional variations in colour and growth rate suggest that adult sea perch are unlikely to undertake extensive movements (Paul 1998; Paul & Horn 2009), implying that their larval stage could be more important for maintaining population connectivity. In Fiordland, however, larval dispersal is likely to be restricted because of estuarine circulation (Ostrow et al. 2001; Sköld et al. 2003; Perrin et al. 2003, 2004), raising the interesting question of whether the subdivided nature of basin habitats within the New Zealand fjords harbour distinct groups of *H. percoides*.

The objective of this study was to determine whether populations of sea perch from subtidal benthic habitats in four fjords in southern Fiordland comprise discrete subpopulations, using four proxies for population isolation. Lack of homogeneity in each of these proxies would provide support for population segregation of adults. Morphological measurements of adult sea perch were used to determine if there were any consistent differences in growth form among the four populations. $\delta^{13}C$ and $\delta^{15}N$ of muscle tissue and length-at-age were used to determine if basal carbon source pools and growth were homogeneous. Length-at-age analyses were used to test for differences in growth among sites. Finally, trace elemental composition of whole otoliths was compared among sites to test for population-specific elemental fingerprints. Results from these analyses were used to evaluate whether these sites are likely to harbour distinct sub-populations of sea perch. As sea perch are thought to be vulnerable to localised depletion (Paul 1998) and there are currently no published studies on Fiordland populations, knowledge of their stock structure is vital for their successful management and conservation within the context of a newly implemented system of spatial management of marine resources in Fiordland.

Materials and methods

Sample collection

Adult *H. percoides* were collected using artificial lures from 10 to 30 m over a 2-week period in November 2004 and frozen until they could be processed. In total, 84 fish of similar size were collected from four different sites in Fiordland: 21 from Shark Cove, Dusky Sound (total length, TL, 17.8–28.3 cm), 22 from the Only Islands, Long Sound (TL 22.0–27.0 cm), 21 from Vancouver Arm, Breaksea Sound (TL 21.7–28.3 cm) and 20 from Deep Cove, Doubtful Sound (TL 19.1–27.2 cm) (Fig. 1).

Morphometric analysis

Five morphometric measurements were made to 0.01 mm using electronic Vernier callipers (Fig. 2), and standard and total lengths were measured to the nearest millimetre. All measurements were taken on thawed specimens, so any 'freezing effects' were assumed to be constant among samples.

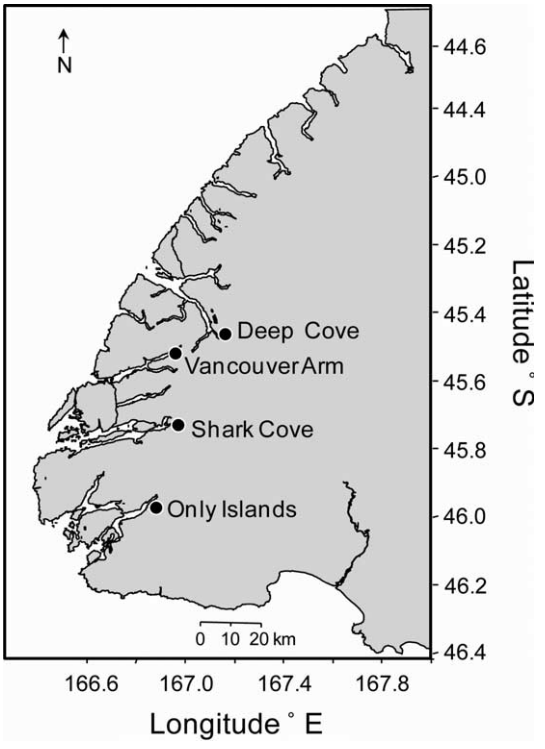

Fig. 1 Location of sampling sites in Fiordland, New Zealand: Deep Cove, Doubtful Sound, Vancouver Arm, Breaksea Sound, Shark Cove, Dusky Sound; and Only Islands, Long Sound.

Linear regression analyses were conducted for each morphometric character against the standard length to test for an allometric effect of size. To avoid possible biases produced by length on morphometric variables, each character was standardised by:

$$\hat{Y} = 10^{K} \quad (1)$$

where the exponent K was the $\log_{10}$ of the adjusted measurement e derived from:

$$e = \log_{10} Y - \beta(\log_{10} X - \log_{10} X_{\mathrm{STL}}) \quad (2)$$

where e was the size-adjusted measurement, Y was the original measurement, β was the common regression slope of $\log_{10} Y$ against $\log_{10} X$, X was the standard length of the individual, and X_{STL} was the overall mean standard length. This transformation best reflected shape variation among groups independently of size (Reist 1985). The standardised morphometric characters showed no significant correlation with standard length ($P > 0.05$ for all characters), indicating that the allometric transformation had successfully removed the length effects.

To identify whether there were any statistically significant differences among populations for each character, Bonferroni corrected one-way analysis of variance (ANOVA) or Kruskal–Wallis tests (when data did not satisfy the assumptions of ANOVA) were performed on each standardised morphometric character. Standardised morphometric characters were analysed using stepwise and jack-knifed quadratic discriminant function analysis (QDFA) in JMP version 7.0.1 (SAS Institute Inc., Cary, NC, USA) and MYSTAT version 12.02.00 (SYSTAT Software Inc., Chicago, IL, USA) respectively. Performance of the QDFA was assessed using Wilk's λ and percentage correct classifications.

Stable isotope analysis

$\delta^{13}C$ and $\delta^{15}N$ were measured from samples of dorsal muscle taken from each fish, placed in sterile Eppendorf tubes and dehydrated in an oven at 60°C for 48 h. Dried tissues were ground into a fine powder using a mortar and pestle, which was thoroughly washed between samples using Milli-Q water and lint free paper; 0.001 g of each powdered sample was weighed into a tin capsule, and analysed with a Europa 20–20 update stable isotope mass spectrometer (Europa Scientific, Crewe, UK) interfaced to a Carlo Erba elemental analyser (NA1500, Carlo Erba, Milan, Italy) in continuous-flow mode (lowest accepted precision: 0.2‰ for $\delta^{13}C$, 0.35‰ for $\delta^{15}N$). Analysis was calibrated to EDTA laboratory standard reference (Elemental Microanalysis, Cheshire, UK) and standardised against international standards (IAEACH-6 for carbon, IAEAN1 and IAEAN2 for nitrogen). Results are expressed in the standard delta notation

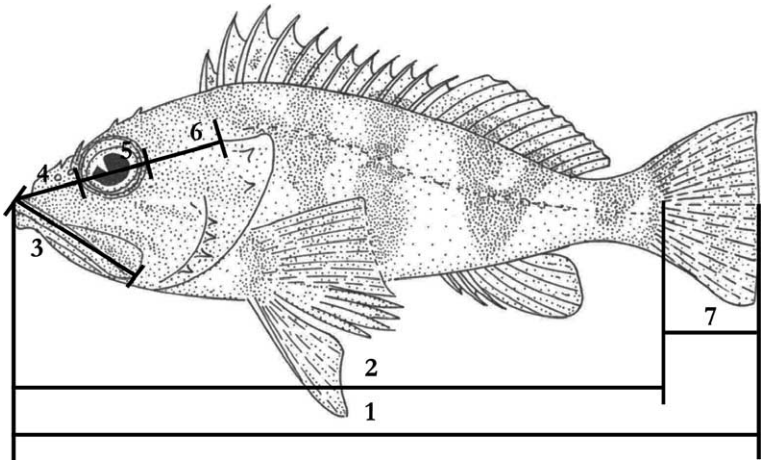

Fig. 2 Morphometric characters measured in sea perch specimens: (1) total length (TL)—snout tip to caudal tip; (2) standard length (SL)—snout tip to caudal base; (3) maxillary length—snout tip to maxillary tip; (4) snout length—snout tip to inner orbital edge; (5) orbital diameter—widest distance across the orbital; and (6) head length—snout tip to innermost point of gill slip. Inter-orbital distance (not displayed) was measured as the shortest distance between the orbitals (image adapted from Paulin et al. 2001).

(e.g. Peterson & Fry 1987). As the data were not normally distributed (Anderson Darling test for normality, $P < 0.05$), Kruskal–Wallis tests and Mann–Whitney U tests were used to test for differences in $\delta^{13}C$ and $\delta^{15}N$ between sites.

Age and growth

To test for differences in growth among study sites, sagittal otoliths were removed from all 84 fish, placed in sterile Eppendorf tubes and left to air dry at room temperature for 3 days. One saggita of the pair was randomly chosen for age analysis. Once dry, otoliths were embedded in a clear epoxy resin (Nuplex K142 resin and hardener) and sectioned across the dorso-ventral axis through the nucleus. Sections 0.6 mm thick were cut using a Buehler Isomet low-speed saw with a diamond-wafering blade (series 15 LC diamond, 0.15 mm × 7.6 cm) and Buehler isocut fluid. Sections were mounted on glass slides using epoxy resin, sanded to approximately 0.4 mm thick using 1500-grade sandpaper, then polished with 15-μm and 1-μm diamond abrasive lapping film (South Bay Technology Inc., San Clemente, CA, USA). Under transmitted light, otolith sections showed a series of alternating light and dark growth zones. Two previous age validation studies have supported the assumption that a single pair of light and dark growth zones is laid down each year in male and female sea perch (Lewis 2008; Paul & Horn 2009). Accordingly, a single light and dark ring was counted as 1 year, starting from the first dark ring after the dense core area.

Three readers independently aged the otoliths, with each otolith being read a total of five times. Reader 1 aged all otoliths twice, 3 months apart; Reader 2 aged them twice, 1 year apart and Reader 3 aged them once. All otoliths were aged in a random order, with no knowledge of the length, weight or sex of the fish. As there were some cases of significant between-reader differences in ring counts, final ages were assigned to each fish according to the following rules: where two independent readers estimated identical ages for a single fish, this age was used; otherwise, an average of all estimates not more than 3 years apart was used. Relative bias and precision were assessed

using age bias plots and standard error among readers against age (Campana 1995).

Because of limited sample sizes, growth curves were not fitted to the data and average length-at-age was compared among sites using 3-year age bins (8–10 years, 11–13 years etc). Only the 11–13 and 14–16-year bins had sufficient sample sizes to compare statistically among sites, although Shark Cove was removed from the latter (14–16-years age bin) because of a lack of samples. One-way ANOVAs indicated that there was no significant difference in the age of individuals between sites for each age bin: 11–13 years ($F_{3,36} = 1.246$, $P = 0.31$) and 14–16 years ($F_{2,21} = 1.071$, $P = 0.36$). Both length-at-age datasets were normally distributed, but the 11–13-year age class could not be transformed to give equal variance, so was log-transformed and compared by Welch ANOVA (JMP 7.0.1), which does not assume equal standard deviation among groups.

Otolith microchemistry

A total of 66 sagittal otoliths were used for elemental analysis (20 from Deep Cove, 16 from Vancouver Arm, 15 from Shark Cove and 15 from Only Island). Samples were washed for 8–10 s in 1% HNO_3 (65% Merck Suprapure®) then rinsed three times in Milli-Q water, placed in labelled, acid washed in 10-ml polypropylene centrifuge tubes and dried in a class-100 laminar flow cabinet. Once dry, otoliths were weighed to the nearest 0.01 mg, then dissolved in 1.5 ml of 20% HNO_3 (65% Merck Suprapure®) inside a laminar flow cabinet and made up to a final volume of 8 ml with Milli-Q water. To analyse for trace elements, a sub-sample of this solution was taken (0.5 or 1 ml, depending on otolith weight) and made up to 10 ml with 1% HNO_3 (65% Merck Suprapure®). A further 100 × dilution was required to measure Sr. Procedural blanks were prepared in the same manner, but contained no otoliths.

Trace element concentrations (Li, Mg, Mn, Fe, Cu, Cd, Ba, Pb) were determined by inductively coupled plasma-mass spectrometry (ICPMS; Varian UltraMass 700) and Sr by inductively coupled plasma-atomic emission spectrometry (ICPAES; Varian Liberty Series II). Both systems were calibrated using certified reference material NIES 22 (Fish Otolith Certified Reference Materials from Japan National Institute for Environmental Studies) and matrix-matched calibration solutions made of matrix otolith and a multi-element standard solution supplied by Choice Analytical Pty Ltd. Concentrations were blank subtracted and converted into otolith concentrations (expressed as μg/g dry wt) based on otolith dry weight and dilution factor. Limits of detection (LOD), calculated as three times the standard deviation of the blanks (after Gillanders & Kingsford 2000), were: 0.005 μg/g (Li), 0.439 μg/g (Mg), 0.252 μg/g (Mn), 1.737 μg/g (Fe), 0.101 μg/g (Cu), 0.023 μg/g (Cd), 1.021 μg/g (Sr), 0.018 μg/g (Ba) and 0.043 μg/g (Pb). Values below LOD were truncated to zero; as Cd, Pb and Mn concentrations were mostly below LOD, they were excluded from subsequent analyses. Fe was also removed because of spectral interference. Precision estimates of the remaining five elements, based on the relative standard deviation of replicate measurements of a spiked otolith sample, were: 18.12% (Li), 7.31% (Mg), 10.12% (Cu), 2.91% (Sr) and 5.33% (Ba). There were no significant correlations between element concentrations and otolith weight.

Concentrations were log-transformed [$\log(y+1)$] to minimise the importance of outliers, tested for equal variance (Brown–Forsythe test), then compared among sites using one-way ANOVA or Welch ANOVA when transformed data remained heterogeneous.

Stepwise and jack-knifed QDFAs were performed in JMP version 7.0.1 and MYSTAT version 12.02.00 respectively, and used to investigate differences in otolith elemental fingerprints between sites, as quadratic

functions do not assume equal covariance matrices (Gillanders & Kingsford 2000). Performance of the QDFAs was assessed using Wilk's λ and percentage correct classifications.

Results

Morphometric analysis

Univariate analyses indicated that standardised morphometric characters differed significantly among sites (Table 1). Individuals from the Only Islands had larger heads and longer tails, while individuals from Shark Cove had shorter maxillaries and shorter snouts compared to other sites. Orbital size was largest in individuals from both Shark Cove and Only Islands, and smallest in individuals from Vancouver Arm. Inter-orbital distance was greatest in fish from Deep Cove compared to the other three sites. Stepwise QDFA indicated that there was significant separation among sites based on morphometric characters. The first discriminant (DF) accounted for 65% of the total variance and the second and third DFs for 28% and 7%, respectively. The group centroids for each site differed significantly (Wilk's $\lambda = 0.248$, $F_{18,212} = 7.5214$, $P < 0.0001$) and 95% confidence ellipses around the group centroids indicated that populations from Shark Cove and the Only Islands were significantly different from those at all other sites (Fig. 3). Jack-knifed QDFA correctly assigned 63% of individual specimens to their collection site. Classification success was highest for specimens from the Only Islands and lowest for specimens from Deep Cove (68% and 55%, respectively) (Table 2). Classification success rates were significantly greater than those expected by chance (chi-square test, $\chi^2 = 41.0$, df = 3, $P < 0.00001$).

Table 1 Results of one-way analyses of variance (ANOVAs) and Kruskal–Wallis tests for differences in individual standardised morphometric characters of sea perch from four sites in Fiordland, New Zealand.

Character	Test statistic	df	*P*	Statistical test
Snout length	$H = 15.12$	3	**0.002**	Kruskal–Wallis
Maxillary length	$F = 10.55$	3	**<0.001**	One-way ANOVA
Orbital width	$F = 24.56$	3	**<0.001**	One-way ANOVA
Head	$H = 23.35$	3	**<0.001**	Kruskal–Wallis
Tail length	$F = 4.54$	3	**0.005**	One-way ANOVA
Inter-orbital distance	$F = 6.01$	3	**0.001**	One-way ANOVA

According to Bonferroni corrections, tests are significant, in bold, at $P < 0.008$.

Stable isotope analysis

There was a significant difference in $\delta^{13}C$ ($H = 14.44$, $P = 0.002$) among sites (Fig. 4). Pair-wise Mann–Whitney *U* tests identified

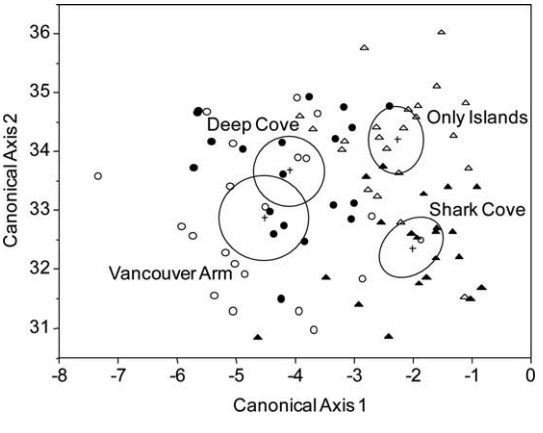

Fig. 3 Canonical plot scores and 95% confidence ellipses from stepwise quadratic discriminant function analysis of six morphometric characteristics of sea perch from four sites in Fiordland, New Zealand (Deep Cove, Doubtful Sound (closed circles); Vancouver Arm, Breaksea Sound (open circles); Only Islands, Long Sound (open triangles); Shark Cove, Dusky Sound (closed triangles)). Site labelled circles correspond to a 95% confidence limit for the group's multivariate mean. Significantly different groups have non-intersecting circles.

Table 2 Classification results of jack-knifed discriminant function analysis assigning sea perch to four different sites in Fiordland, New Zealand, based on six morphometric characteristics.

	Deep Cove		Only Islands		Shark Cove		Vancouver Arm	
	Count	(%)	Count	(%)	Count	(%)	Count	(%)
Deep Cove	**11**	**(55)**	4	(20)	1	(5)	4	(20)
Only Islands	2	(9)	**15**	**(68)**	3	(13)	2	(9)
Shark Cove	2	(9)	5	(24)	**12**	**(57)**	2	(10)
Vancouver Arm	7	(33)	1	(5)	1	(5)	**12**	**(57)**

Rows denote collection site of specimens; columns indicate analysis classifications. Correct classifications are in bold.

the population from Only Islands as being significantly different from the populations from Deep Cove and Vancouver Arm, while the population from Shark Cove was significantly different from the population from Deep Cove (Table 3). $\delta^{15}N$ also differed significantly among sites ($H = 32.15$, $P < 0.001$) (Fig. 4). Pairwise Mann–Whitney U tests identified the population from Only Islands as being significantly different from the other three sites (Table 3).

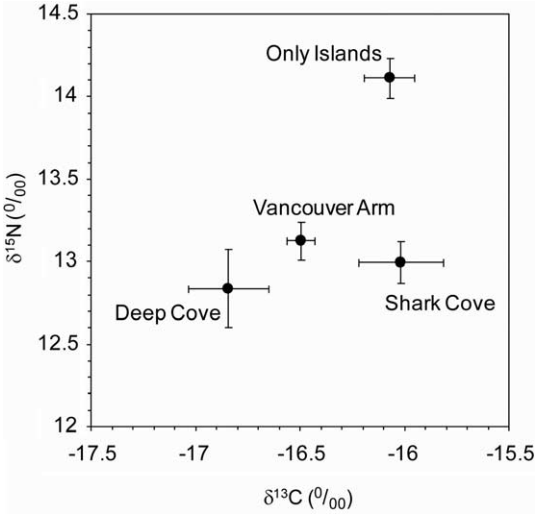

Fig. 4 Mean $\delta^{15}N$ and $\delta^{13}C$ values measured in dorsal muscle tissue of sea perch from four sites in Fiordland, New Zealand. Error bars are 1 standard error.

Age and growth

Age estimates ranged from 8 to 25 years, with a median age of 13.4 years. Similarity in the range of ages estimated in this study to those reported elsewhere for sea perch in New Zealand (Paul & Horn 2009) and related species of Scorpaenids (e.g. Kelly et al. 1999) suggests that the ages estimated here were reasonably accurate.

Ageing precision was variable both within and between readers, however, all fish in this study had age estimates assigned to them by at least two different readers that differed by fewer than 3 years, and 67% had identical ages assigned by at least two different readers. The average coefficient of variation (CV) for all fish, based on all five readings, was 13.0. This

Table 3 Results of Mann–Whitney U pair-wise comparisons of $\delta^{13}C$ and $\delta^{15}N$ of sea perch.

	Vancouver Arm	Shark Cove	Only Islands
$\delta^{13}C$			
Deep Cove	0.32	**0.04**	**<0.001**
Vancouver Arm		0.19	**<0.001**
Shark Cove			0.85
$\delta^{15}N$			
Deep Cove	0.99	0.70	**<0.0001**
Vancouver Arm		0.40	**<0.0001**
Shark Cove			**<0.0001**

Significant P-values are in bold.

value included some apparently anomalous readings (for example where four of the five readings differed from each other by no more than 3 years but one reading differed by more than 10 years). When age estimates were 'filtered' according to the rules described in the methods above, the average CV was 2.78.

There is no *a priori* precision value that is acceptable for ageing studies, however, a CV of 5% has been suggested informally as a reference point for many fishes of moderate longevity and reading complexity (Campana 2001). Although some CVs for individual fish in this study were higher than this recommended value, because of the difficulty in ageing sea perch otoliths (Paul & Horn 2009), the precision of age estimates was judged acceptable. Age bias plots indicated that while there was some variation between readers in age estimates for individual fish, there were no overall biases. There was a significant difference in length-at-age between sites for both age classes: 11–13 years ($F_{3,36} = 9.69$, $P = 0.01$) and 14–16 years ($F_{2,21} = 4.29$, $P = 0.029$). Length-at-age was greatest for individuals from Vancouver Arm and smallest for individuals from Deep Cove for both the 11–13 year and the 14–16 year age bins (Fig. 5).

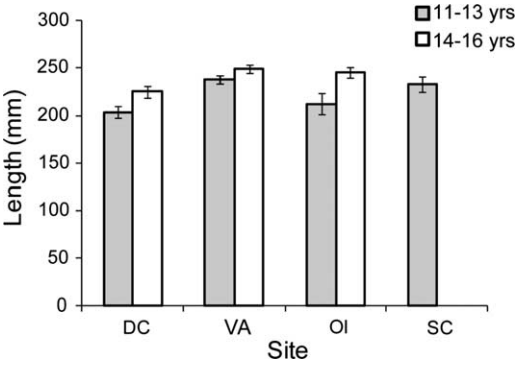

Fig. 5 Mean length-at-age for sea perch of two age groups from four sites in Fiordland (Deep Cove, Doubtful Sound (DC); Vancouver Arm, Breaksea Sound (VA); Only Islands, Long Sound (OI); Shark Cove, Dusky Sound (SC)). Error bars are 1 standard error.

Otolith microchemistry

Univariate analyses indicated significant differences in log-transformed whole otolith concentrations of Li ($F_{3,62} = 9.82$, $P < 0.001$), Mg ($F_{3,62} = 8.81$, $P < 0.001$), Sr ($F_{3,62} = 4.28$, $P = 0.008$) and Ba ($F_{3,62} = 5.20$, $P = 0.003$) among sites, but no difference in Cu (Fig. 6). Concentrations of Li could not be transformed to give equal variance; however, the

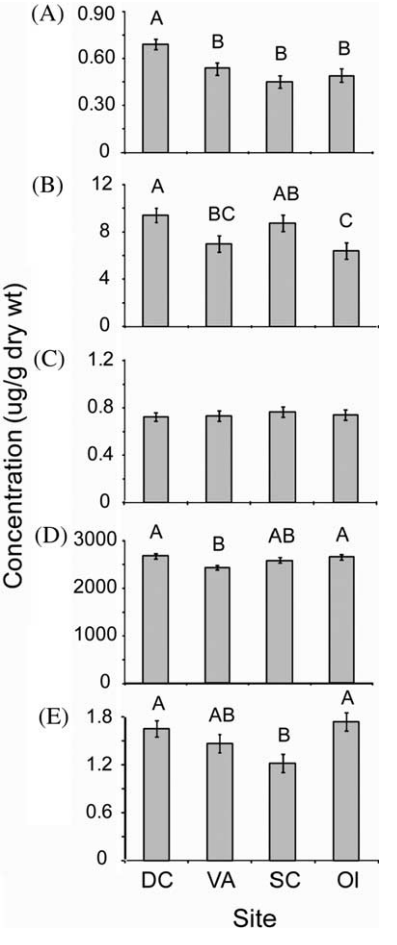

Fig. 6 Mean whole otolith concentrations of (a) Li, (b) Mg, (c) Cu, (d) Sr and (d) Ba for sea perch from four sites in Fiordland (Deep Cove, Doubtful Sound (DC); Vancouver Arm, Breaksea Sound (VA); Only Islands, Long Sound (OI); Shark Cove, Dusky Sound (SC)). Letters indicate significant differences from a Tukey's pairwise test. Error bars are 1 standard error.

same result was obtained using both Welch ANOVA and standard ANOVA, so results from the latter are displayed.

Stepwise QDFA indicated that there was significant separation among sites based on whole otolith concentrations of Li, Ba, Sr and Mg. The first DF accounted for 55% of the total variance and the second and third DFs for 31% and 14%, respectively. The group centroids for each site differed significantly (Wilk's $\lambda = 0.346$, $F_{12,156} = 6.426$, $P < 0.0001$) and 95% confidence ellipses around the group centroids indicated that whole otolith elemental fingerprints of sea perch from Deep Cove and Shark Cove were significantly different from those of fish from the other two sites (Fig. 7). *F-to-remove* values were highest for Li and Mg, suggesting that these elements were most useful for separating sites, with concentrations of Li highest in Deep Cove and Mg lowest in Long and Breaksea Sounds ($P < 0.05$, Tukeys) (Fig. 6 and 7). Jack-knifed QDFA correctly assigned 76% of otoliths to collection site. Classification success was highest for individuals from Only Islands and lowest for Shark Cove (80% and 73% respectively) (Table 4). Classification success rates were significantly greater than those expected by chance (chi-square test, $\chi^2 = 61.2$, df = 3, $P < 0.00001$).

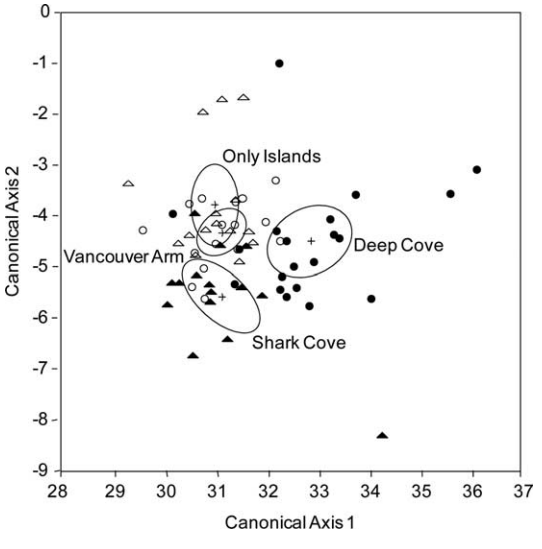

Fig. 7 Canonical plot scores and 95% confidence ellipses from stepwise quadratic discriminant function analysis of whole otolith elemental fingerprints of sea perch from four sites in Fiordland, New Zealand (Deep Cove, Doubtful Sound (closed circles); Vancouver Arm, Breaksea Sound (open circles); Only Islands, Long Sound (open triangles); Shark Cove, Dusky Sound (closed triangles)). Site labelled circles correspond to a 95% confidence limit for the group's multivariate mean. Significantly different groups have non-intersecting circles.

Discussion

The results of this study indicate that adult sea perch, *H. percoides*, comprise relatively distinct subpopulations within the inner fjord basins of Doubtful, Breaksea, Dusky and Long Sounds. The resulting pattern in subpopulation structure suggests that, as adults, sea perch probably have fidelity for habitats in individual fjord basins and may be subject to energetic differences among local food webs. This divided stock structure is revealed by distinct patterns in morphology, length-at-age, $\delta^{13}C$ and $\delta^{15}N$ of muscle tissue, and trace elemental fingerprints of whole otoliths, at the scale of individual fjords.

The subpopulations from the Only Islands in Long Sound and from Shark Cove in Dusky Sound were characterised by distinctly larger eyes than those at the other two sites. Subpopulations from the Only Islands also had larger heads compared to other sites. Subpopulations in these regions also have particularly depressed growth conditions with relatively small asymptotic sizes (Lewis 2008). This pattern is consistent with local conditions at these two extremely inner fjord sites where nutritional inputs to the food web may be dominated by estuarine algae and recycling of forest litter (McLeod & Wing 2007; Wing et al. 2008). Isotopic analysis indicates that sea perch from the Only Islands and Shark Cove had $\delta^{13}C$ that is relatively enriched in ^{13}C, a pattern consistent with large inputs of estuarine algae. $\delta^{15}N$ is significantly enriched in ^{15}N in the Only Islands site, a pattern consistent with a higher

Table 4 Classification results of jack-knifed quadratic discriminant function analysis assigning sea perch to four sites in Fiordland, New Zealand based on whole otolith concentrations of Li, Mg, Ba and Sr.

	Deep Cove		Only Islands		Shark Cove		Vancouver Arm	
	Count	(%)	Count	(%)	Count	(%)	Count	(%)
Deep Cove	**15**	**(75)**	1	(5)	3	(15)	1	(5)
Only Islands	1	(7)	**12**	**(80)**	1	(7)	1	(7)
Shark Cove	2	(13)	1	(7)	**11**	**(73)**	1	(7)
Vancouver Arm	2	(13)	1	(6)	1	(6)	**12**	**(75)**

Rows denote collection site of specimens; columns indicate analysis classifications. Correct classifications are in bold.

trophic position and distinct structure of the local food web (Wing et al. 2004). Because $\delta^{13}C$ and $\delta^{15}N$ have half-lives of the order of years for marine fish (Suring & Wing 2009), spatial patterns in these markers can indicate site fidelity or lack of strong mixing for these populations (Hobson 1999; Rodgers & Wing 2008). Significant differences in the stable isotopic composition of sea perch from Only Islands to those from Deep Cove and Vancouver Arm suggest that there is little exchange of individuals between these sites over relatively short time scales. Similarities in the stable isotopic composition of sea perch between the remaining sites could suggest that there is movement of adults between these sites, but may also be reflective of similarities in the isotopic composition of food sources at these sites.

The observed plasticity in growth and morphology among adjacent populations of sea perch highlights the importance of local habitat quality and food resources for subpopulation structure (Paul & Horn 2009). These differences probably reflect extrinsic environmental influences on growth that act over the lifetime of the individual, rather than genetic divergence among substocks (Lewis 2008). High rates of classification success for morphology and trace elemental composition of whole otoliths, suggest that on the temporal scale of the lifetime of a sea perch (10–20 years) there is relatively little mixing of adults among these four fjord basin habitats. In support of this, tagged adults have been caught from within 20 m of their release site in Deep Cove, after more than 2 years at liberty (Lewis 2008). Unique whole otolith elemental fingerprints, such as those for Deep Cove and Shark Cove, imply that these fish have been exposed to similar environmental conditions for a large proportion of their lifetime (Campana et al. 2000), suggesting long-term site fidelity. While overlapping fingerprints, such as for Vancouver Arm and Only Islands, could indicate exchange between these areas, given the differences observed in the other three markers studied, it is more probably related to a lack of long-term differences in water chemistry at these sites.

The coincidence of fjord level differences in growth, morphology, $\delta^{13}C$ and $\delta^{15}N$ of muscle tissue and whole otolith elemental fingerprints provides strong evidence that there is limited exchange of adults between these four sites over both short and long time scales. These results highlight the probable subdivided nature of adult sea perch populations within Fiordland and raise several key questions about stock structure and management of this important member of the Fiordland reef fish fauna.

Marine protected areas with exclusion of commercial fishing, implemented in the Fiordland Marine Management Act 2005, were designed to include regions in each of the fjords to account for the likely subdivided nature of populations among fjords across the landscape. There are 14 commercial exclusion zones within Fiordland with a total area of 46,002 ha or 59% of inner fjord habitat (Wing et al. 2004). Additionally 10 marine reserves are distributed

throughout the fjords with a total area of 10,241 ha or 13% of inner fjord habitat (Wing et al. 2004). Areas outside of the marine reserves and commercial exclusion zones are designated 'fully fished' and are managed under the individual transferable quota system and special regulations on recreational take within the Fiordland Marine Area. The present study demonstrates that sea perch, *H. percoides*, probably exist as relatively discrete subpopulations among fjord basins and therefore are encompassed by the spatial management units within the Fiordland system. Long Sound is a fully protected marine reserve, while the inner reaches of Doubtful Sound shares this status. The inner basins of Dusky and Breaksea Sounds are designated commercial exclusion zones, but recreational and customary fishing is allowed. The dynamics and persistence of each of the observed discrete adult subpopulations of *H percoides* is probably influenced by patterns in connectivity via dispersal of larvae or juveniles. However, as larval dispersal from sources outside each fjord basin is expected to be low (Ostrow et al. 2001; Sköld et al. 2003; Perrin et al. 2003, 2004), these sub-populations may be highly vulnerable to localised depletion.

The present study provides an important example of divided adult population structure within a coastal marine fish with high potential for both adult and larval dispersal. This finding deviates strongly from the assumption that coastal marine fish populations are made up of dynamic pools of individuals and that their population dynamics, and responses to management, reflect large-scale population units or stocks (Kritzer & Sale 2006). In this case, adult sea perch are probably long-term, or permanent, residents within individual fjord basins and their energetics, as revealed by patterns in growth and morphology, are strongly linked to the local environment. A corollary is that differences in habitat quality and local food web structure will most likely strongly influence vital rates, and yield, of these individual subpopulations. This system highlights the important influence of adult site fidelity and the energetics of local food webs for maintenance of abundance and distribution across subdivided landscapes, which has important general consequences for understanding the effects of spatial management of both fisheries and biological diversity within Fiordland, and other coastal systems.

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References

Agnew DJ 1988. Evidence for the existence of two populations of Irish Sea cod (*Gadus morhua* L.) from consideration of growth rates. International Council for the Exploration of the Seas, No. CM 1988/G:65, Copenhagen.

Begg G, Campana SE, Fowler A, Suthers I 2005. Otolith research and application: current directions in innovation and implementation. Marine and Freshwater Research 56: 477–483.

Begg G, Waldman J 1999. A holistic approach to fish stock identification. Fishery Research 43: 35–44.

Block BA, Teo SLH, Walli A, Boustany A, Stokesbury MJW, Farwell CJ, Weng KC, Dewar H, Williams TD 2005. Electronic tagging and population structure of Atlantic bluefin tuna. Nature 434: 1121–1127.

Bolle L, Hunter E, Rijnsdorp A, Pastoors M, Metcalfe J, Reynolds J 2005. Do tagging experiments tell the truth? Using electronic tags to evaluate conventional tagging data. ICES Journal of Marine Science 62: 236–246.

Campana SE 1995. Graphical and statistical methods for determining the consistency of age determinations. Transactions of the American Fisheries Society 124: 131–138.

Campana SE 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine Ecology Progress Series 188: 263–297.

Campana SE 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology 59: 197–242.

Campana SE, Chouinard G, Hanson J, Frechet A, Brattey J 2000. Otolith elemental fingerprints as biological tracers of fish stocks. Fishery Research 46: 343–357.

Carbines G, McKenzie J 2004. Movement patterns and stock mixing of blue cod in Dusky Sound in 2002. Wellington, Ministry of Fisheries.

Cowen R, Lwiza K, Spongaugle S, Paris C, Olson D 2000. Connectivity of marine populations: open or closed? Science 287: 857–859.

Crowder L, Lyman S, Figuerina W, Priddy J 2000. Source-sink population dynamics and the problem of siting marine reserves. Bulletin of Marine Science 66: 799–820.

DeNiro M, Epstein S 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42: 495–506.

DeNiro M, Epstein S 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta 45: 341–351.

Forgarty M 1998. Implications of migration and larval interchange in American lobster (*Horus americanus*) stocks: spatial structure and resilience. In: Jamieson G, Campbell A eds North Pacific Symposium on Invertebrate Stock Assessment and Management, Naniamo. Pp. 273–283.

Francis M, Ling N 1985. Abundance of reef fish in Doubtful and Thompson Sounds, Fiordland. Wellington, New Zealand Oceanographic Institute, Fisheries Resource Division.

Gillanders B, Kingsford M 2000. Elemental fingerprints of otoliths of fish may distinguish estuarine 'nursery' habitats. Marine Ecology Progress Series 201: 273–286.

Grange K, Singleton R, Richardson J, Hill P, del Main W 1981. Shallow rock-wall biological associations of some southern fiords of New Zealand. New Zealand Journal of Zoology 8: 209–227.

Hesslein R, Hallard K, Ramlal P 1993. Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (Coregonus nasus) in response to a change in diet traced by $\delta^{34}S$, $\delta^{13}C$ and $\delta^{15}N$. Canadian Journal of Fisheries and Aquatic Sciences 50: 2071–2076.

Hixon M, Pacala S, Sandin S 2002. Population regulation: historical context and contemporary challenges of open vs. closed systems. Ecology 83: 1490–1508.

Hobson K 1999. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120: 314–326.

Jones G, Milicich M, Emslie M, Lunow C 1999. Self-recruitment in a coral reef fish population. Nature 402: 802–804.

Kalish J 1991. Determinants of otolith chemistry: seasonal variation in the composition of blood plasma, endolymph and otoliths of bearded rock cod *Pseudophysis barbatus*. Marine Ecology Progress Series 74: 137–159.

Kelly C, Connolly P, Bracken J 1999. Age estimation, growth, maturity, and distribution of the bluemouth rockfish *Helicolenus d. dactylopterus* (Delaroche 1809) from the Rockall Trough. ICES Journal of Marine Science 56: 61–74.

Kritzer J, Sale P 2006. Marine metapopulations. London, Elsevier Academic Press.

Lewis AM 2008. Connectivity of sea perch, *Helicolenus percoides*, populations in southern New Zealand. MSc thesis, University of Otago, Dunedin.

McLeod R, Wing S 2007. Hagfish in the New Zealand fjords are supported by chemoautotrophy of forest carbon. Ecology 88: 809–816.

Ostrow D, Wing S, Mladenov P, Roy M 2001. Genetic differentiation of *Terebratella sanguinea* in the New Zealand fjords: a dispersal barrier in the marine environment? In: Howard C, Brunton C, Robin L, Cocks M, Long S ed. Brachiopods past and present. London, The Natural History Museum.

Paul L 1998. A summary of biology and commercial landings, and a stock assessment of sea perches, *Helicolenus* spp. (Scorpaenidae) in New Zealand waters. Wellington, Ministry of Fisheries.

Paul L, Horn P 2009. Age and growth of sea perch (*Helicolenus percoides*) from two adjacent areas off the east coast of South Island, New Zealand. Fisheries Research 95: 169–180.

Paulin CD, Stewart A, Roberts C, McMillan P 2001. New Zealand fish: a complete guide. Wellington, Te Papa Press. P. 279.

Perrin C, Roy M, Wing S 2003. Genetic differentiation amongst populations of the sea urchin Evechinus chloroticus and the sea star *Coscinasterias muricata* in New Zealand's fjords. In: Féral J, David B ed. Echinoderm research. Rotterdam, Balkema.

Perrin C, Wing S, Roy M 2004. Population genetic structure amongst populations of the sea star *Coscinasterias muricata* in the New Zealand fjords. Molecular Ecology 13: 2183–2195.

Peterson B, Fry B 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18: 293–320.

Reist J 1985. An empirical evaluation of several univariate methods that adjust for size variation in morphometric data. Canadian Journal of Zoology 63: 1429–1439.

Rodgers K, Wing S 2008. Spatial structure and movement of blue cod (*Parapercis colias*) in Doubtful Sound, New Zealand, inferred from $\delta^{13}C$ and $\delta^{15}N$. Marine Ecology Progress Series 359: 239–248.

Roelke L, Cifuentes L 1997. Use of stable isotopes to assess groups of king mackerel, *Scomberomorus cavalla*, in the Gulf of Mexico and southeastern Florida. Fishery Bulletin 95: 540–551.

Sale P, Cowen R, Danilowicz B, Jones G, Kritzer J, Lindeman K, Planes S, Polunin N, Russ G, Sadovy Y, Steneck R 2005. Critical science gaps impede use of no-take fishery reserves. Trends in Ecology and Evolution 20: 74–80.

Silva A 2003. Morphometric variation among sardine (*Sardina pilchardus*) populations from northeastern Atlantic and western Mediterranean. ICES Journal of Marine Science 60: 1352–1360.

Sköld M, Wing S, Mladenov P 2003. Genetic subdivision of the sea star *Coscinasterias muricata* in the fjords of New Zealand. Marine Ecology Progress Series 250: 163–174.

Stanton B, Pickard G 1981. Physical oceanography of the New Zealand fiords. New Zealand Oceanographic Institute Memoir 88: 3–37.

Suring E, Wing S 2009. Isotopic turnover rate and fractionation in multiple tissues of red rock lobster (*Jasus edwardsii*) and blue cod (*Pararcis colias*): Consequences for ecological studies. Journal of Experimental Marine Biology and Ecology (in press).

Swearer SE, Shima JS, Hellberg ME, Thorrold SR, Jones GP, Robertson DR, Morgan SG, Selkoe KA, Ruiz GM, Warner RR 2002. Evidence of self-recruitment in demersal marine populations. Bulletin of Marine Science 70: 251–271.

Taylor M, Hellberg M 2003. Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. Science 299: 107–109.

Thorrold S, Latkoczy C, Swart P, Jones C 2001. Natal homing in a marine fish metapopulation. Science 291: 297–299.

Thresher R 1999. Elemental composition of otoliths as a stock delineator in fishes. Fisheries Research 43: 165–204.

Wing S, Bowman M, Smith F, Rutger S 2004. Analysis of biodiversity patterns and management decision making processes to support stewardship of marine resources and biodiversity in Fiordland—a case study (Report 2 of 3). Wellington, Ministry for the Environment.

Wing S, McLeod R, Clark K, Frew R 2008. Plasticity in diet of two echinoderm species across an ecotone: microbial recycling of forest litter and bottom-up forcing of population structure. Marine Ecology Progress Series 360: 115–123.