Variation with Depth in Temperate Seagrass-Associated Fish Assemblages in Southern Victoria, Australia

Neil Hutchinson · Gregory P Jenkins · Andrew Brown · Timothy M Smith

Abstract Variability in the abundance and distribution of seagrass-associated fish assemblages was examined at different depths in a temperate bay in southern Australia. Depth differences in seagrass-associated fish assemblages are poorly known but this information is critical given that seagrass loss can occur at specific depths depending on the cause. Overall, 69 species of fish from 26 families were recorded, with higher species richness in shallow than deep beds, with 12 species found only in deep beds and 22 species found only in shallow beds. While the total fish abundance (i.e. abundance of all species recorded) varied between years and seasons, and to some extent between sites, it was significantly higher in shallow than deep seagrass beds in the majority of cases. Although there was some variation between sites, seagrass tended to be longer and have a higher biomass in shallow than deep beds during both spring and autumn throughout the study. A positive relationship between seagrass biomass/length and total fish abundance/species richness was apparent. Assemblage structure tended to be distinct at each depth, with the largest species recorded in shallow seagrass. Large numbers of small schooling fish, such as atherinids, dominated in shallow seagrass but were not found in deep seagrass. Loss of seagrass could therefore have varying implications for distinct assemblages found at different depths.

Keywords Seagrass habitats · *Zostera nigricaulis* · Port Phillip Bay · Nursery

Communicated by David J. Booth

N. Hutchinson · G. P. Jenkins · A. Brown · T. M. Smith
Fisheries Research Branch, Department of Primary Industries, DPI Queenscliff centre, P.O. Box 114, Queenscliff 3225, VIC, Australia

N. Hutchinson (✉)
JCU Singapore, TropWATER—Centre for Tropical Water and Aquatic Ecosystem Research, James Cook University, 600 Upper Thomson Road, Singapore, Singapore 574421
e-mail: neil.hutchinson@jcu.edu.au

G. P. Jenkins
Department of Zoology, University of Melbourne, Melbourne 3010, VIC, Australia

T. M. Smith
Centre for Integrative Ecology, Deakin University, Waurn Ponds, VIC, Australia

Present Address:
A. Brown
Fish Ageing Services, 28 Swanston St, Queenscliff 3225, VIC, Australia

Introduction

Much has been written on the significance of seagrass beds (Larkum et al. 2006), including their provision of important ecosystem services (Barbier et al. 2011; Lavery et al. 2013). Costanza et al. (1998) proposed that, globally, seagrass beds provide ecosystem services equivalent to over US$28,000 ha^{-1} year^{-1} and estimates in South Australia (McArthur and Boland 2006) suggest that secondary production contributions from seagrass in the State's gulf waters alone contribute over AU$134,000,000 year^{-1} to the economy (values quoted for these two studies have been updated taking inflation up to 2012 into account). Seagrass is, however, disappearing from coastal areas at an increasingly rapid rate (Waycott et al. 2009). This is of particular concern given that seagrass beds provide important habitat and food resources for a wide and diverse range of organisms in both temperate and tropical regions (Howard et al. 1989; Bologna and Heck 1999; Jackson et al. 2006a).

Approximately one third of the world's seagrass is thought to have disappeared over the last 130 years (Waycott et al. 2009). This is due to a wide range of natural and anthropogenic

impacts such as declining water quality and coastal development (Shepherd et al. 1989; Orth et al. 2006; Grech et al. 2012) and loss of seagrass beds is expected to be further exacerbated by climate change (Connolly 2009).

It is important to understand how declines in seagrass at different depths will impact associated assemblages, given their ecological and economic importance (Duarte 2002). A wide range of fish species utilise seagrass beds, some residing in them throughout their life history, and others only using them during specific life stages or as food patches in a mosaic of varied habitat types (Pollard 1984). This results in seagrass ecosystems often supporting disproportionally higher abundances and diversity of fish than adjacent coastal habitats (Travers and Potter 2002).

As concern grows about potential impacts of habitat loss on fish populations (Engel et al. 1999), more evidence is emerging on specific impacts of seagrass loss, indicating that in many cases these fish are unable to adapt to such changes (Hughes et al. 2002; Nakamura 2010). In the event of seagrass loss, it is likely that impacts on fish assemblages will be variable and dependent on a range of factors including the depth of seagrass beds that they utilise and the extent to which species are tied to only one habitat type (Jenkins and Wheatley 1998; Jenkins and Hamer 2001; Deudero et al. 2008). Those studies that have examined variation in assemblage structure

of seagrass-associated fish at different depths (e.g. Francour 1997; Travers and Potter 2002; Heithaus 2004; Pogoreutz et al. 2012) have, however, tended to identify one of two contrasting patterns. Either abundance, diversity and size are found to be higher in deep seagrass beds (Bell et al. 1992; Heithaus 2004; Jackson et al. 2006b) or the opposite scenario is described where they are higher in shallow seagrass beds (Francour 1997; Travers and Potter 2002). Information regarding such depth differences in cool, temperate systems is sparse (Jackson et al. 2006b; Smith et al. 2011).

In the present study, we investigate patterns of spatial and temporal variation in fish assemblages associated with beds of *Zostera nigricaulis* in Port Phillip Bay, south-eastern Australia. The main aim of the work presented is to identify the extent to which these assemblages vary with depth. Additional, limited analysis is also performed to identify potential relationships between fish abundance/species richness and seagrass length/biomass.

Materials and Methods

Study Site Fish assemblages (finfish and elasmobranchs) were sampled around low tide, slack water in shallow (<1 m) and

Fig. 1 Map of sampling sites for seagrass-associated fish in Port Phillip Bay, Victoria, Southern Australia
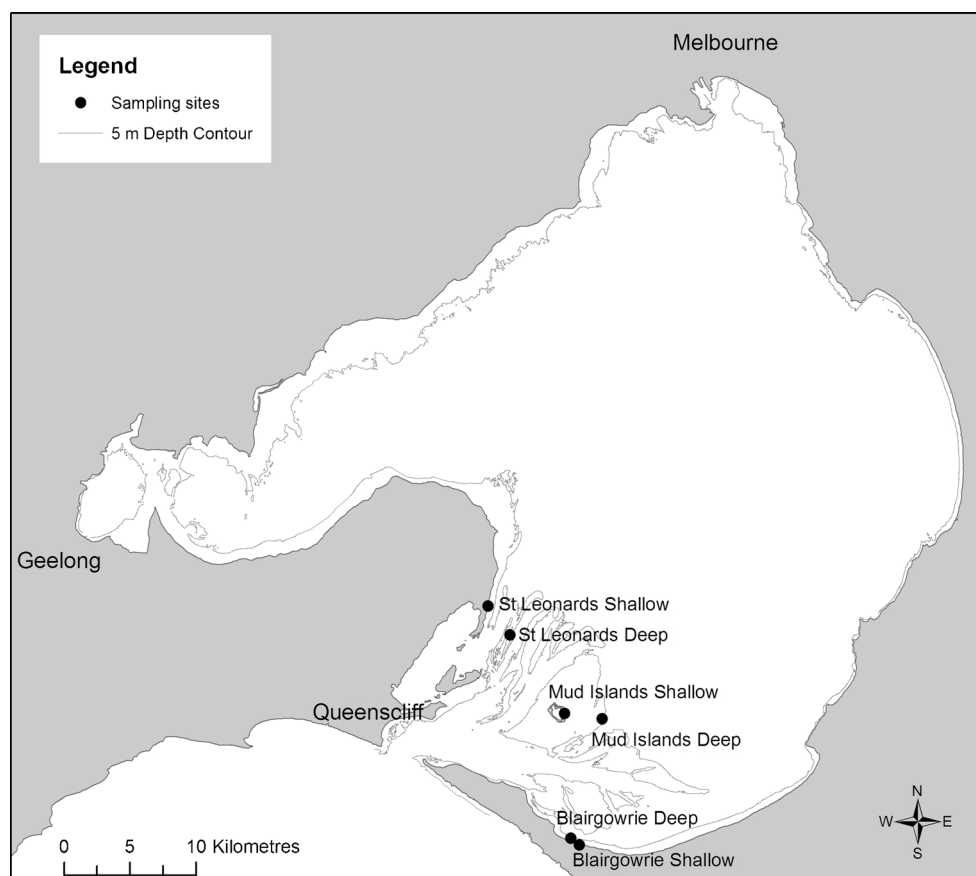
Table 1 Summary of mean values (± SE) of fish abundance and species richness by depth, site and sampling time

		Autumn 2008	Spring 2008	Autumn 2009	Spring 2009	Autumn 2010	Spring 2010
Total fish abundance							
Deep	Blairgowrie	24.25±3.82	18±2.89	26.75±14.29	14.25±6.41	13.25±8.06	45.25±23.53
	Mud Islands	21.25±11.10	21.25±4.19	21±8.06	10±5.017	4.75±2.32	3.75±1.75
	St Leonards	21.25±7.92	12±3.34	12.25±1.89	60.25±21.42	5.5±1.85	3.25±0.63
Shallow	Blairgowrie	122±43.84	488.25±309.73	161.25±57.92	142±49.64	216.5±31.01	56.25±16.85
	Mud Islands	327±20.22	38.5±6.06	121.75±53.44	130.75±61.08	259.25±74.22	288.25±43.17
	St Leonards	53.5±16.14	75.25±44.63	580.75±140.24	21.75±6.38	169.25±53.51	51.25±11.46
S. nigra abundance							
Deep	Blairgowrie	6.25±2.016	3.5±0.65	4.75±3.15	0.75±0.48	7±6.014	1±0.71
	Mud Islands	4±1.35	0	5.75±1.89	0	2.25±1.11	0
	St Leonards	3±1.73	0.5±0.29	9.75±5.66	8.25±4.50	0.5±0.29	0.5±0.5
Shallow	Blairgowrie	55.75±31.55	20.5±8.01	53±13.20	4±2.45	5.5±2.40	17±7.63
	Mud Islands	119±62.97	3±1.58	92.5±31.34	35.75±24.06	76.75±26.19	1.25±0.48
	St Leonards	22.75±11.03	0.75±0.25	78.75±42.49	2.25±1.60	123±46.72	5.75±2.50
A. spilomelanurus abundance							
Deep	Blairgowrie	0.25±0.25	4±1.78	0	8.5±4.03	0	34±19.74
	Mud Islands	7.25±4.23	19.25±4.09	2±1.41	6.25±3.94	0	1.75±1.11
	St Leonards	4.75±2.75	0±0	0±0	1.25±1.25	1.25±0.95	0.5±0.5
Shallow	Blairgowrie	1±0.41	2.75±1.38	5.5±2.90	0	6.25±3.71	1.5±0.96
	Mud Islands	0.75±0.75	0	2.5±2.18	0.75±0.48	1±1	0
	St Leonards	0.25±0.25	0	0.25±0.25	0	1.25±0.75	0
Species richness							
Deep	Blairgowrie	7.25±0.85	8.25±0.95	3.5±1.26	4.25±0.489	3±1.08	4.75±1.38
	Mud Islands	6.25±1.65	2.25±0.25	6±1.58	3.25±0.48	2±0.91	2±0.71
	St Leonards	6.75±1.49	4.75±0.75	5.25±0.48	9.5±1.5	3.75±1.11	2.75±0.25
Shallow	Blairgowrie	10±1.47	11±1.83	8.5±1.19	10.5±1.32	13.25±2.06	10.25±1.31
	Mud Islands	7.25±0.85	5.5±0.87	10.5±1.85	13±2.04	13.75±1.11	7.5±0.5
	St Leonards	7±0	7.5±1.66	8±0.41	9±2.04	16±1.78	9.5±0.87

deep (2–8 m) seagrass beds at Blairgowrie (38°21′S, 144°46′E), Mud Islands (38°16′S, 144°44′E) and St Leonards (38°11′S, 144°42′E); three sites situated in the south of Port Phillip Bay, a large embayment in Victoria, temperate southern Australia (Fig. 1). The bay is characterised by mean annual surface seawater temperatures from ~10–20 °C, a tidal range of ~0.9–1.7 m and mean annual salinities of ~35.5 g kg^{-1} (King 1970; Blake and Ball 2001; Lee et al. 2012). Patches of the seagrass *Z. nigricaulis* grew as a dense monoculture at these sites as is characteristic of the genus (Moore and Short 2006), with mean canopy heights ranging from ~50–250 mm in deep beds to ~150–400 mm in shallow beds. Total seagrass coverage varied with ~6 % cover at Blairgowrie, ~41 % at Mud Islands and ~13 % at St Leonards (Ball et al. 2009). These beds are known to harbour a wide variety of fish species (Jenkins et al. 1997a, b; Hindell et al. 2001; Smith et al. 2010).

Fish Sampling Fish assemblages were sampled on a biannual basis in autumn (April–May) and spring (November) from 2008–2010. Samples were collected at each site using a bottom weighted, 15×3 m seine net, with wings of 3 mm mesh, a cod-end of 1 mm mesh, and 15 m ropes at each end. The net,

Table 2 Comparison using Kolmogorov–Smirnov two sample tests of length frequency data between fish in shallow and deep seagrass beds during each visit

|	Total fish		*S. nigra*		*A. spilomelanurus*	
	D	P	D	P	D	P
Autumn 2008	0.804	*<0.001*	0.162	0.152	0.403	0.217
Spring 2008	0.786	*<0.001*	0.455	*0.006*	0.624	*0.001*
Autumn 2009	0.890	*0.003*	0.248	*<0.001*	0.667	*0.004*
Spring 2009	0.478	*<0.001*	0.292	*0.014*	0.438	0.719
Autumn 2010	0.967	*<0.001*	0.668	*<0.001*	0.682	*0.022*
Spring 2010	0.962	*<0.001*	0.323	0.574	0.571	*0.045*

Significant differences (*P*<0.05) are shown in italics

stowed in a fish-bin, was carried 15 m from an anchored research vessel to which one of the end ropes was attached, by either wading if in shallow seagrass beds or from a small tender when in deep beds. After setting the net in a straight line perpendicular to the boat, the second end rope was returned to the research vessel. At shallow sites, the net was then hauled in slowly with the bottom of the net dragging along the substrate and the floats at the surface. A modified technique was used for deep beds, with additional weights attached to the net bridles and tow ropes to allow the net to be hauled along the substrate prior to it reaching the surface. It should be noted that when sampling deep beds, the floats at the top of the net were initially in mid water rather than at the surface due to the height of the net. At both depths, the net was hauled in such a way that the bridles were ~4 m apart until the mouth of the net was lifted from the water surface. The sweep area of one trawl was estimated to be approximately 100 m^2. Four random, replicate hauls were taken at both depths at each site. Where possible, fish were identified, measured (standard length) and released in the field. Unidentified

individuals were euthanized by over-anaesthesia in a seawater bath containing 200 mg/L of isoeugenol (AQUI-S™), and preserved in 95 % ethanol for laboratory analysis. All atherinids, potentially including *Atherinosoma microstoma*, *Kestratherina brevirostris*, *Kestratherina esox* and *Leptatherina presbyteroides*, were grouped due to issues related to sorting and identification of large numbers in the field.

Seagrass Sampling Six, 0.25 m^2 quadrats were randomly placed in seagrass beds at both depths at each site, by snorkel or SCUBA where appropriate, and all seagrass was cut at the sediment surface and removed. Average stem/leaf length for each quadrat was determined by randomly measuring the length of ten plants. Biomass was determined after drying seagrass for 2 days at 60 °C.

Data Analysis Spatial and temporal variation in total fish abundance (i.e., abundance of all species recorded), abundance of individual fish species, fish species richness, seagrass length,

Fig. 2 Total proportion of finfish arranged by size class (millimeters) in deep (*dark*) and shallow (*grey*) seagrass beds from Autumn 2008 to Spring 2010. *Stars* indicate the largest size class
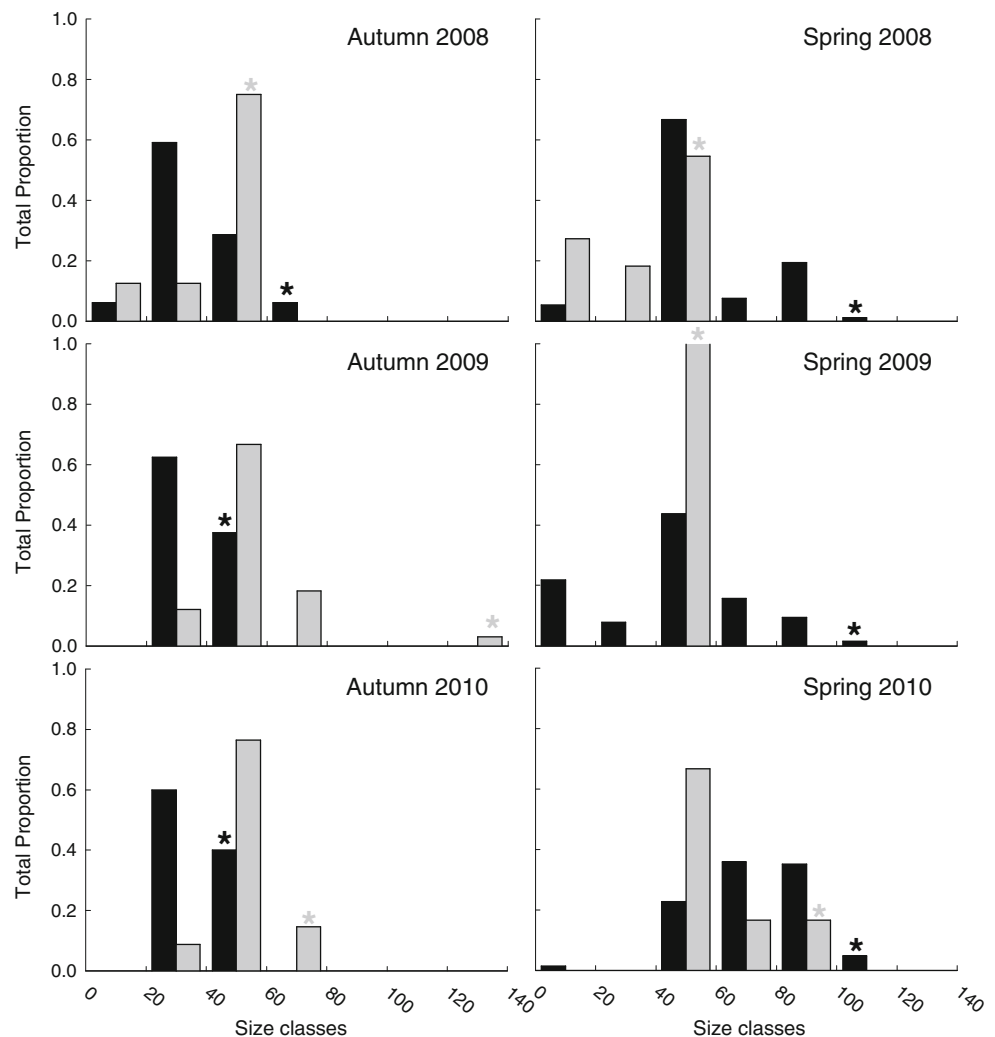
and seagrass biomass were analysed with a four-factor mixed-model ANOVA (Underwood 1997). This analysis was used to examine variation with year (three levels; fixed and orthogonal; i.e. 2008, 2009 and 2010), season (two levels; fixed and orthogonal; i.e. autumn and spring), site (three levels; random and orthogonal; i.e. Blairgowrie, Mud Islands and St Leonards) and depth (two levels; fixed and orthogonal; i.e. shallow and deep). Data for all analyses were checked for homogeneity of variances (Cochran's *C* test) and log transformed where appropriate to satisfy assumptions of homogeneity of variances. Significant differences for fixed factors were examined further, where appropriate, using SNK multiple comparison tests (Zar 1996).

The same four-factor model was applied to a mixed-model permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) to identify significant differences in fish assemblage structure. This analysis was run with 9,999 permutations per test, on data obtained through Bray–Curtis measures of dissimilarity on square root transformed abundance data. Pooling was conducted on terms where the model gave negative estimates for components of

variation. Our primary interests were differences in assemblage structure between depths and between seasons and therefore only those pairwise comparisons that specifically address these factors are reported for the appropriate interactions.

Subsequent relationships were visualised with non-metric multidimensional scaling (nMDS) ordinations, and while stress levels obtained through this analysis should ideally be <0.1, values <0.2 are thought to still provide an adequate representation of relationships between replicates (Clarke 1993). A SIMPER procedure was then used to identify the species that contributed most to treatment differences (Clarke 1993). Further analysis was conducted on raw data using Kolmogorov–Smirnov two sample tests to identify differences in the size frequencies of finfish with depth and season.

To examine relationships between seagrass and fish, data from the whole study was pooled and linear regression analysis was employed to assess any influence of seagrass length and biomass on fish abundance, species richness and common species.

Fig. 3 Total proportion of *S. nigra* arranged by size class (millimeters) in deep (*dark*) and shallow (*grey*) seagrass beds from Autumn 2008 to Spring 2010. *Stars* indicate the largest size class
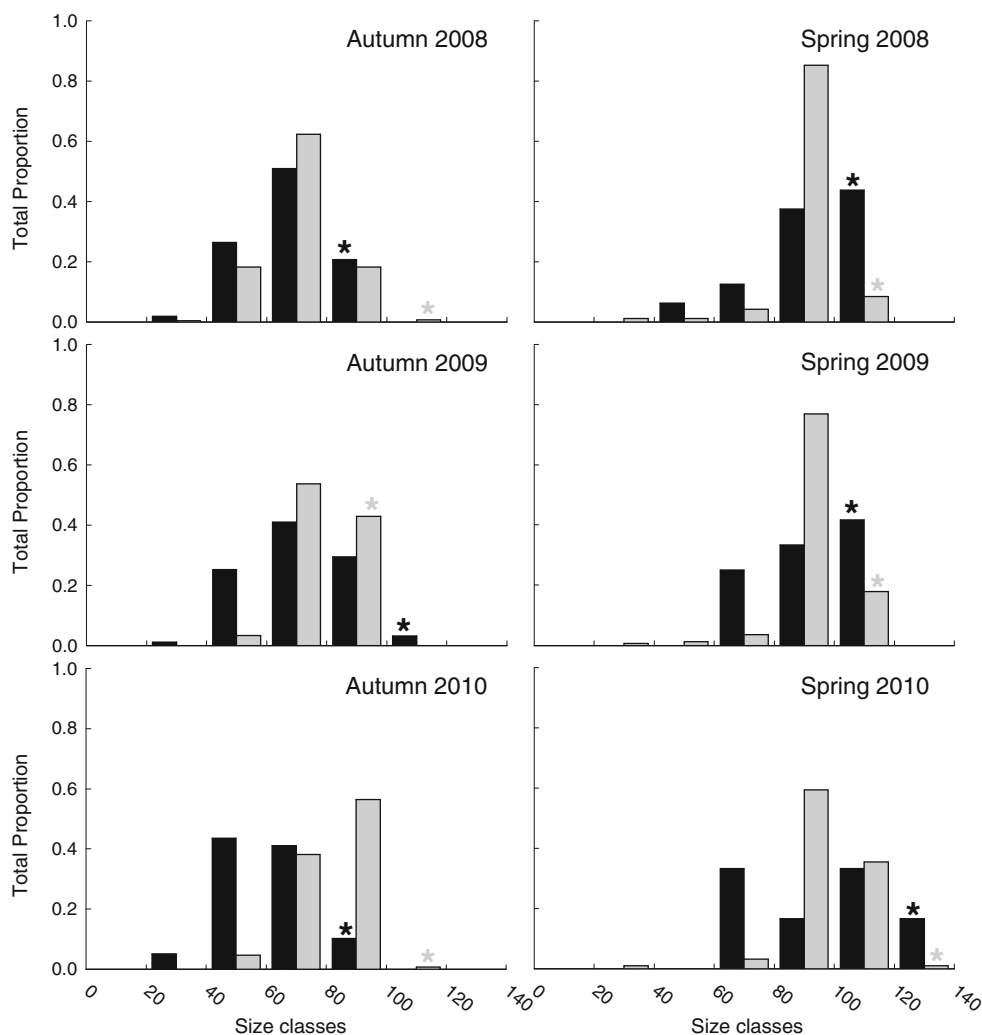
Results

A rich and diverse fish assemblage was found in all seagrass beds that were sampled. Sixty nine species from 26 families were recorded throughout the study, the majority from multiple samples, with 12 found only in deep beds (e.g. the callionymids *Eoacallionymus papilio* and *Foetorepus calauropomus*), 22 found only in shallow beds (e.g. the Gobiid *Afurcagobius tamarensis* and the sillaginid *Sillaginodes punctatus*), and 35 found at both depths (e.g. the monacanthid *Acanthaluteres spilomelanurus* and the sygnathid *Stigmatopora nigra*). While some of the species occurred at only one or two of the sites, the majority of common species were found at all sites (see additional data in Online Resource).

Fish Abundance and Species Richness

Total fish abundance was significantly higher in shallow than deep seagrass beds during the majority of visits (except at St Leonards in autumn 2008 and spring 2009, Mud Islands in spring 2008 and Blairgowrie in spring 2010; Table 1; Online Resource; $Y \times Se \times Si \times De$: $F_{4, 108} = 3.9144$, $P < 0.001$). Seasonal differences in total fish abundance in shallow seagrass beds were inconsistent, but in all cases where significant differences occurred (at Mud Islands in 2008, St Leonards in 2009, and Blairgowrie in 2010) abundances were higher in autumn than in spring (Table 1). Total fish abundance in deep seagrass beds did not vary seasonally except on rare occasions (Table 1). Significant differences in abundance between years were infrequent, with no consistent pattern, and are not discussed any further (Table 1).

Variation in abundance with depth could also be seen for individual species. The commonly occurring sygnathid, *S. nigra* ($Y \times Se \times Si \times De$: $F_{4, 108} = 2.79$, $P = 0.030$) was significantly more abundant in shallow than deep beds at the majority of sites during autumn of each year (except at Blairgowrie in 2010; Table 1) and less commonly during spring (only at Blairgowrie in 2008 and 2010, and at Mud Islands in 2009). Abundances of *S. nigra* were significantly higher in autumn

Fig. 4 Total proportion of *A. spilomelanurus* arranged by size class (millimeters) in deep (*dark*) and shallow (*grey*) seagrass beds from Autumn 2008 to Spring 2010. *Stars* indicate the largest size class
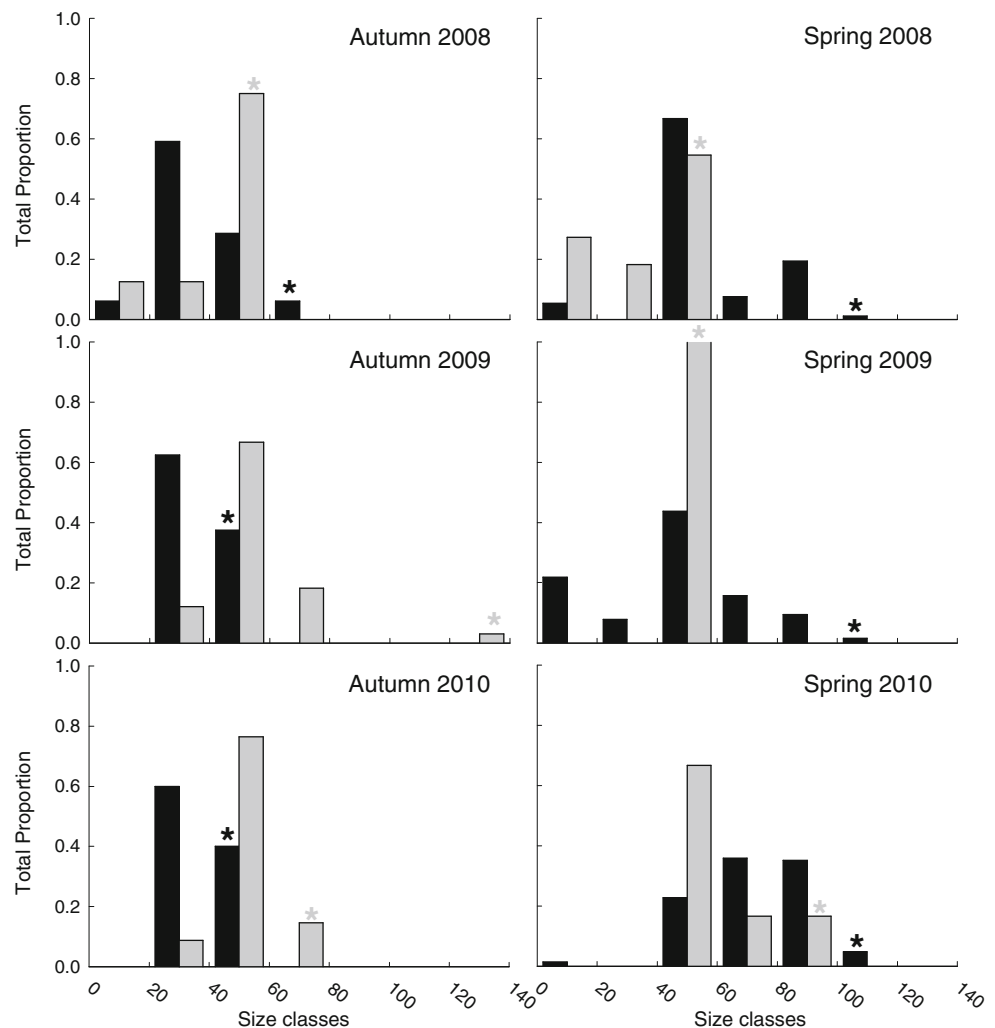
than spring each year in the majority of shallow seagrass beds (except at Blairgowrie in 2008 and 2010), but rarely in deep beds (only at Mud Islands in 2009). Significant differences in abundance between years, where they occurred, were inconsistent with no apparent pattern.

Abundances of the monocanthid, *A. spilomelanurus* (Se× Si×De: $F_{2, 108}$=8.63, P <0.001; Table 1) also varied significantly with depth and season but patterns were inconsistent (Table 1). This species was more abundant in shallow than deep beds at Blairgowrie during autumn and more abundant in deep than shallow beds in spring at Blairgowrie and Mud Islands (Table 1). Abundances were significantly higher in autumn than spring in deep beds at Blairgowrie (Table 1) and more abundant in spring than autumn in shallow beds at Blairgowrie and Mud Islands (Table 1).

Species richness rarely showed any significant difference between depths, seasons or years (Y×Se×Si×De: $F_{4, 108}$= 2.72, P =0.033; Table 1).

Fish Size Frequencies

Fish lengths were significantly different between shallow and deep seagrass beds during all visits (Table 2). These differences were predominantly due to two observed patterns. Firstly, the largest fish recorded tended to be caught by the seine nets in shallow seagrass (Fig. 2), and included a range of species such as the monacanthid, *Meuschenia freycineti*, and the tetraodontids *Contusus brevicaudus* and *Tetractenos glaber*. Other large species including platycephalids, rhombosolids and several species of elasmobranch tended to be few in number and were recorded infrequently at both depths. Secondly, certain species occurred at one depth only. For example, during spring 2008 and 2009 large numbers of *S. punctatus* recruited only to shallow seagrass beds, as indicated by a peak of fish in the 20–40 mm size range while during the same periods the gobiesocid, *Genus C sp. 1* (Gomon et al. 2008) generally recorded in the 0–20 mm size class, was found only in deep seagrass beds.

Differences were also apparent for individual species. For example, *A. spilomelanurus* and *S. nigra* showed significant differences between depths on the majority of visits (Table 2; Figs. 3 and 4). In autumn each year, a higher proportion of larger *A. spilomelanurus* (over 40 mm) were found in shallow than deep seagrass, whereas in spring the largest individuals (over 80 mm) were recorded in deep seagrass, where a broader size range including new recruits (<20 mm) were also found. While *S. nigra* did not show such distinct differences, in autumn each year, a higher proportion of larger individuals (80 mm and over) tended to be recorded in shallow than deep seagrass. In spring each year, the highest proportion of individuals in shallow seagrass were in the 80–100 mm size range, while in deeper seagrass there was a higher proportion of individuals over 100 mm.

Assemblage Structure

Fish assemblage structure was significantly different between shallow and deep seagrass beds in each season and year for the three sites (Table 3). Four-factor PERMANOVA revealed significant interactions between year, season, site and depth, with pairwise comparisons indicating that assemblage structure was significantly different between autumn and spring at both depths during each year for the majority of sites (except in deep seagrass in 2010 at Mud Islands and in 2008 and 2010 at St Leonards; Table 3). nMDS ordinations for each individual sampling visit also showed clear differences in the similarity of assemblage structure in deep and shallow seagrass beds throughout the study, with differences between sites in some instances, e.g. autumn 2010 (Fig. 5). Further analysis with SIMPER indicated that the species which made the greatest contributions to variation between depths varied between sampling visits and included: the gobiids *A. tamarensis* and *Nesogobius maccullochi*, atherinids, the sygnathids *Stigmatopora argus* and *S. nigra*, the monacanthid *A. spilomelanurus*, the tetradontid *C. brevicaudus*, the clinid *Heteroclinus perspicillatus*, and the sillaginid *S. punctatus* (Table 4).

Seagrass bed Dynamics and Possible Relationship with Fish

In most cases, seagrass was significantly longer in shallow than deep beds at each site during autumn, but only

Table 3 Summary of four-factor PERMANOVA results based on Bray–Curtis distances, comparing fish assemblage structure across years (Y), seasons (Se), sites (Si) and depths (De)

Source	df	MS	Pseudo-F	p(perm)
Y	2	4,840.3	0.9601	0.566
Se	1	22,484	3.2990	0.108
Si	2	9,889.9	7.2504	*0.001*
De	1	69,307	7.4669	0.1
Y×Se	2	3,341.8	1.0634	0.459
Y×Si	4	5,041.6	3.6960	*0.001*
Ye×De	2	6,793.1	1.7254	0.166
Se×Si	2	6,815.4	4.9965	*0.001*
Se×De	1	14,338	2.2328	0.209
Si×De	2	9,281.8	6.8046	*0.001*
Y×Se×Si	4	3,142.6	2.3039	*0.001*
Y×Si×De	4	3,937.1	2.8863	*0.001*
Se×Si×De	2	6,421.7	4.7079	*0.001*
Pooled	6	3,685.9	2.7022	*0.001*
Res	108	1,364		

Significant differences (P <0.05) are shown in italics

Estimates of components of variation were greatest at the level of individual replicate samples (36.9 %) followed by depth (28.9 %)

occasionally during spring each year (Y×Se×Si×De: $F_{4, 180}$=5.94, P<0.001; Fig. 6). In the majority of cases, there were no significant differences in seagrass length between seasons or years at either depth (Fig. 6).

Seagrass biomass was also significantly higher in shallow than deep beds in the majority of cases (Y×Si×De: $F_{4, 180}$=5.19, P<0.001; Se×Si×De: $F_{2, 180}$=6.66, P=0.002; Fig. 7). There were few seasonal differences in biomass in shallow or deep beds, but where such differences were recorded, biomass was higher in spring than autumn (Fig. 7). There were no consistent differences in biomass between years.

Significant positive relationships between species richness and both seagrass length ($F_{1, 34}$=7.770, P=0.009, r^2=0.186) and seagrass biomass ($F_{1, 34}$=6.978, P=0.012, r^2=0.170) were identified using regression analysis on data pooled across depths. Analysis also indicated significant positive relationships between total fish abundance and both seagrass length ($F_{1, 34}$=11.100, P=0.002, r^2=0.237) and seagrass biomass ($F_{1, 34}$=13.290, P<0.001, r^2=0.275).

Discussion

The range of fish species recorded during the present study is typical of seagrass beds in the region (Edgar and Shaw 1995; Jenkins et al. 1997a, b; Smith et al. 2008). Assemblages at both depths included fish that can be considered residents in seagrass such as several species of sygnathids, monacanthids and odacids (Jenkins and Wheatley 1998). The majority of the other species recorded were also closely associated with seagrass and surrounding sand patches (Gomon et al. 2008; Froese and Pauly 2012).

There were clear differences in seagrass-associated fish assemblages with depth, supporting the initial findings of a concurrent study that utilised underwater video technology at Mud Islands (Smith et al. 2012). While we identified depth differences in the distribution patterns of many species, these were more complex for species found at both depths and further work is needed on individual species to examine why depth differences occur. Distinct differences in total fish

Fig. 5 nMDS ordination comparing the similarity of fish assemblages in deep and shallow seagrass beds at three sites. Increasing distance between points indicates decreasing similarity
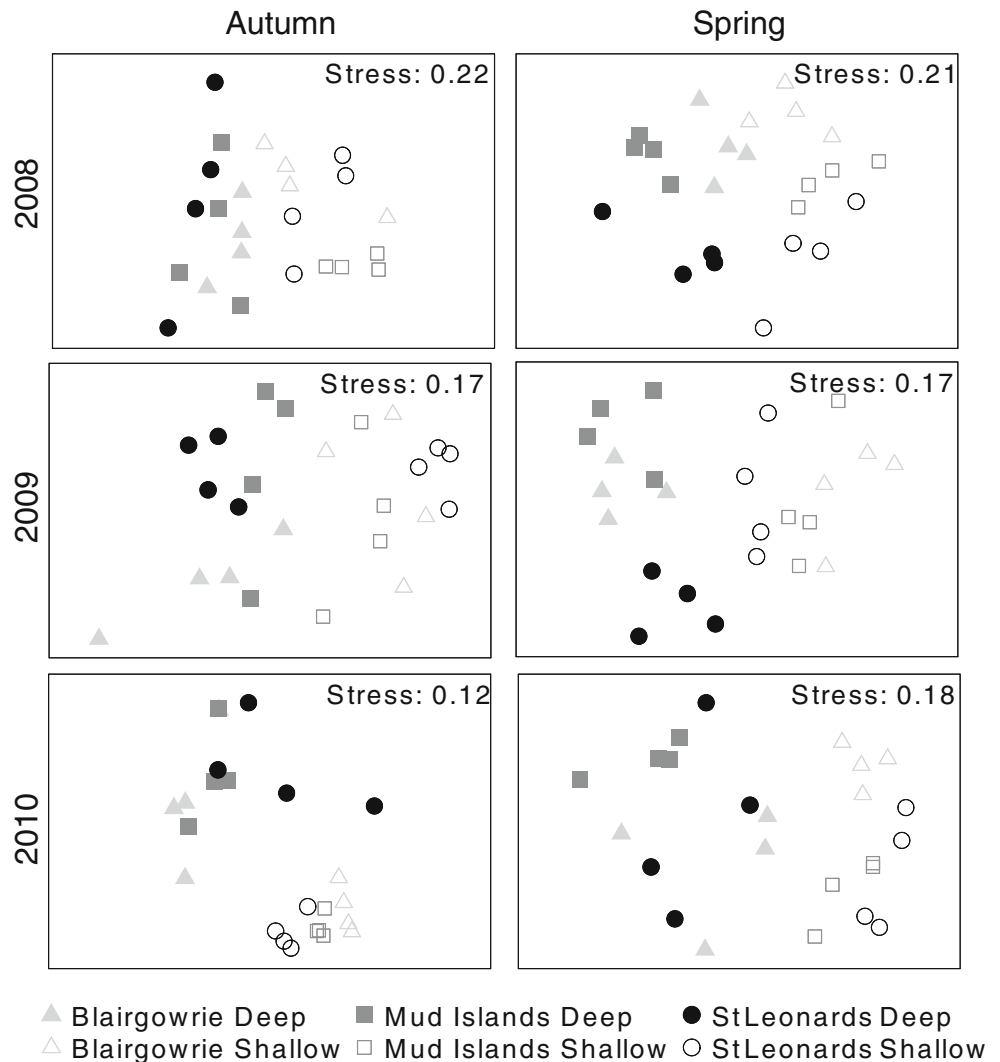
abundance and species richness could also be seen between depths at the surveyed sites, with approximately half of the species recorded in either shallow or deep seagrass beds alone. Total fish abundance and species richness both tended to be higher in shallow (<1 m) than deep (2–8 m) seagrass beds as previously indicated by Smith et al. (2012). While this pattern is similar to those shown by Francour (1997) and Travers and Potter (2002), it contrasts with other studies (e.g. Heithaus 2004; Jackson et al. 2006b). As discussed by Smith et al. (2012), this may partly be due to a lack of consistency in sampling strategy and habitat definition, and large scale studies using standardised methods may be required to identify any general patterns.

Differences in the length frequency distributions of fish caught in shallow and deep seagrass beds during the present study supported those found by Smith et al. (2012) and other studies at similar depths (e.g. Blaber et al. 1992). The differences that we identified were primarily driven by the presence of larger fish such as *M. freycineti*, *Arripis* spp., and *Haletta semifasciata* exclusively in shallow seagrass beds. The presence of these species in shallow seagrass emphasises that shallow seagrass functions not only as a nursery habitat but

is also important for adult species. In spring, recruitment processes resulted in smaller overall fish lengths in shallow seagrass, for example with *S. punctatus* recruiting in shallow seagrass only (also see Jenkins and May (1994)). This occurrence of small individuals in shallow seagrass beds is widely reported in terms of nursery ground function in both temperate and tropical systems (Jackson et al. 2001; Gullstrom et al. 2002; Ford et al. 2010) and in Port Phillip Bay recruitment of *S. punctatus* to shallow seagrass beds is thought to be related to food availability (Jenkins et al. 1996; Jenkins and Hamer 2001), larval dispersal patterns (e.g. Jenkins et al. 1998) and the avoidance of predators (Smith et al. 2011).

Compared to the video results of Smith et al. (2012), we recorded a limited number of large fish in deep seagrass, indicating that there were probable gear selectivity differences between the two techniques, interacting with depth. It is likely that the net sweep area in our study varied with depth and that the net was more efficient in shallow than deep areas. This may partly explain why we only caught larger fish and some pelagics such as the atherinids and *Enoplosus armatus* in shallow water. The seine nets were, however, more successful in capturing the incidence of small individuals than the

Table 4 Summary of SIMPER analyses indicating the relative importance of individual species contributing most to 50 % depth differences during each sampling time (1=most important), pooled for all sites

Numbers in superscript indicate percentage contribution

Species	Autumn 2008	Spring 2008	Autumn 2009	Spring 2009	Autumn 2010	Spring 2010
Acanthaluteres spilomelanurus	5 $^{5.23}$	2 $^{7.79}$	6 $^{4.34}$	2 $^{7.02}$	9 $^{3.48}$	4 $^{6.96}$
Acanthaluteres vittiger			20 $^{1.89}$			
Afurcagobius tamarensis	3 $^{6.56}$	8 $^{3.68}$	18 $^{2.29}$	8 $^{4.52}$		
Arenigobius frenatus			23 $^{1.39}$			
Atherinidae	1 $^{10.04}$	1 $^{8.33}$	1 $^{14.82}$	6 $^{4.96}$	1 $^{12.11}$	1 $^{12.81}$
Brachaluteres jacksonianus	7 $^{4.95}$		11 $^{3.39}$			
Contusus brevicaudus			4 $^{4.85}$	7 $^{4.8}$		6 $^{5.69}$
Enoplosus armatus			10 $^{4.06}$		6 $^{4.73}$	
Genus C Sp. 1		9 $^{3.56}$	19 $^{2.13}$	9 $^{4.35}$		
Gymnapistes marmoratus			12 $^{3.2}$			
Haletta semifasciata					8 $^{3.58}$	
Heteroclinus adelaide			13 $^{3.11}$		4 $^{5.43}$	
Heteroclinus kuiteri			22 $^{1.75}$			
Heteroclinus perspicillatus		3 $^{7.27}$	7 $^{4.29}$	3 $^{6.04}$		
Meuschenia freycineti			15 $^{2.81}$			
Neoodax balteatus	6 $^{5.03}$	7 $^{3.75}$	9 $^{4.11}$		3 $^{6.29}$	5 $^{6.37}$
Nesogobius greeni			8 $^{4.28}$			
Nesogobius maccullochi			3 $^{5.29}$			
Nesogobius pulchellus			17 $^{2.3}$			
Nesogobius sp2	4 $^{5.24}$		5 $^{4.78}$			
Parablennius tasmanianus			21 $^{1.87}$			
Sillaginodes punctatus		4 $^{7.13}$		1 $^{10.38}$		
Stigmatopora argus	8 $^{4.77}$	6 $^{5.36}$	16 $^{2.33}$	5 $^{5.98}$	5 $^{4.75}$	2 $^{9.49}$
Stigmatopora nigra	2 $^{9.39}$	5 $^{6.1}$	2 $^{8.9}$	4 $^{6.01}$	2 $^{8.09}$	3 $^{8.73}$
Vanacampus phillipi			14 $^{2.88}$		7 $^{4.51}$	

cameras used by Smith et al. (2012). For example, while they only found adult *A. spilomelanurus* in deep seagrass, our netting showed that juveniles were present at both depths. Given that recorded depth differences were consistent between the two studies, we are confident that such differences were real rather than an artefact of sampling, but suggest that it would be appropriate to use an amalgamation of these techniques in future studies (Murphy and Jenkins 2010).

While we have discussed our findings in terms of differences in fish assemblages with depth, there is a widespread issue with this and similar studies in the literature, in that depth may be confounded with seagrass structure. Given limited resources, we were unable to address this in the present study but it could be resolved through the use of standardised artificial seagrass beds. While the positive relationships we identified between seagrass length or biomass and fish abundance or species richness should, therefore, be viewed with

some caution given that data was pooled across depths, the physical structure of seagrass beds is likely to be an important factor governing the distribution pattern of fish at different depths in the present study. Numerous other studies have shown that seagrass structure, including length and biomass, influence the distribution of fish in seagrass (e.g. Orth et al. 1984; Bell and Westoby 1986; Jackson et al. 2006b) yet there is still some disparity between studies as to the impact of the physical structure of seagrass beds on fish abundances (Bell and Westoby 1986; Bell et al. 1992). This most likely depends on whether fish are using the seagrass as a food resource, or whether it serves predominantly as a refuge, and at what spatial scale these factors are most important. For example, a recent study on the parrotfish *Leptoscarus vaigiensis* in Tanzania found that seagrass complexity and feeding mode were important at small spatial scales whereas at larger spatial scales the distribution of predators was more important, with

Fig. 6 Average (+SE) stem/leaf length recorded in seagrass beds at each site from Autumn 2008 to Spring 2010
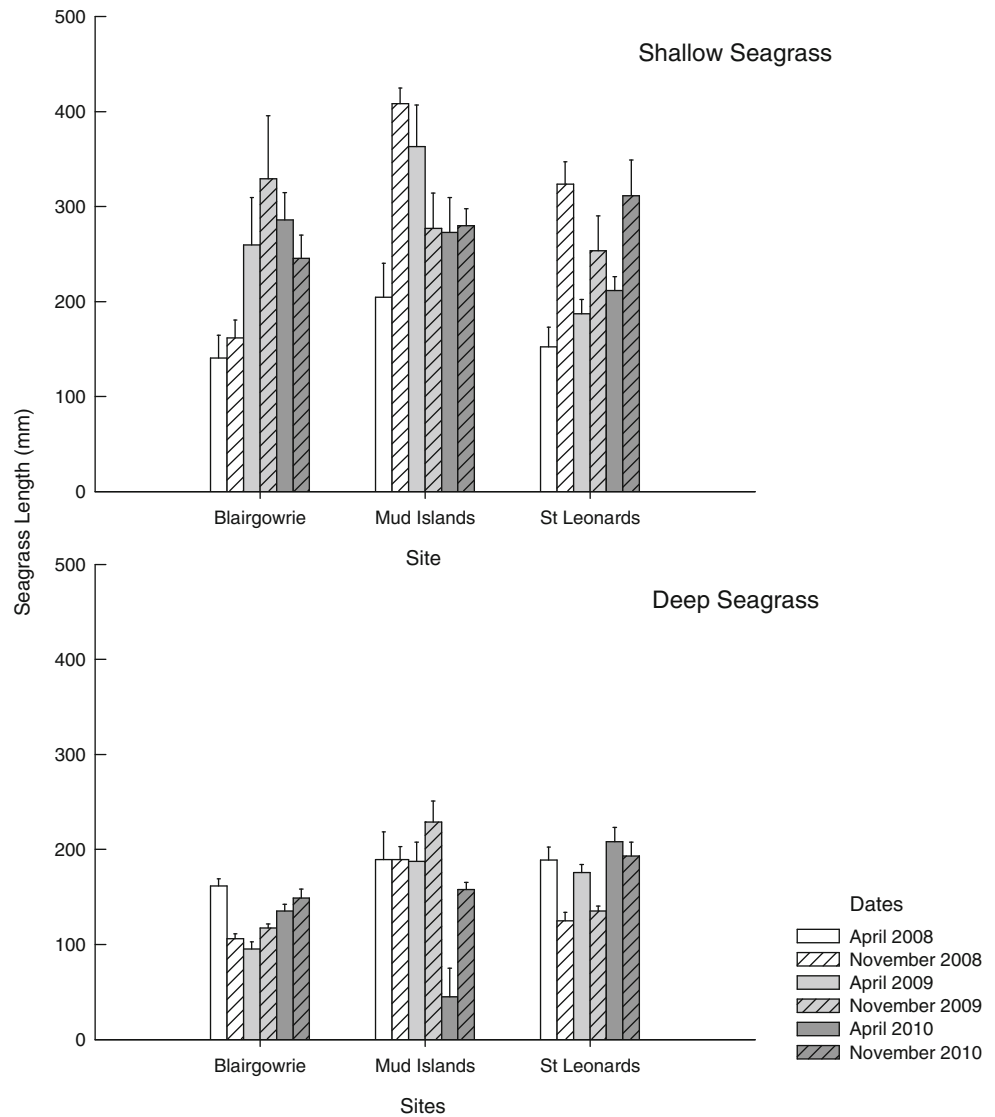
seagrass canopy height as the main predictor explaining variability in the density and biomass of predators (Gullstrom et al. 2011).

As in our study, Kendrick and Hyndes (2003) found differences in the depth distribution of sygnathids. They suggested that differences in abundances between depths were partly due to changes in seagrass cover and seagrass species. While the seagrass beds examined at each depth during the present study were monocultures of the same species, *Z. nigricaulis*, differences in seagrass cover may interact with a range of other factors that are known to play an important role in governing the distribution of *S. nigra* and other seagrass-associated fish including patch size, variation in the ratio of area to edge perimeter, and habitat fragmentation (Smith et al. 2008; Macreadie et al. 2009, 2010a, b).

While impacts of climate change on seagrass beds are difficult to predict (Short and Neckles 1999) they may result

in seagrass being more susceptible to loss at different depths. For example, newly inundated shallow water areas created by sea-level rise may lack suitable substrate for seagrass growth, impacting the upper limits of seagrass distribution, while increased turbidity may impact lower depth limits (e.g. Saunders et al. 2013). The limited work that has been conducted examining the impact of seagrass loss on faunal assemblages at specific depths has concentrated on changes that occur in adjacent intertidal and shallow subtidal seagrass communities (Micheli et al. 2008) rather than shallow and deep subtidal seagrass-associated fish assemblages. Yet the factors responsible for recovery or adaptation are likely to be similarly complex. In a situation such as that described in the present study, where deep and shallow seagrass habitat are distinct, it is likely that fish which utilise both depths of seagrass, such as shoaling species that are able to move large distances, may be better able to adapt to loss at either depth.

Fig. 7 Average (+SE) biomass of seagrass recorded in seagrass beds at each site from Autumn 2008 to Spring 2010
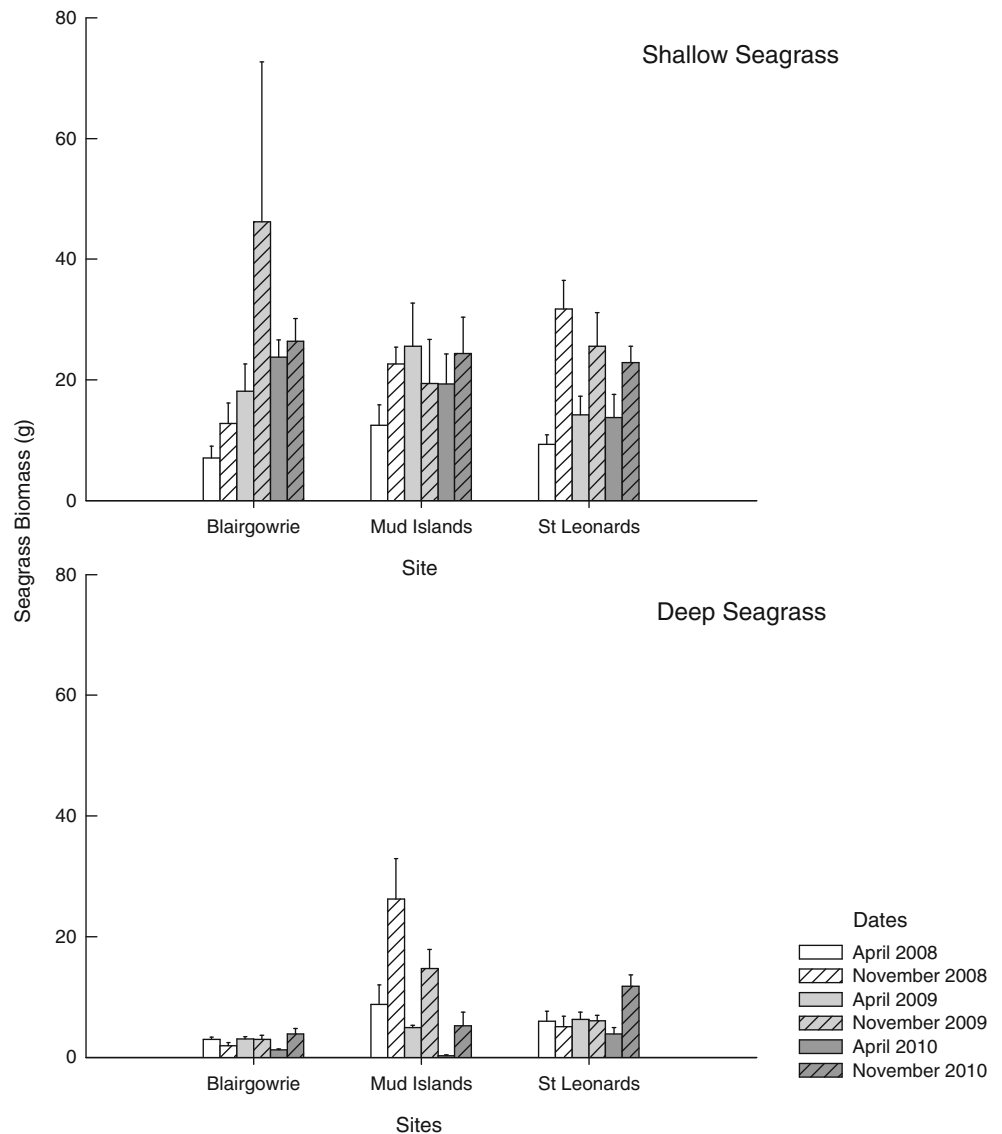
However, those found at only one depth are less likely to be able to do so unless they are able to adapt to different conditions or make use of alternative habitats and food sources.

Seagrass beds are known to provide a wide range of resources to fish in both temperate and tropical systems (Boström et al. 2006). These can include cryptic habitat that offers shelter from predators as well as food, in the form of the seagrass itself, epiphytes and an assorted range of invertebrate and vertebrate prey that also inhabit these systems (Klumpp et al. 1989). As such, loss of seagrass may have a dramatic impact on fisheries (Bell and Pollard 1989; Gillanders 2005; Waycott et al. 2009) with a large percentage of seagrass-dependent species either disappearing completely (Vanderklift and Jacoby 2003; Nakamura 2010) or becoming much less abundant (Hughes et al. 2002). Our results indicate that the fish species and assemblages impacted by seagrass loss will depend on the depth range affected, which in turn depends on the cause of loss. Further study is required on a species by species basis to fully appreciate whether fish populations exhibit the behavioural plasticity that will allow them to utilise seagrass outside of their current depth distribution.

References

Anderson, M.J. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26: 32–46.

Ball, D., M. Soto-Berelov, P. Young and A. Coots. 2009. Baywide seagrass monitoring program—historical seagrass mapping. Fisheries Victoria Technical Report Series No. 70. Department of Primary Industries, Queenscliff, Victoria, Australia. http://www.oem.vic.gov.au/Assets/809/1/HistoricalSeagrassMapping_10Dec09_Final.pdf. Accessed 22 October 2013.

Barbier, E.B., S.D. Hacker, C. Kennedy, E.W. Koch, A.C. Stier, and B.R. Silliman. 2011. The value of estuarine and coastal ecosystem services. *Ecological Monographs* 81: 169–193.

Bell, J.D., and D.A. Pollard. 1989. Ecology of fish assemblages and fisheries associated with seagrasses. In *Biology of seagrasses: a treatise on the biology of seagrasses with special reference to the Australian region*, ed. A.W.D. Larkum, A.J. McCoomb, and S.A. Shepherd, 565–609. Amsterdam: Elsevier.

Bell, J.D., and M. Westoby. 1986. Importance of local changes in leaf height and density to fish and decapods associated with seagrasses. *Journal of Experimental Marine Biology and Ecology* 104: 249–274.

Bell, J.D., D.J. Ferrell, S.E. McNeill, and D.G. Worthington. 1992. Variation in assemblages of fish associated with deep and shallow margins of the seagrass *Posidonia australis*. *Marine Biology* 114: 667–676.

Blaber, S.J.M., D.T. Brewer, J.P. Salini, J.D. Kerr, and C. Conacher. 1992. Species composition and biomasses of fishes in tropical seagrasses at Groote Eylandt, Northern Australia. *Estuarine, Coastal and Shelf Science* 35: 605–620.

Blake, S. and D. Ball. 2001. Victorian marine habitat database: seagrass mapping of Port Phillip Bay. Geospatial Systems Section, Marine and Freshwater Resources Institute Report No. 39. Marine and Freshwater Resources Institute: Queenscliff. Accessed 22 October 2013. http://www.dse.vic.gov.au/__data/assets/pdf_file/0007/97153/PPB_Seagrass_Report.pdf. Accessed 22 October 2013.

Bologna, P.A.X., and K.L. Heck. 1999. Macrofaunal associations with seagrass epiphytes—relative importance of trophic and structural characteristics. *Journal of Experimental Marine Biology and Ecology* 242: 21–39.

Boström, C., E.L. Jackson, and C.A. Simenstad. 2006. Seagrass landscapes and their effects on associated fauna: a review. *Estuarine, Coastal & Shelf Science* 68: 383–403.

Clarke, K.R. 1993. Non-parametric mutivariate analyses of changes in community structure. *Australian Journal of Ecology* 18: 117–143.

Connolly, R.M. 2009. Seagrass. In *A marine climate change impacts and adaptation report card for Australia 2009*, eds. Poloczanska, E.S., A.J. Hobday and A.J. Richardson. NCCARF Publication 05/09.

Costanza, R., R. d'Arge, R. de Groot, S. Farber, M. Grasso, K. Hannon, K. Limburg, S. Naeem, R.V. O'Neill, J. Paruelo, R.G. Raskin, P. Sutton, and M. van den Belt. 1998. The value of the world's ecosystem services and natural capital (Reprinted from Nature, vol 387, pg 253, 1997). *Ecological Economics* 25: 3–15.

Deudero, S., G. Morey, A. Frau, J. Moranta, and I. Moreno. 2008. Temporal trends of littoral fishes at deep *Posidonia oceanica* seagrass meadows in a temperate coastal zone. *Journal of Marine Systems* 70: 182–195.

Duarte, C.M. 2002. The future of seagrass meadows. *Environmental Conservation* 29: 192–206.

Edgar, G.J., and C. Shaw. 1995. The production and trophic ecology of shallow-water fish assemblages in southern Australia III. General relationships between sediments, seagrasses, invertebrates and fishes. *Journal of Experimental Marine Biology and Ecology* 194: 107–131.

Engel, D.W., G.W. Thayer, and D.W. Evans. 1999. Linkages between fishery habitat quality, stressors, and fishery populations. *Environmental Science & Policy* 2: 465–475.

Ford, J.R., R.J. Williams, A.M. Fowler, D.R. Cox, and I.M. Suthers. 2010. Identifying critical estuarine seagrass habitat for settlement of coastally spawned fish. *Marine Ecology Progress Series* 408: 181–193.

Francour, P. 1997. Fish assemblages of *Posidonia oceanica* beds at port-Cros (France, NW Mediterranean): assessment of composition and long-term fluctuations by visual census. *Marine Ecology* 18: 157–173.

Froese, R. and D. Pauly. 2012. Fishbase www.fishbase.org. Accessed 8 June 2012

Gillanders, B.M. 2005. Seagrasses, fish, and fisheries. In *Seagrasses: biology, ecology, and their conservation*, ed. A.W.D. Larkum, R.J. Orth, and C.M. Duarte, 503–536. Berlin: Springer.

Gomon, M.F., D.J. Bray, and R.H. Kuiter. 2008. *Fishes of Australia's southern coast*. Sydney: Reed New Holland.

Grech, A., K. Chartrand-Miller, P. Erftemeijer, M. Fonseca, L. McKenzie, M. Rasheed, H. Taylor, and R. Coles. 2012. A comparison of threats, vulnerabilities and management approaches in global seagrass bio-regions. *Environmental Research Letters* 7(2): 1–8.

Gullstrom, M., M. de la Torre Castro, S.O. Bandeira, M. Bjork, M. Dahlberg, N. Kautsky, P. Ronnback, and M.C. Ohman. 2002. Seagrass ecosystems in the Western Indian Ocean. *Ambio* 31: 588–596.

Gullstrom, M., C. Berkstrom, M.C. Ohman, M. Bodin, and M. Dahlberg. 2011. Scale-dependent patterns of variability of a grazing parrotfish

(*Leptoscarus vaigiensis*) in a tropical seagrass-dominated seascape. *Marine Biology* 158: 1483–1495.

Heithaus, M.R. 2004. Fish communities of subtropical seagrass meadows and associated habitats in Shark Bay, western Australia. *Bulletin of Marine Science* 75: 79–99.

Hindell, J.S., G.P. Jenkins, and M.J. Keough. 2001. Spatial and temporal variability in the effects of fish predation on macrofauna in relation to habitat complexity and cage effects. *Marine Ecology Progress Series* 224: 231–250.

Howard, R.K., G.J. Edgar, and P.A. Hutchings. 1989. Faunal assemblages of seagrass beds. In *Biology of seagrasses*, ed. A.W.D. Larkum, A.J. McComb, and S.A. Shepard, 536–564. Amsterdam: Elsevier.

Hughes, J., L. Deegan, J. Wyda, M. Weaver, and A. Wright. 2002. The effects of eelgrass habitat loss on estuarine fish communities of southern New England. *Estuaries and Coasts* 25: 235–249.

Jackson, E.L., A.A. Rowden, M.J. Attrill, S.J. Bossey, and M.B. Jones. 2001. The importance of seagrass beds as a habitat for fishery species. *Oceanography and Marine Biology. Annual Review* 39: 269–303.

Jackson, E.L., M.J. Attrill, and M.B. Jones. 2006a. Habitat characteristics and spatial arrangement affecting the diversity fish and decapod assemblages of seagrass (*Zostera marina*) beds around the coast of Jersey (English Channel). *Estuarine, Coastal & Shelf Science* 68: 421–432.

Jackson, E.L., M.J. Atrill, A.A. Rowden, and M.B. Jones. 2006b. Seagrass complexity hierarchies: influence on fish groups around the coast of Jersey (English Channel). *Journal of Experimental Marine Biology and Ecology* 330: 38–54.

Jenkins, G.P., and P.A. Hamer. 2001. Spatial variation in the use of seagrass and unvegetated habitats by post-settlement King George whiting (Percoidei: Sillaginidae) in relation to meiofaunal distribution and macrophyte structure. *Marine Ecology Progress Series* 224: 219–229.

Jenkins, G.P., and H.M.A. May. 1994. Variation in settlement and larval duration of King George whiting, *Sillaginodes punctata* (Sillaginidae), in Swan Bay, Victoria, Australia. *Bulletin of Marine Science* 54: 281–296.

Jenkins, G.P., and M.J. Wheatley. 1998. The influence of habitat structure on nearshore fish assemblages in a southern Australian embayment: comparison of shallow seagrass, reef algal, and unvegetated habitats, with emphasis on their importance to recruitment. *Journal of Experimental Marine Biology and Ecology* 221: 147–172.

Jenkins, G.P., M.J. Wheatley, and A.G.B. Poore. 1996. Spatial variation in recruitment, growth and feeding of post-settlement King George whiting, *Sillaginodes punctata*, associated with seagrass beds of Port Phillip Bay, Australia. *Canadian Journal of Fisheries and Aquatic Sciences* 53: 96–105.

Jenkins, G.P., K.P. Black, M.J. Wheatley, and D.N. Hatton. 1997a. Temporal and spatial variability in recruitment of a temperate, seagrass-associated fish is largely determined by physical processes in the pre- and post-settlement phases. *Marine Ecology Progress Series* 148: 23–35.

Jenkins, G.P., H.M.A. May, M.J. Wheatley, and M.G. Holloway. 1997b. Comparison of fish assemblages associated with seagrass and adjacent unvegetated habitats of Port Phillip Bay and Corner Inlet, Victoria, Australia, with emphasis on commercial species. *Estuarine, Coastal and Shelf Science* 44: 569–588.

Jenkins, G.P., M.J. Keough, and P.A. Hamer. 1998. The contributions of habitat structure and larval supply to broad-scale recruitment variability in a temperate zone, seagrass-associated fish. *Journal of Experimental Marine Biology and Ecology* 226: 259–278.

Kendrick, A.J., and G.A. Hyndes. 2003. Patterns in the abundance and size-distribution of syngnathid fishes among habitats in a seagrass-dominated marine environment. *Estuarine Coastal and Shelf Science* 57: 631–640.

King, R.J. 1970. Surface sea-water temperatures at Port Phillip Bay Heads, Victoria. *Australian Journal of Marine and Freshwater Research* 21: 47–50.

Klumpp, D.W., R.K. Howard, and D.A. Pollard. 1989. Trophodynamics and nutritional ecology of seagrass communities. In *Biology of seagrasses*, ed. A.W.D. Larkum, A.J. McComb, and S.A. Shepard, 394–457. Amsterdam: Elsevier.

Larkum, W.D., R.J. Orth, and C.M. Duarte (eds.). 2006. *Seagrasses: biology, ecology and conservation*. The Netherlands: Springer.

Lavery, P.S., M.-Á. Mateo, O. Serrano, and M. Rozaimi. 2013. Variability in the carbon storage of seagrass habitats and its implications for global estimates of blue carbon ecosystem service. *PLoS ONE* 8(9): e73748. doi:10.1371/journal.pone.0073748.

Lee, R.S., K.P. Black, C. Bosserel, and D. Greer. 2012. Present and future prolonged drought impacts on a large temperate embayment: Port Phillip Bay, Australia. *Ocean Dynamics* 62(6): 907–922.

Macreadie, P.I., J.S. Hindell, G.P. Jenkins, R.M. Connolly, and M.J. Keough. 2009. Fish responses to experimental fragmentation of seagrass habitat. *Conservation Biology* 23: 644–652.

Macreadie, P.I., R.M. Connolly, M.J. Keough, G.P. Jenkins, and J.S. Hindell. 2010a. Short-term differences in animal assemblages in patches formed by loss and growth of habitat. *Austral Ecology* 35: 515–521.

Macreadie, P.I., J.S. Hindell, M.J. Keough, G.P. Jenkins, and R.M. Connolly. 2010b. Resource distribution influences positive edge effects in a seagrass fish. *Ecology* 91: 2013–2021.

McArthur, L.C., and J.W. Boland. 2006. The economic contribution of seagrass to secondary production in South Australia. *Ecological Modelling* 196: 163–172.

Micheli, F., M.J. Bishop, C.H. Peterson, and J. Rivera. 2008. Alteration of seagrass species composition and function over two decades. *Ecological Monographs* 78: 225–244.

Moore, K.A., and F.T. Short. 2006. Zostera: biology, ecology and management. In *Seagrasses: biology, ecology and conservation*, ed. W.D. Larkum, R.J. Orth, and C.M. Duarte, 361–386. The Netherlands: Springer.

Murphy, H.M., and G.P. Jenkins. 2010. Observational methods used in marine spatial monitoring of fishes and associated habitats: a review. *Marine and Freshwater Research* 61: 236–252.

Nakamura, Y. 2010. Patterns in fish response to seagrass bed loss at the southern Ryukyu Islands, Japan. *Marine Biology* 157: 2397–2406.

Orth, R.J., K.L. Heck, and J. Van Montfrans. 1984. Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator–prey relationships. *Estuaries* 7A: 339–350.

Orth, R.J., T.J.B. Carruthers, W.C. Dennison, C.M. Duarte, J.W. Fourqurean, K.L. Heck, A.R. Hughes, G.A. Kendrick, W.J. Kenworthy, S. Olyarnik, F.T. Short, M. Waycott, and S.L. Williams. 2006. A global crisis for seagrass ecosystems. *Bioscience* 56: 987–996.

Pogoreutz, C., D. Kneer, M. Litaay, H. Asmus, and H. Ahnelt. 2012. The influence of canopy structure and tidal level on fish assemblages in tropical Southeast Asian seagrass meadows. *Estuarine, Coastal and Shelf Science* 107: 58–68.

Pollard, D.A. 1984. A review of ecological studies on seagrass-fish communities, with particular reference to recent studies in Australia. *Aquatic Botany* 18: 3–42.

Saunders, M.I., J. Leon, S.R. Phinn, D.P. Callaghan, K.R. O'Brien, C.M. Roelfsema, C.E. Lovelock, M.B. Lyons, and P.J. Mumby. 2013. Coastal retreat and improved water quality mitigate losses of seagrass from sea level rise. *Global change biology* 19: 2569–2583.

Shepherd, S.A., A.J. McComb, D.A. Bulthius, V. Neverauskas, D.A. Steffensen, and R. West. 1989. Decline of seagrasses. In *Biology of seagrasses*, ed. A.W.D. Larkum, A.J. McComb, and S.A. Shepherd, 346–393. Amsterdam: Elsevier.

Short, F.T., and H.A. Neckles. 1999. The effects of global climate change on seagrasses. *Aquatic Botany* 63(3): 169–196.

Smith, T.M., J.S. Hindell, G.P. Jenkins, and R.M. Connolly. 2008. Edge effects on fish associated with seagrass and sand patches. *Marine Ecology Progress Series* 359: 203–213.

Smith, T.M., J.S. Hindell, G.P. Jenkins, and R.M. Connolly. 2010. Seagrass patch size affects fish responses to edges. *Journal of Animal Ecology* 79: 275–281.

Smith, T.M., J.S. Hindell, G.P. Jenkins, R.M. Connolly, and M.J. Keough. 2011. Edge effects in patchy seagrass landscapes: the role of predation in determining fish distribution. *Journal of Experimental Marine Biology and Ecology* 399: 8–16.

Smith, T.M., G.P. Jenkins, and N. Hutchinson. 2012. Seagrass edge effects on fish assemblages in deep and shallow habitats. *Estuarine, Coastal and Shelf Science* 115: 291–299.

Travers, M.J., and I.C. Potter. 2002. Factors influencing the characteristics of fish assemblages in a large subtropical marine embayment. *Journal of Fish Biology* 61: 764–784.

Underwood, A.J. 1997. *Experiments in ecology*. Cambridge: Cambridge University Press.

Vanderklift, M.A., and C.A. Jacoby. 2003. Patterns in fish assemblages 25 years after major seagrass loss. *Marine Ecology Progress Series* 247: 225–235.

Waycott, M., C.M. Duarte, T.J.B. Carruthers, R.J. Orth, W.C. Dennison, S. Olyarnik, A. Calladine, J.W. Fourqurean, K.L. Heck, A.R. Hughes, G.A. Kendrick, W.J. Kenworthy, F.T. Short, and S.L. Williams. 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences* 106: 12377–12381.

Zar, J.H. 1996. *Biostatistical analysis*. London: Prentice-Hall.