

A.H. Baird · A. Salih · A. Trevor-Jones

## Fluorescence census techniques for the early detection of coral recruits

Received: 27 July 2005 / Accepted: 19 October 2005 / Published online: 17 November 2005  
© Springer-Verlag 2005

**Abstract** Many coral recruits are very small and often cryptic at settlement making them difficult to detect with normal census techniques. Here we show that fluorescence census techniques can increase the accuracy of juvenile coral counts in highly fluorescent taxa. Using fluorescent filters at night, counts of coral recruits were 20–50% higher than during the day. *Acropora* abundances were up to 300% higher, the difference being made up of cryptic individuals, and individuals that were too small to see during the day. Fluorescence techniques will be particularly useful in regions where fluorescent taxa are dominant, such as most Indo-Pacific reefs. The technique offers particular promise to determine the influence of early post-settlement mortality on the ecology of fluorescent taxa, because corals can be detected at the size at which they settle.

**Keywords** Coral reefs · *Acropora* · Fluorescence · Recruitment · Settlement · Mortality

### Introduction

Processes active early in coral life histories may play an important role in determining the distribution and abundance of many species (Hughes et al. 1999; Baird

et al. 2003). However, the study of these processes has been hindered by difficulties in observing young coral recruits on the reef substratum (Babcock et al. 2003). Coral recruits are very small at settlement and growth is slow, with the result that it may take up to a year before a coral juvenile becomes easily visible under white light (Wallace and Bull 1981). During this period mortality is typically high (Rylandsdam 1983; Babcock 1985; Wallace et al. 1986). A delay between settlement and the point at which a coral becomes detectable can have a number of important consequences for ecological research. For example, post-settlement processes during this period may be missed (e.g. density dependent mortality), leading to false conclusions regarding mechanisms of population regulation or spatial pattern of adults (Keough and Downes 1982).

Fluorescence census techniques have recently been described which make coral juveniles much easier to observe than under standard white light census (Piniak et al. 2005). Fluorescence techniques depend on the high abundance of fluorescent pigments in many coral juveniles (Papina et al. 2002). These pigments emit intense blue-green to orange wavelengths when illuminated in the dark by Ultra Violet (UV), blue or green light (Mazel 1995). Even very small fluorescent organisms become prominently visible when viewed against a dark background (Mazel 1995). Piniak et al. (2005) concluded that while coral recruits were more easily observed via fluorescence than under white light, abundance estimates were comparable. Here, we show that when fluorescent taxa are common, the fluorescence census techniques can greatly increase the counts of coral recruits.

### Methods

We compared the abundance and taxonomic composition of coral juveniles on the reef substratum at Ribbon Reef no.15-072 (Lat 15.30.766, Long 145.46.643), a mid-shelf reef in the Cairns Section of the Great Barrier Reef

Communicated by Ecological Editor P.J. Mumby

A.H. Baird (✉)  
Centre for Coral Reef Biodiversity,  
School of Marine Biology and Aquaculture,  
James Cook University, 4811 Townsville, Qld, Australia  
E-mail: andrew.baird@jcu.edu.au  
Tel.: +61-617-47814857  
Fax: +61-617-47251570

A. Salih  
The Australian Key Centre for Microscopy and Microanalysis,  
The University of Sydney, 2006 Sydney, Australia

A. Trevor-Jones  
173, 2773 Glenbrook, NSW, Australia

Marine Park, Australia using both normal white light census and the fluorescence technique described by Piniak et al. (2005). We used dead *Acropora hyacinthus* colonies as replicate well defined areas of natural substratum and censused colonies during the day and at again at night using a blue exciter filter mounted in front of an Underwater Kinetics Light Cannon and a yellow barrier filter (both filters from NightSea LLC) placed over the divers mask.

## Results and discussion

The total count of coral recruits was 20–50% higher using fluorescence census techniques than during the day (Table 1). The increase in abundance was highest in the genus *Acropora*, where the fluorescence counts were 20–300% higher than counts during the day (Table 1). In contrast, the number of poritid recruits was substantially lower at night: 23 poritid juveniles were recorded during the day versus only four at night (Table 1), reflecting the fact that many poritid species are non-fluorescent (see Fig. 1 and Salih et al. 2000).

The additional *Acropora* recruits detected at night were either cryptic individuals difficult to spot during the day, or were very small. The majority of the recruits recorded during the day exceeded 5 mm maximal diameter, a resolution size limit typical of daytime reef recruit surveys (Edmunds et al. 1998; Mumby 1999). In contrast, many of the recruits we detected at night were as small as 1 mm diameter (Fig. 2), which is the approximate size at settlement of *Acropora* recruits (Babcock et al. 2003). The increased capacity to detect recruits is due to the contrast provided by a fluorescing organism against a generally non-fluorescent (dark) background (Fig. 2).

In the initial description of this technique, Piniak et al. (2005) found that while coral recruits were more easily observed via fluorescence than under white light, abundance estimates were not significantly different. The most likely reason for the discrepancies between our studies is the pronounced difference in assemblage structure between Indo-Pacific reefs and those of the western Atlantic. Piniak et al.'s (2005) study site was at

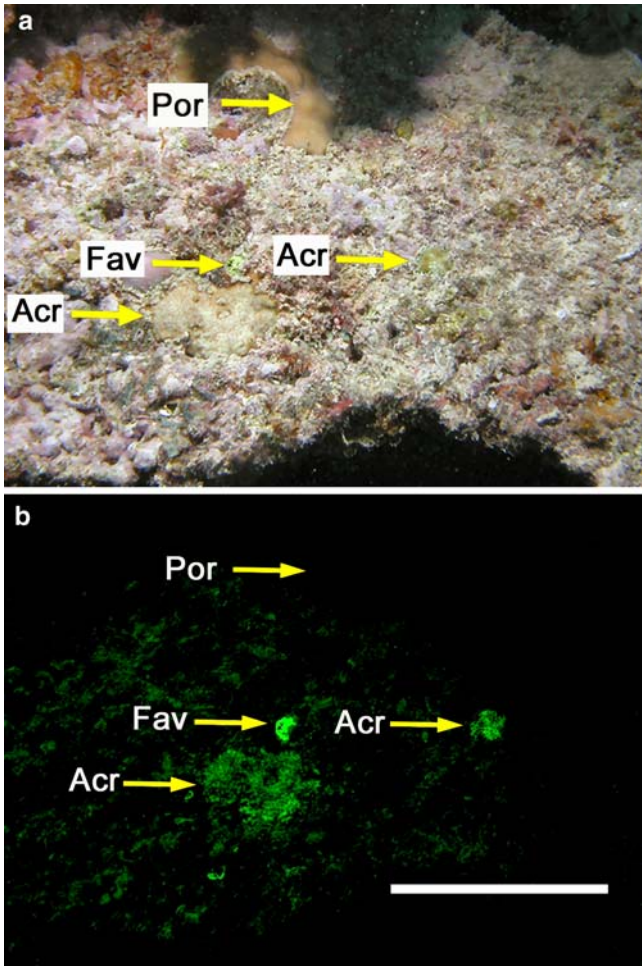
Key Largo, Florida, and the recruit assemblage was dominated by *Porites sp.*, *Agaricia sp.* and *Siderastrea siderea* (Piniak et al. 2005). These taxa are frequently weakly fluorescent, eg. *Porites* (Salih et al. 1998 and see Fig. 2), or are relatively large at settlement, eg. *Porites* and *Agaricia* (both these genera brood their larvae in the Caribbean (van Moorsel 1983; Szmant 1986). While this assemblage is certainly typical of many modern sites in the Caribbean (Chiappone and Sullivan 1996), it is very different to most Indo-Pacific recruit assemblages, where early recruit assemblages are dominated by taxa from the family Acroporidae (Sammarco 1985), which are generally highly fluorescent (Salih et al. 2000; Dove et al. 2001; Papina et al. 2002) and small at settlement, at least when compared to most brooders (Babcock et al. 2003).

As noted by Piniak et al. (2005), the taxonomic resolution was lower using the fluorescence technique than during the day: the number of unidentifiable recruits increased from zero to four and we could not distinguish *Acropora* from *Montipora* recruits at night (Table 1). This is largely because many of the features used to identify coral juveniles, such as colour, and morphological detail such as exert septa, are not evident under fluorescent lights. However, we found that this problem was minimised by the use of a strong white light at night, in addition to the fluorescence-excitation irradiation. Furthermore, poor taxonomic resolution is not limited to fluorescence techniques and even microscopy has clear limits to which taxa can be consistently distinguished (Baird and Babcock 2000; Babcock et al. 2003). While fluorescent lights are much more effective in discovering small and cryptic fluorescent taxa, white light is also necessary in order to maximise the taxonomic resolution and the two techniques should be used in tandem.

Another potential problem with the fluorescence techniques identified by Piniak et al. (2005) was false positives caused by a number of other organisms, which fluoresce. We found that the number of false positives could also be minimised with the use of strong white light at night. Furthermore, many taxa have distinct patterns of fluorescence, which allow them to be distinguished from scleractinian recruits. For example, some *Palythoa* spp. have thin tentacles which emerge

**Table 1** The number of coral juveniles on the upper surface of three dead *A. hyacinthus* colonies censused during the day and at night using fluorescence techniques

Taxa	Dead Colony 1		Dead Colony 2		Dead Colony 3	
	Day	Night	Day	Night	Day	Night
<i>Acropora</i>	26	50	4	5	32	94
<i>Montipora</i>					3	
<i>Pocillopora</i>	2	1				
<i>Goniastrea</i>	1	1			2	2
<i>Porites</i>					22	4
<i>Favia</i>					1	1
<i>Favites</i>					1	1
<i>Stylocoeniella</i>					1	1
Unknown		4				1
Mussidae						1
Total	29	56	4	5	62	105



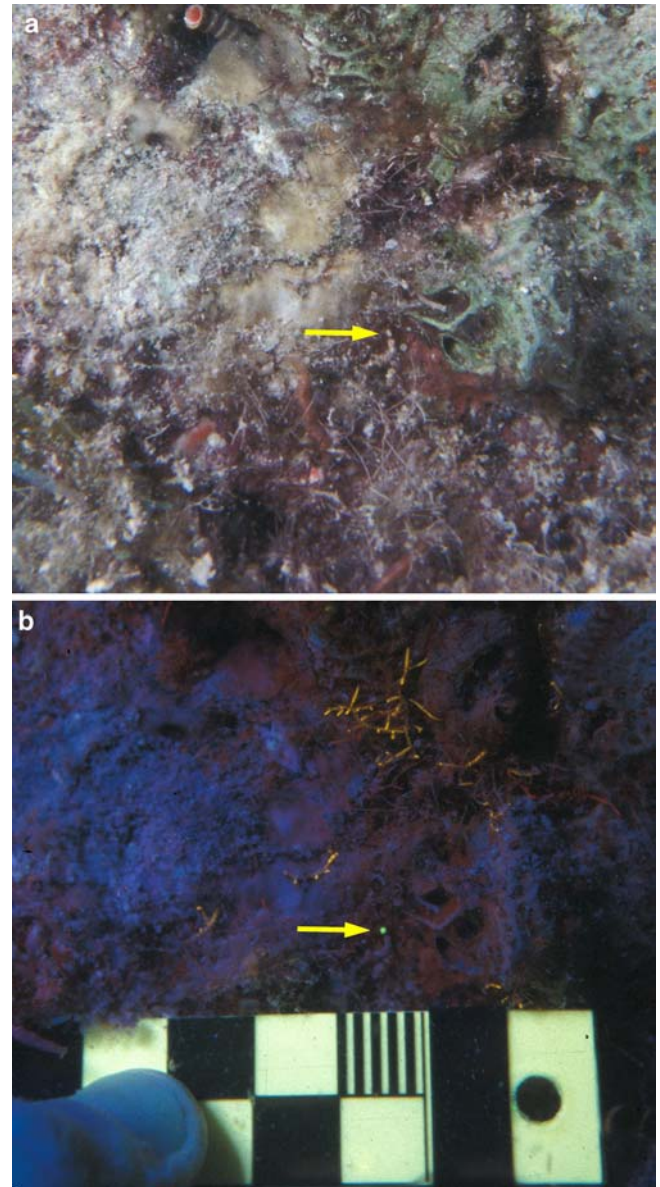
**Fig. 1** **a** White-light excitation-light flash photograph of the surface of a dead *A. hyacinthus* table at a Ribbon Reef Central Section of the Great Barrier Reef. **b** The identical area of substratum photographed with the NightSea Filter set. Fluorescent juveniles that are almost invisible when photographed with white light are now prominently visible. Also note the *Porites* sp. recruit, which does not exhibit any fluorescence. Acr *Acropora*, Fav *Favia*, Por *Porites*. Scale bar = 5 cm

from a non-fluorescent oral disc. In addition, most non-scleractinian fluorescent taxa are soft to the touch.

Fluorescence photo quadrant imaging is also feasible during the day if the relative influence of ambient sunlight is significantly reduced during the imaging procedure. We captured fluorescence images during the day with a digital camera (Nikon Coolpix 4500) in an underwater housing (Ikelite) (Fig. 1). A yellow barrier filter was fitted to the dome port of the housing and an underwater strobe (Ikelite DS50) was fitted with a blue exciter filter as the fluorescence excitation source. The shutter speed of the camera was set to 1/125 s and the strobe was set to maximum power permitting the aperture of the lens to be small enough to reduce the influence of sunlight (Fig. 1). Shutter speeds greater than 1/125 s would further reduce the influence of the ambient light. This technique can also be used with film based cameras, such as the Nikonos used by Piniak et al.

(2005), however, the inability to synchronise the strobe at faster shutter speeds would limit daylight fluorescence photography to deeper water unless a very powerful strobe was used.

Fluorescence techniques have the potential to greatly increase the speed and accuracy of juvenile coral counts in highly fluorescent taxa and will be particularly useful in regions where these recruits are dominant. The technique offers particular promise to test for the influence of early post-settlement mortality on the regulation of populations of fluorescent taxa, because the technique allows corals to be detected at the size at which they settle.



**Fig. 2** A coral recruit, approximately 0.5 mm diameter; **a** almost invisible under white light as indicated by the arrow, but **b** brightly fluorescent and clearly visible when photographed using the NightSea filter set

**Acknowledgements** This work was supported by ARC Fellowships to A. Baird and A. Salih and Undersea Explorer (UE). We thank UE crew and sponsors (Carl Zeiss Pty Ltd. & Varian) of the Coral Light and Life Workshop, and C. Mazel for comments on the manuscript.

## References

- Babcock RC (1985) Growth and mortality in juvenile corals *Goniastrea*, *Platygyra* and *Acropora* in the first year. Proc fifth Int Coral Reef Congr 4:355–360
- Babcock RC, Baird AH, Piromvaragorn S, Thomson DP, Willis BL (2003) Identification of scleractinian coral recruits from Indo-Pacific reefs. Zool Stud 42:211–226
- Baird AH, Babcock RC (2000) Morphological differences among three species of newly settled pocilloporid coral recruits. Coral Reefs 19:179–183
- Baird AH, Babcock RC, Mundy CP (2003) Habitat selection by larvae influences the depth distribution of six common coral species. Mar Ecol Prog Ser 252:289–293
- Chiappone M, Sullivan K (1996) Distribution, abundance and species composition of juvenile scleractinian corals in the Florida Reef Tract. Bull Mar Sci 58:555–569
- Dove SG, Hoegh-Guldberg O, Ranganathan S (2001) Major colour patterns of reef-building corals are due to a family of GFP-like proteins. Coral Reefs 19:197–204
- Edmunds PJ, Aronson RB, Swanson DW, Levitan DR, Precht WF (1998) Photographic versus visual census techniques for the quantification of juvenile corals. Bull Mar Sci 62:937–946
- Hughes TP, Baird AH, Dinsdale EA, Moltschaniwskyj NA, Pratchett MS, Tanner JE, Willis BL (1999) Patterns of recruitment and abundance of corals along the Great Barrier Reef. Nature 397:59–63
- Keough MJ, Downes BJ (1982) Recruitment of marine invertebrates: the role of active larval choices and early mortality. Oecologia 54:348–352
- Mazel CH (1995) Spectral measurements of fluorescence emission in Caribbean cnidarians. Mar Ecol Prog Ser 120:185–191
- Mumby PJ (1999) Bleaching and hurricane disturbances to populations of coral recruits in Belize. Mar Ecol Prog Ser 190:27–35
- Papina M, Sakihama Y, Bena C, van Woesik R, Yamasaki H (2002) Separation of highly fluorescent proteins by SDS-PAGE in Acroporidae corals. Comp Biochem Physiol B-Biochem Mol Biol 131:767–774
- Piniak GA, Fogarty ND, Addison CM, Kenworthy WJ (2005) Fluorescence census techniques for coral recruits. Coral Reefs DOI:10.1007/s00338-00005-00495-00331
- Rylaarsdam KW (1983) Life history and abundance of colonial corals on Jamaican reefs. Mar Ecol Prog Ser 13:249–260
- Salih A, Hoegh-Guldberg O, Cox G (1998) Photoprotection of symbiotic dinoflagellates by fluorescent pigments in reef corals. In: Greenwood JG, Hall NJ (eds) Australian coral reef society 75th anniversary conference. The University of Queensland, Brisbane, pp 217–230
- Salih A, Larkum A, Cox G, Kuehl M, Hoegh-Guldberg O (2000) Fluorescent pigments in corals are photoprotective. Nature 408:850–853
- Sammarco PW (1985) The great barrier reef vs. the Caribbean: comparisons of grazers, coral recruitment patterns and reef recovery. Proc fifth Int Coral Reef Congr 4:391–398
- Szmant AM (1986) Reproductive ecology of Caribbean reef corals. Coral Reefs 5:43–54
- Van Moorsel GWMN (1983) Reproductive strategies in two closely related stony corals (*Agaricia*, *Scleractinia*). Mar Ecol Prog Ser 13:273–283
- Wallace CC, Bull GD (1981) Patterns of juvenile coral recruitment on a reef front during a spring-summer spawning period. Proc fourth Int Coral Reef Symp 2:345–350
- Wallace CC, Watt A, Bull GD (1986) Recruitment of juvenile corals onto coral tables preyed upon by *Acanthaster planci*. Mar Ecol Prog Ser 32:299–306