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## Effect of biological fouling on passive collectors used to estimate fish recruitment

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Evidence is provided that biofouling of artificial substrata for estimating recruitment of nearshore reef fishes influences recruitment, and it is recommended that investigators consider the effects of fouling when estimating recruitment over space and time.

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Accurately estimating spatial and temporal patterns of variation in larval recruitment is essential to understanding the distribution, abundance and population dynamics of marine organisms (Morgan, 2001; Underwood & Keough, 2001; Strathmann *et al.*, 2002). Larval recruitment has been estimated using towed nets and underwater visual surveys (Ebeling & Hixon, 1991; Larson *et al.*, 1994), but the expense and labour-intensive nature of these methods usually precludes the frequent sampling needed to accurately estimate the high variation of recruits in space and time (Gaines & Bertness, 1993). Light traps and fixed nets offer the advantage of continuously monitoring recruitment at multiple sites. Light traps, however, are limited to nocturnal sampling of phototactic species (Dixon *et al.*, 1999), and fixed nets are biased by variation in current velocity (Kingsford, 2001). Alternatively, artificial settlement substrata have long been used to monitor the larval supply of invertebrates over large areas, because they provide a continuous estimate of recruitment with minimal cost and effort (Young, 1990; Rabalais *et al.*, 1995). Passive collectors have recently been adapted to monitor recruitment of temperate and tropical nearshore reef fishes by capitalizing on the strong affinity of competent fish larvae for physical structures (Findlay & Allen, 2002; Steele *et al.*, 2002; Ammann, 2004; Ben-David & Kritzer, 2005; Valles *et al.*, 2006).

A recent study concluded that a passive collector known as the ‘standard monitoring unit for the recruitment of fishes’ (SMURF) is an accurate and efficient tool for indexing the relative rates of delivery of competent juvenile fishes to temperate rocky reef habitats (Ammann, 2004). Subsequent studies using SMURFs

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have adapted this experimental design to a variety of different species, geographic locations and research goals (Ammann, 2001, 2004; Ben-David & Kritzer, 2005; Valles *et al.*, 2006). Comparisons among collectors, however, depend on maintaining similar attractiveness to recruits. Although construction of SMURF is easily standardized (Ammann, 2001, 2004), they could become fouled by plant and animal settlers after deployment, which may bias estimates of fish recruitment (Ammann, 2001, 2004; Valles *et al.*, 2006).

The focus of this study was to determine whether differences in biofouling among adjacent SMURF may influence recruitment by temperate reef fishes, as previously suggested by Ammann (2001). Each SMURF consisted of a plastic mesh cylinder (1.0 × 0.35 m in diameter) as described by Ammann (2001). Three SMURF were moored 7 m apart over sandy substratum in 4.5 m of water off Doran Beach in Bodega Bay, California, U.S.A. (38° 18' N; 123° 04' W). Recruitment was evaluated among SMURF in close proximity to control for spatial variation in delivery of recruiting fishes to different treatments (Valles *et al.*, 2006). SMURF were attached to cement anchors using a 7.8 m nylon line. A float was secured to the line at 3.7 m and to the free end of the line. A weight was attached between the floats to keep the line vertical as tides ebbed and flowed. SMURF were attached using longline clips and were permitted to float vertically in the water column with the shallowest end being situated 1.5 m below the sea surface.

One year in advance of this study, three SMURF were deployed in Bodega Harbor during which time two were lost before the start of the field trials. Recruitment of fishes to the remaining heavily fouled SMURF and two lightly fouled SMURF, which had been immersed in a flow-through outdoor tank at the Bodega Marine Laboratory for less than a week, were compared by deploying them on separate moorings from 17 to 26 May 2004. The heavily fouled SMURF was densely covered by algae and invertebrates, whereas lightly fouled SMURF accumulated only a thin film of algae. SMURF were sampled every third day for nine days. Many more fishes appeared to recruit to the heavily than the lightly fouled SMURF. These data were not analysed statistically because only one heavily fouled SMURF was available for this initial trial. The results, however, prompted the continuation of examining the effect of fouling on settlement preferences, and these data later were combined with the results of a second field trial for analysis. The second field trial was immediately conducted from 26 May to 4 June 2004 by pairing fouled SMURFs from the first trial with unfouled SMURF on each mooring. SMURF were sampled again every third day for nine days. The new design was chosen for two reasons. First, strong upwelling reduced visibility to <1 m during the first trial as is typical for this region, thereby diminishing the ability of recruits to discriminate visually between treatments on separate moorings. Second, investigators that place SMURF close to each other (Valles *et al.*, 2006) or immediately adjacent to natural habitats (Ben-David & Kritzer, 2005) might underestimate recruitment if recruits prefer fouled substrata. Although pairing fouled and unfouled SMURF on the same mooring can be considered to be non-independent, this design is needed to evaluate the ability of fishes to choose between treatments while controlling for spatial variation in delivery.

During both field trials, SMURF were sampled by free-diving and the order that they were sampled was alternated between sampling dates. Each SMURF was enveloped in a Binke net as described by Ammann (2001). SMURF were anchored

at a single end so that the net could slip over it without requiring handling. The netted SMURF was doused with sea water and shaken over an aerated tub to remove recruits. Fishes were counted and released in eelgrass *Zostera marina* beds of Bodega Harbor. Unfouled SMURF were thoroughly cleaned using a freshwater spray nozzle.

The mean abundance of recruits to different levels of fouling on SMURF was analysed using repeated-measures regression models that incorporated variation among collection dates, and replicates including the increase in variation during large recruitment events (Diggle *et al.*, 2002). A  $\log_{10}$  linear relationship between abundance and predictors was assumed, with a general model of the form:  $\log_{10}A = D + F + R$ , where  $A$  represents abundance,  $D$  is the date effect,  $F$  is the fouling effect and  $R$  is the effect of random variation among replicates. Two hypotheses were tested: (1) the overall hypothesis that fouling changes the average abundance and (2) the sub-hypothesis that heavy fouling has a greater effect, than light fouling. Two different estimation strategies were used. First, a standard general linear model was used to analyse  $\log_{10}$ -transformed data, which enabled the inclusion of data from both field trials. Second, a standard general linear mixed model that estimated log abundance while assuming a Poisson distribution was used. This approach required

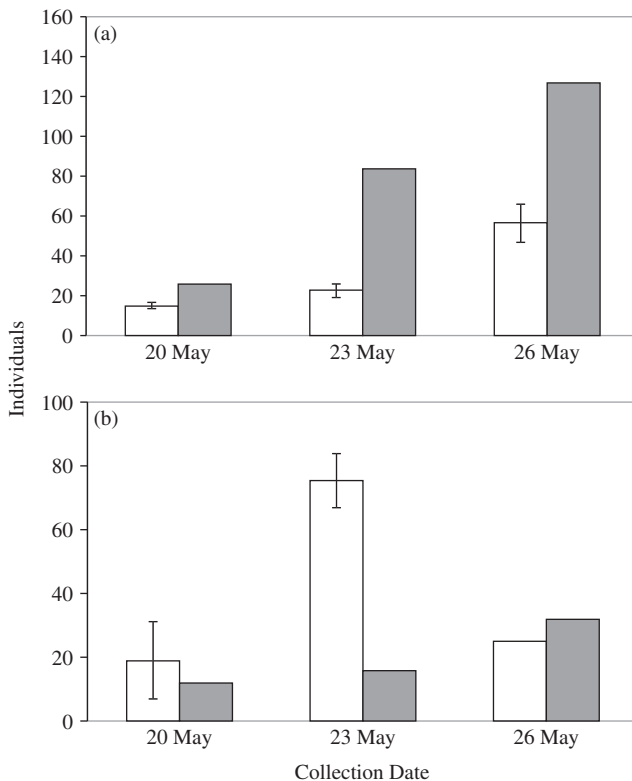


FIG. 1. Mean  $\pm$  95% CI recruitment of (a) 416 *Sebastes melanops* and (b) 288 *Sebastes caurinus* to two lightly ( $\square$ ) and one heavily fouled ( $\blacksquare$ ) standard monitoring unit for recruitment of fishes (SMURF) moored 7 m apart. SMURF were sampled every third day from 17 to 26 May 2004. Note the scale change for the two species.

at least two observations per mooring, which limited its application to the second field trial. All tests were two-sided at a level of 0.05 and were analysed using SAS version 9.1 (www.sas.com).

Two species of fishes recruited in sufficient numbers to be analysed during the first trial, and the relationship between them was determined using a Pearson correlation. The number of invertebrates inhabiting each SMURF was quantified at the end of the field trial by sieving contents through a plankton net (335 µm mesh) and viewed under a dissecting microscope to classify them into taxonomic categories.

During the first trial, 416 *Sebastes melanops* Girard and 288 *Sebastes caurinus* Richardson recruited to SMURF, and recruitment of the two species was negatively correlated (Fig. 1; Pearson correlation,  $n = 1, -0.264$ ). *Sebastes melanops* appeared to be more abundant in the heavily fouled SMURF across all sampling dates and recruitment appeared to increase over time. In contrast, *S. caurinus* did not appear to be more abundant in the heavily fouled SMURF. *Sebastes caurinus* appeared to recruit in similar numbers on both types of collectors on two sampling dates and in much greater numbers in the lightly fouled SMURF on one date.

During the second trial, 5436 *S. melanops* and few *S. caurinus* recruited to collectors (Fig. 2). Fouled SMURF attracted an estimated 51% more *S. melanops* recruits across all dates than did unfouled SMURF (Table I). Two more interesting patterns were observed. The unfouled SMURF that was deployed in tandem with the heavily fouled SMURF appeared to consistently accumulate more *S. melanops* recruits than the other lightly fouled SMURF. Recruitment also appeared to steadily increase during the course of the trial, as it did for *S. melanops* during the first trial.

When recruitment during both trials was combined, light fouling increased recruitment by an estimated 43%, and heavy fouling increased abundance by an estimated 59% relative to clean SMURF (Table II). The alternate  $\log_{10}$ -transformation model for the entire dataset yielded similar estimates.

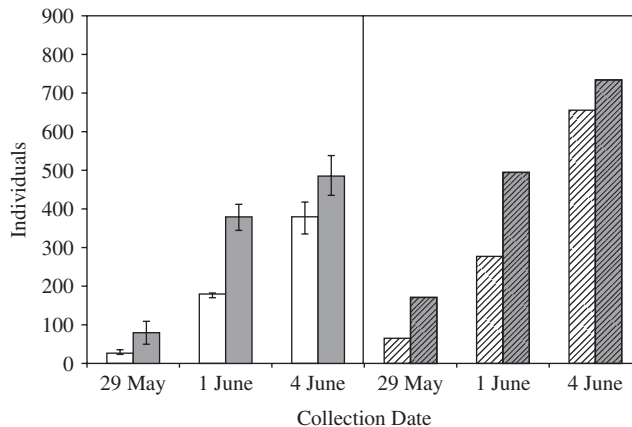


FIG. 2. Mean  $\pm$  95% CI recruitment of 5436 *Sebastes melanops* on (a) a pair of unfouled (□) and lightly fouled (■) standard monitoring unit for recruitment of fishes (SMURF) that were attached to each of two moorings and (b) a pair of unfouled (▨) and heavily fouled (■) SMURF that were attached to a third mooring. SMURF were sampled every third day from 26 May to 4 June 2004.

TABLE I. Generalized linear model of *Sebastes melanops* recruitment during the second field trial using unfouled standard monitoring unit for the recruitment of fishes (SMURF) on 4 June 2004 as reference populations relative to fouled SMURF and assuming a Poisson distribution

Variable	Estimate*	S.E.	P	% increase
Intercept	6.451	0.124	<0.001	
29 May	-1.908	0.154	<0.001	
1 June	-0.500	0.085	<0.001	
Fouled	-0.415	0.054	<0.001	51 <sup>†</sup> (95% CI 36–69)

\*Coefficients are the expected values of the log of abundance by sampling date and presence of fouling.

<sup>†</sup>The abundance of fish in fouled SMURFs across all days was estimated to be 51% higher (with 95% CI 36–69%) compared with unfouled SMURF ( $P < 0.001$ ).

TABLE II. Generalized linear model of *S. melanops* recruitment during both field trials using unfouled SMURF on 4 June 2004 as reference populations relative to lightly and heavily fouled SMURF and assuming a Poisson distribution

Variable	Estimate*	S.E.	P value	% Increase <sup>†</sup>
Intercept	6.0267	0.0831	<0.001	
May 29	1.9204	0.1539	<0.001	
June 1	0.5026	0.0854	<0.001	
Fouled	0.3603	0.0918	<0.001	43 (95% CI 20–72)
Heavily fouled	0.1488	0.0106	<0.001	59 (95% CI 34–90)

\*Coefficients are the expected values of the log of abundance by sampling date, light fouling and heavy fouling.

<sup>†</sup>Light fouling increased abundance by an estimated 43% and heavy seasoning by an additional 16%.

A wide variety of invertebrates recruited to the SMURF during the study. Small numbers of polychaetes, mussels, nudibranchs, barnacles and holothuroids settled on all SMURF. Lightly fouled SMURF contained more bryozoans, gastropods, copepods and amphipods than did unfouled SMURF that were deployed for 3 days (Fig. 3). The heavily fouled SMURF was covered with crabs, barnacles, limpets, tunicates, hydroids, echinoids, polychaetes and an algal mat, and its wet mass was 4.4 kg greater than clean SMURF.

Biofouling of SMURF was highly significantly associated with recruitment of *S. melanops*. Many more of the 5852 *S. melanops* recruited to SMURF that were most fouled, and results were consistent across all sampling dates. A clear preference of *S. melanops* for fouled SMURF was evident despite the brevity and low replication of the study, because sampling coincided with a large recruitment event. The regression analyses showed that biofouling of SMURF was associated with higher abundance of *S. melanops* across all dates. This pattern probably was not generated by spatial variation in the delivery of recruits, because SMURF were placed in close proximity to one another and the trend occurred across all sampling dates. *S. melanops* may have been attracted to fouled SMURF for several reasons. First, fouled SMURF contained more small invertebrates, a potential food source, than lightly or unfouled SMURF (Fig. 3). Second, macroalgae on fouled SMURF may have provided additional structure for *S. melanops* recruits (Carr, 1991; Anderson,

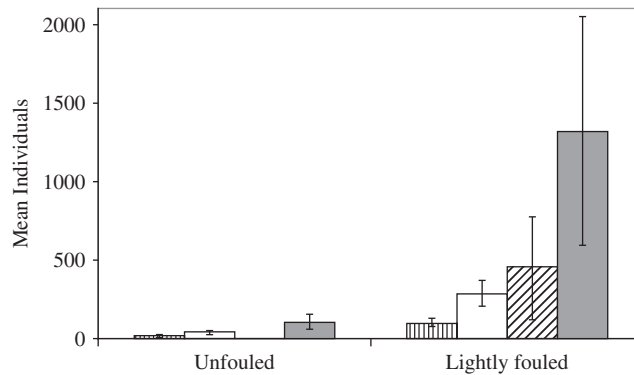


FIG. 3. Mean  $\pm$  s.d. number of snails (▨), amphipods (□), bryozoans (▧) and copepods (■) colonizing unfouled and lightly fouled standard monitoring unit for recruitment of fishes (SMURF) that were deployed for 3 days.

1994; Levin & Hay, 1996). Third, chemical cues emitted by biotic fouling may have attracted and induced reef fishes to settle (Lecchini *et al.*, 2005).

Intra and interspecific interactions may have modified settlement patterns. Intraspecific interactions among *S. melanops* may explain why recruits were more abundant on SMURF that were deployed next to the more attractive heavily fouled SMURF. Fishes attracted to the heavily fouled SMURF may have ‘spilled over’ to less fouled, less attractive neighbouring SMURF due to crowding of the heavily fouled, heavily populated SMURF. This was particularly apparent during a large recruitment pulse when the heavily fouled SMURF sheltered >700 fish (Fig. 2). Interspecific interactions may explain why *S. caurinus* did not recruit more to fouled than to unfouled SMURF. Larger *S. melanops* may have displaced *S. caurinus* to less attractive substrata. Inhibition and predation by species that are relatively large at settlement has been suggested previously to reduce recruitment of species that settle at smaller sizes on SMURF (Ammann, 2001). Other factors that can affect estimates of recruitment include the timing of recruitment between sampling periods and the arrival order of species (Sutherland, 1974).

The present study suggests that biofouling can influence recruitment of fishes even when SMURF are deployed in close proximity to unfouled SMURF and when SMURF have been fouled for only 3 days. Consequently, investigators may wish to consider standardizing fouling levels to ensure accurate estimates of recruitment over space and time, as previously recommended by Ammann (2001). Daily replacement of SMURF with clean ones minimizes biofouling and provides an unbiased estimate of recruitment. This will often be impractical in long-term studies, however, and weekly or monthly cleaning and sampling intervals may be necessary. In this case, visually ranking the level of biofouling can account for the effect of biofouling on fish recruitment (Ammann, 2001). Investigators also may wish to capitalize on the attractiveness of biofouling to increase recruitment when SMURF are deployed near potentially more attractive natural habitat (Ben-David & Kritzer, 2005). In this case, time could be treated as a covariate to account for the increasing attractiveness of SMURF as they become increasingly fouled during the course of a long-term study.



Estimates of recruitment using this approach could vary spatially if the composition of fouling communities on SMURF changes across the study area.

Artificial substrata have been important for estimating larval recruitment of invertebrates for decades and the application of this approach in estimating recruitment of reef fishes is an important advance. The approach can be particularly effective in determining the relative importance of potential transport mechanisms regulating larval delivery to shore in time and space. The present brief study reinforces the recommendation by Ammann (2001) that biofouling should be considered when estimating larval recruitment. After all, it has become increasingly apparent that reef fishes settle into preferred natural habitats, producing much of the structure that is evident in reef communities (Booth & Wellington, 1998; Guitierrez, 1998). The next step is to determine the generality of these findings by conducting more extensive studies with other species at other locations.

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