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Variations in sampling procedure and frequency affect estimates of recruitment of barnacles

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ABSTRACT: The influence of variations in sampling procedure and frequency on estimates of recruitment of the barnacle *Semibalanus balanoides* (L.) on an intertidal rocky shore at Sandy Cove, Nova Scotia, Canada was determined from approximately daily monitoring of recently settled individuals. The effect of sampling procedure (removing or not removing barnacles after sampling) on estimates of recruitment varied with intertidal height. There was no effect of sampling procedure in the mid-intertidal zone, but in the low zone, where recruitment was on average 4 times larger, the estimate of recruitment was significantly greater from quadrats where barnacles had been removed after sampling. Estimates of recruitment and post-settlement mortality decreased exponentially as the sampling frequency decreased. In both the mid- and low-intertidal zones, significantly larger estimates of recruitment and post-settlement mortality were obtained when sampling every 1.3 d compared to sampling ca every 2 d. These findings indicate that comparisons of results between studies will be difficult if estimates of recruitment or post-settlement mortality are confounded by variations in sampling frequency. Unless individuals can be tracked over time, estimates of recruitment made by sampling without removal of recruits will tend to underestimate settlement by incorporating some post-settlement mortality, the magnitude of which will be directly related to the interval between samples. Without quantitative data on the effect of sampling frequency on estimates of recruitment and post-settlement mortality, results of tests of hypotheses requiring accurate estimates of recruitment or post-settlement mortality may be compromised if sampling is not done as frequently as possible.

INTRODUCTION

Renewed interest in variations in recruitment as a primary determinant of population structure in marine organisms with planktonic larvae (Connell 1985, Lewin 1986, Young 1987, Underwood & Fairweather 1989) has led to increased sampling of the distribution and abundance of early stages of life-history. Accurate estimation of recruitment is particularly important in those studies that measure recruitment as a predictor of the distribution and abundance of adult populations. This requires careful sampling at frequent intervals due to the small size of recently settled individuals and the fast rate of mortality which may occur shortly after settlement. Unfortunately, limitations of sampling in the field (e.g. constraints imposed by weather, availability of personnel, and/or funding) often preclude frequent sampling and lead to methodological differences among studies that may hamper comparisons of recruitment [see examples in Connell (1985) for hard-substratum organisms, Butman (1987) for soft-sediment organisms, and Booth (1991) for damselfish]. Also, recruitment is commonly estimated by destructive sampling (i.e. by removing individuals) although this may yield estimates which differ from those obtained by non-destructive sampling. An understanding of the consequences of different sampling methods in estimating recruitment is necessary to evaluate the validity of predictive models and general theories based upon them.

Recruitment of sessile marine invertebrates with planktonic larvae is generally defined as the number of individuals that have survived some time after set-

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tlement, i.e. until the investigator has sampled the population (Keough & Downes 1982, Underwood & Denley 1984, Connell 1985). As such, recruitment is a purely operational term that incorporates settlement (the attachment of larvae to the substratum) and post-settlement mortality (Keough & Downes 1982, Underwood & Denley 1984, Connell 1985). The magnitude of recruitment, therefore, often depends on how long after settlement the animals are counted. Settlement, a biological process, is difficult, if not impossible, to measure directly in the field, but may be estimated if sampling is frequent enough so that post-settlement mortality is negligible. To avoid inconsistencies in usage of the terms 'settlement' and 'recruitment' (see Connell 1985), it may be more practical to consider all estimates of the number of recently attached individuals as recruitment and to specify the sampling interval over which these estimates are made.

In this paper, we demonstrate how variations in procedure and frequency of sampling can significantly influence estimates of recruitment of a barnacle on an intertidal rocky shore. Further, we show how small differences in frequency of sampling can produce conflicting conclusions about differences in post-settlement mortality of barnacles between 2 heights on a shore, a discrepancy which could influence the construction of predictive models of the structure of adult populations.

METHODS

Records of recruitment for the barnacle *Semibalanus balanoides* (L.) used in this study are extracted from a larger data set collected at Sandy Cove, Nova Scotia, Canada in 1988 (Minchinton & Scheibling 1991). Approximately daily (occasionally every 2 d and once after 3 d) rates of recruitment were monitored at low tide in 12 randomly located quadrats (10 × 10 cm) in the mid- and low-intertidal zones. Immediately before the onset of barnacle settlement in 1988, the macrobenthic community was manually removed from all quadrats and the substratum was burned using a propane torch to remove algal films and crusts. Two procedures of sampling were used to compare estimates of recruitment in each zone: (1) barnacles on 6 of the 12 quadrats were removed after counting, hereafter called 'cleared' quadrats; and (2) barnacles on the other 6 quadrats were not removed after counting, hereafter called 'uncleared' quadrats.

For the cleared quadrats, recruitment over a given sampling period was estimated as the sum of the number of cyprids (settled larvae) and recently metamorphosed juveniles removed at each sampling date during that sampling period. For the uncleared quadrats, recruitment over a given sampling period was estimated as the sum of the increases in the number of barnacles between successive sampling dates during that sampling period. An increase in the number of barnacles between successive sampling dates was calculated by adding the number of cyprids counted on one sampling day to the difference between the number of metamorphosed individuals counted on that sampling day and the number of cyprids plus metamorphosed individuals counted on the previous sampling day. This method of estimating recruitment on uncleared quadrats may not be as accurate as mapping the location of individuals since it assumes that cyprids counted on one sampling date either metamorphosed or were dislodged or killed by the next sampling date, an assumption which may not always be true (Connell 1985).

The effect of sampling frequency on estimates of recruitment was assessed by purposefully excluding some sampling dates from the approximately daily records of recruitment on the uncleared quadrats. For example, estimates of recruitment would be calculated by using every second or every third date from the record. This generated a range of sampling frequencies and corresponding estimates of recruitment. Post-settlement mortality for each sampling frequency was estimated as the percentage difference between the estimate of recruitment for a given sampling frequency and the estimate of recruitment made once for the entire sampling period. Although the term mortality is used, it is possible that some cyprids may have resuspended themselves or were dislodged on the incoming tide and subsequently settled in other areas and may not have actually died. This estimate of post-settlement mortality can also be viewed as a direct measure of the percentage of the estimate of recruitment for a given sampling frequency that would otherwise go undetected if recruitment had only been measured once for the entire sampling period.

The effect of sampling frequency on estimates of recruitment and post-settlement mortality was determined from recruitment records for 2 sampling periods: (1) a 44 d period (with samples taken on 35 d) from 26 April to 8 June 1988 in the mid-intertidal zone only; and (2) a 21 d period (with samples taken on 17 d) from 7 to 27 May 1988 in the mid- and low-intertidal zones. Identical sampling dates were used for comparisons between intertidal zones during the 21 d period. These sampling periods corresponded with times of greatest recruitment in 1988 (Minchinton & Scheibling 1991). Sampling frequency is expressed as its inverse, using an average sampling interval, and is calculated as the number of days divided by the number of sampling intervals during a sampling period.

RESULTS

Effect of sampling procedure

Sampling procedure had a variable effect on the estimate of recruitment depending on the sampling location (i.e. whether in the mid or low zone). For samples taken at intervals of 1.3 d from the mid-intertidal zone during the 44 d period, the estimate of recruitment was not significantly different between cleared and uncleared quadrats (*t*-test, $t = -0.82$, $p > 0.05$; variances homogeneous according to F_{max}-test, $F_{max} = 2.15$, $p > 0.05$; untransformed data) (Fig. 1). For samples taken at intervals of 1.3 d from the mid- and low-intertidal zones during the 21 d period, there was a significant interaction between zone and sampling procedure (2-factor analysis of variance, $F_{1,20} = 13.39$, $p < 0.01$; variances homogeneous according to Cochran's test, $C = 0.57$, $p > 0.05$; untransformed data) (Fig. 1). The estimate of recruitment was significantly greater in the low-intertidal zone than in the mid zone regardless of whether quadrats were cleared or uncleared. In the mid-intertidal zone, the estimate of recruitment was not significantly different between cleared and uncleared quadrats, whereas in the low zone there was significantly greater recruitment on cleared than on uncleared quadrats (Student-Newman-Keuls procedure, $\alpha = 0.05$).

Effect of sampling frequency

Estimates of recruitment and post-settlement mortality on uncleared quadrats decreased exponentially as the sampling interval increased (Figs. 2 & 3). At short sampling intervals (1 to 3 d), small increases in the sampling interval result in a large reduction in the estimates of recruitment and post-settlement mortality, whereas at long sampling intervals (>1 wk), the converse is true (Fig. 2). As the time between samples increased, the number of individuals which settle and die without being sampled increased, until at relatively long sampling intervals (at a ca 2 wk sampling interval in this case), few of these individuals were detected and the estimate of post-settlement mortality approached zero (Fig. 2C).

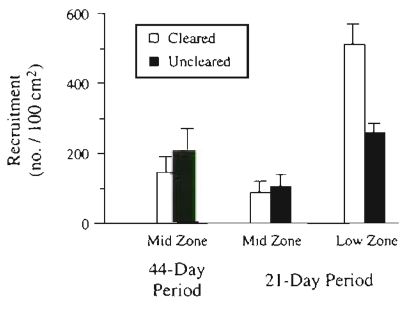

Fig. 1. *Semibalanus balanoides*. Effect of sampling procedure [barnacles removed (cleared) or not removed (uncleared) after counting] on the estimate of recruitment (mean ± 1 SE)

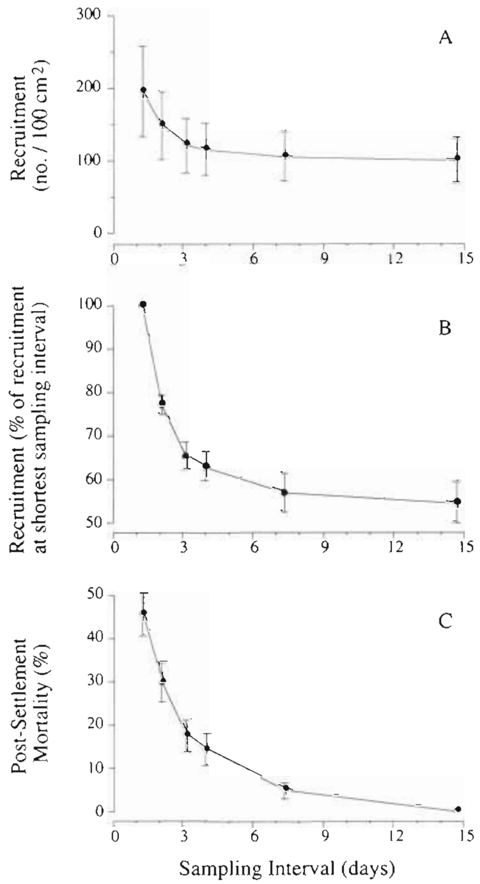

Fig. 2. *Semibalanus balanoides*. Effect of average sampling interval on estimates of: (A) recruitment; (B) recruitment expressed as a percentage of recruitment at the shortest interval (1.3 d); and (C) post-settlement mortality on uncleared quadrats in the mid-intertidal zone for a 44 d sampling period (26 April to 8 June 1988). Values given are means ± 1 SE

Because of the large variation in recruitment among quadrats at each sampling interval (Figs. 2A & 3A), the estimate of recruitment for the shortest sampling interval (1.3 d) was used to standardize estimates for longer intervals (Figs. 2B & 3B). The estimate of recruitment made by sampling every 2.1 d during the 44 d period in the mid-intertidal zone was 77 % of, and significantly smaller than, the estimate made by sampling every 1.3 d (paired *t*-test, $t = 2.90$, $p < 0.05$; untransformed data) (Fig. 2B). Similarly, estimates of recruitment made by sampling every 2.3 d during the 21 d period in the mid- and low-intertidal zones were significantly smaller than estimates made by sampling every 1.3 d (paired *t*-tests: mid zone,

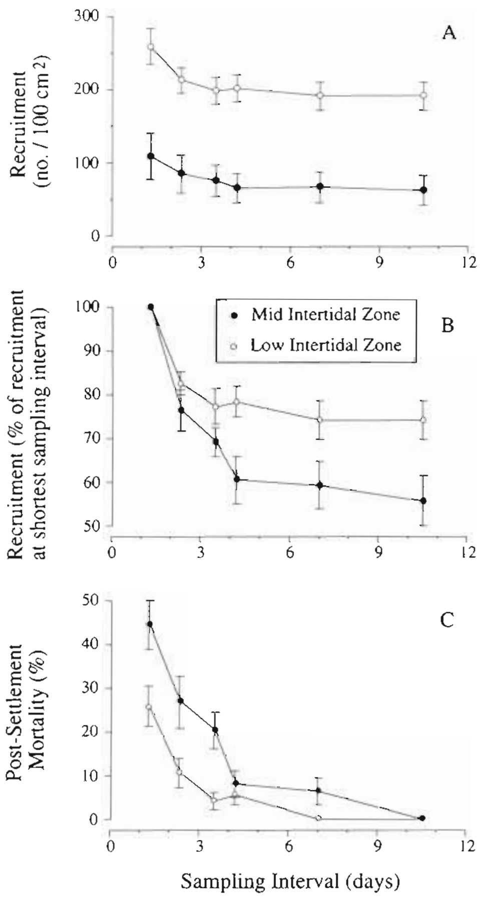

Fig. 3. *Semibalanus balanoides*. As in Fig. 2 but for quadrats in the mid- and low-intertidal zones and a 21 d sampling period (7 to 27 May 1988). Values given are means ± 1 SE

t = 3.34, $p < 0.05$; low zone, t = 5.23, $p < 0.01$; untransformed data) (Fig. 3B). During the 44 d period in the mid-intertidal zone and the 21 d period in the mid- and low-intertidal zones, estimates of post-settlement mortality made by sampling about every 2 d were significantly smaller than estimates made by sampling every 1.3 d (paired t-tests: mid zone during 44 d period, t = 11.67, $p < 0.001$; mid zone during 21 d period, t = 4.06, $p < 0.05$; low zone during 21 d period, t = 6.84, $p < 0.01$; untransformed data) (Figs. 2C & 3C). It should be noted that estimates of recruitment and post-settlement mortality for each sampling interval were made using data from the same quadrat and, therefore, are non-independent. Consequently, if tests were done to compare estimates from several sampling intervals, the non-independence could alter the probability of making a Type I error.

When estimates of recruitment were compared between the mid- and low-intertidal zones for the same sampling interval during the 21 d period, the estimate of recruitment was always significantly greater in the low zone (t-tests, $p < 0.05$; variances for each comparison homogeneous according to F_{max}-test, $p > 0.05$; untransformed data) (Fig. 3A). In contrast, results of comparisons of estimates of post-settlement mortality between zones for the same sampling interval depended on the sampling interval (Fig. 3C). At sampling intervals of 1.3, 2.3 and 3.5 d, post-settlement mortality was significantly greater in the mid-intertidal zone than in the low zone, whereas at a sampling interval of 4.2 d there was no significant difference in post-settlement mortality between the 2 zones (Table 1). The outcome may even be reversed if the 2 intertidal zones are sampled at different sampling frequencies (Fig. 3C). Compared to the estimate of post-settlement mortality for the low-intertidal zone at a sampling interval of 1.3 d, the estimate for the mid zone was significantly greater when sampled every 1.3 d, not significantly different when sampled every 2.3 to 3.5 d, and significantly smaller when sampled every 4.2 d (Table 1).

Interestingly, recruitment and post-settlement mortality in the low intertidal zone was greater at a sampling interval of 4.2 d than at a sampling interval of 3.5 d (Fig. 3). This is an unexpected result of large recruitment and great early mortality on one date that was included in the record for the estimation of recruitment at the 4.2 d sampling interval but not at the 3.5 d sampling interval. Thus, estimates of recruitment may be particularly underestimated when an organism arrives in pulses. Under these circumstances, frequent sampling will be particularly necessary.

DISCUSSION

We have shown that differences in procedure and frequency of sampling can result in significant variation in the estimates of recruitment and post-settle-

Table 1. Matrix of results of t-tests (α = 0.05) for differences in estimates of post-settlement mortality between the low- and mid-intertidal zones (L and M respectively) at various sampling intervals (d). Data are presented in Fig. 3C. Variances for each comparison were homogeneous according to F_{max}-test, $p > 0.05$. Analyses were done on untransformed data

Low-intertidal zone: Sampling interval ↓ / Mid-intertidal zone: Sampling interval →	1.3	2.3	3.5	4.2
1.3	M > L	M = L	M = L	M < L
2.3		M > L		
3.5			M > L	
4.2				M = L

ment mortality of barnacles. These results should not be surprising since different procedures usually do yield different results and more frequent sampling of a transient organism (e.g. a larva that settles and then may die soon after) will generally result in larger estimates of its density and mortality. What is most striking is that significantly larger estimates of recruitment and post-settlement mortality were obtained when the interval between samples was reduced from about every 2 d to every 1.3 d. One should note, however, that this small reduction in sampling interval represents a 60 to 70 % increase in sampling effort. Estimates of recruitment from data collected every tidal cycle may demonstrate that even daily sampling is too infrequent for an accurate estimation of settlement. Booth (1991) also found that sampling frequency influenced estimates of recruitment of damselfish on corals and suggested that, due to great post-settlement mortality, accurate estimates of rates of settlement of these fish would only be obtained at sampling intervals smaller than 2 d.

Although settlement is extremely difficult to measure in the field, it may be estimated by progressively sampling at increasing frequencies (decreasing intervals) until the most practicable sampling frequency is reached (and this will vary depending on available resources and technology). An accurate estimation of settlement thus may be attained when increases in sampling frequency do not result in any significant increase in estimates of recruitment. This approach would give an operational definition of settlement that could be used in a pilot study to assess the potential costs of sampling associated with testing particular hypotheses that require accurate estimates of settlement. If logistical constraints such as funding or time preclude sampling with the necessary frequency to estimate settlement accurately, then the study should not proceed or its hypotheses should be modified.

Estimates of recruitment from the low intertidal zone were significantly greater on cleared than on uncleared quadrats, but the opposite trend was observed in the mid intertidal zone, although the difference was not significant. Removing recruits after sampling also resulted in a greater estimate of recruitment of damselfish on corals compared to when recruits were not removed (Booth 1991). Unless individuals can be tracked over time, estimates of recruitment made by sampling without removal of recruits will tend to underestimate settlement by incorporating some post-settlement mortality, the magnitude of which will be directly related to the interval between samples (Booth 1991).

Recruitment was, on average, about 4 times greater in the low-intertidal zone than in the mid zone, and only in the low zone were significant differences detected between cleared and uncleared quadrats. In other studies in the low-intertidal zone at Sandy Cove, we found that larvae of *Semibalanus balanoides* initially settle in greater densities at uncleared than at cleared sites, but as the settlement period progresses and uncleared sites become more populated, larvae avoid these densely colonised areas and settle in greater densities at sites cleared of organisms (Minchinton & Scheibling 1993; also see Connell 1985, Bertness et al. 1992). Such spatial and temporal variation in habitat selection at settlement makes it difficult to determine the relationship between larval supply and settlement. We found that the availability of barnacle cyprids and the rate of settlement on cleared quadrats were more highly correlated later than earlier in the settlement period, presumably because these quadrats were initially less attractive to earlier-arriving cyprids (Minchinton & Scheibling 1991; also see Bertness et al. 1992). It is likely that interactions among the conditions resulting from the sampling procedure, the magnitude of recruitment to an area, and habitat selection by the larvae could confound estimates of recruitment.

Our results show how differences in the frequency of sampling can reverse the outcome of studies designed to compare the survivorship of recently settled larvae in different intertidal zones. Although researchers may intend to sample all treatments with equal frequency, inclement weather or differential accessibility of sampling units may result in one treatment being sampled more frequently than others during an experiment. More commonly, however, variations in sampling frequency will exist among studies of different researchers. If these researchers are testing similar hypotheses, comparisons of results between studies will be difficult as estimates of recruitment and post-settlement mortality may be confounded by variation in the frequency and other methods of sampling (Underwood & Fairweather 1986, Booth 1991). This could lead to erroneous conclusions regarding the relative importance of settlement versus post-settlement mortality in determining the distribution and abundance of adult populations. Booth (1991) cited the recruitment limitation hypothesis (see Doherty 1982) as one example where actual rates of settlement must be estimated to be able to test the hypothesis. This would necessarily involve frequent sampling.

Although most researchers will recognize this limitation and be careful to report their frequency of sampling and mention that they may be underestimating settlement and early mortality, without quantitative data on the effect of sampling frequency on the variable of interest, it will remain uncertain whether recognition of a potentially serious problem is enough. Ignoring the consequences of variations in sampling on results will impede the refinement of models from which more general predictions can be tested.

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