

**Abstract**—Estimates of larval supply can provide information on year-class strength that is useful for fisheries management. However, larval supply is difficult to monitor because long-term, high-frequency sampling is needed. The purpose of this study was to subsample an 11-year record of daily larval supply of blue crab (*Callinectes sapidus*) to determine the effect of sampling interval on variability in estimates of supply. The coefficient of variation in estimates of supply varied by 0.39 among years at a 2-day sampling interval and 0.84 at a 7-day sampling interval. For 8 of the 11 years, there was a significant correlation between mean daily larval supply and lagged fishery catch per trip (coefficient of correlation  $[r]=0.88$ ). When these 8 years were subsampled, a 2-day sampling interval yielded a significant correlation with fishery data only 64.5% of the time and a 3-day sampling interval never yielded a significant correlation. Therefore, high-frequency sampling (daily or every other day) may be needed to characterize interannual variability in larval supply.

Manuscript submitted 27 January 2012.  
Accepted 24 August 2012.  
Fish. Bull. 110:451–457 (2012).

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## Effect of sampling interval on estimates of larval supply

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Estimation of the supply of larval shellfishes and finfishes entering estuarine nursery habitats can provide important information on variability in year-class strength of many commercially important coastal species. The episodic nature of larval supply is a significant challenge to the generation of accurate estimates of larval supply (e.g., Dixon et al., 1999). Sampling must be conducted frequently enough to capture pulses in larval supply, but frequent sampling is costly and time consuming. Hettler et al. (1997) examined the effect of reduced sampling frequency by subsampling from a data set of daily supply of larval Atlantic menhaden (*Brevoortia tyrannus*) and determined that the variability of annual estimates of larval supply was “acceptable” [their term] on the basis of the standard error of estimates at sampling intervals of 7 days or less. Intervals of 14 and 30 days were deemed to be too long to be useful for estimation of annual larval abundance. Because their analysis was derived from a single year of data on daily larval supply, it did not account for interannual variations in the pattern of daily supply. In a long-term study of settlement of postlarvae (megalopae) (a proxy for larval supply) of blue crab (*Callinectes sapidus*), we observed a difference of 2 orders of magnitude in larval supply among years (Ogburn et al., 2012). This variability was due to changes in 1) the magnitude of daily larval supply, 2)

the length of the larval settlement season, and 3) the number of pulses in settlement that occurred each year. Such interannual variability has the potential to strongly affect estimates of larval supply derived at a sampling interval of 7 days. The purpose of this study was to evaluate the effect of sampling at intervals of 1, 2, 3, 4, 5, and 7 days on the variability of estimates of annual larval supply derived from an 11-year record of blue crab megalopal settlement.

Adult blue crabs live in estuaries of the western Atlantic from Nova Scotia to Argentina. Mature females migrate to coastal areas where they release several clutches of larvae (Carr et al., 2005, Dickenson et al., 2006, Darnell et al., 2009). Planktonic larvae develop in 30–50 days in surface waters of the continental shelf (Costlow and Bookhout, 1959) and return to estuarine nursery habitats as megalopae (Orth and van Montfrans, 1987). The settlement of megalopae in estuaries is higher during nighttime flood tides than during daytime flood tides or during ebb tides (DeVries et al., 1994), but the annual pattern of larval settlement depends on the interaction between atmospheric forcing mechanisms and nighttime flood tides (Little and Epifanio, 1991; Ogburn et al., 2009).

Interannual variability in blue crab larval supply may be an important source of variability in annual fishery landings (Forward et al., 2004;

Ogburn et al., 2012). Therefore, estimates of larval supply could be a useful tool for fisheries managers; however, to aid management, monitoring must be cost effective and time efficient even as it is conducted in ways that minimize sampling error. The objectives of this study were to determine the variation in estimates of annual larval supply at sampling intervals of 1, 2, 3, 4, 5, and 7 days and to compare the utility of estimates of annual larval supply derived from these different sampling intervals for predicting fishery landings.

## Materials and methods

Daily larval supply was determined through the collection of nightly megalopal settlement data at the dock of the Duke University Marine Laboratory in the Newport River estuary, North Carolina. Megalopae were sampled nightly with 3 replicate “hog’s hair” settlement collectors during the period from September to November in each of 8 years from 1993 to 2003; data were not collected in 1997, 1999, or 2001 (Forward et al., 2004). In 2004–06, the other 3 years in our 11-year record, data were collected from June to November (Ogburn et al., 2009). Collectors were rinsed in freshwater to remove megalopae according to standard protocols (Metcalf et al., 1995), and megalopae were identified to genus by following Ogburn et al. (2011). Some megalopae may have been the lesser blue crab (*Callinectes similis*), but these megalopae likely made up <5% of the total number of megalopae collected (Ogburn et al., 2012). Detailed descriptions of the methods that we used in this study can be found in Forward et al. (2004) and Ogburn et al. (2009). Before analysis, gaps in settlement time series were filled by using linear interpolation. Differences in the variability of daily larval supply among years were explored through calculation of the mean number of megalopae collected in each hog’s hair collector for each day (megalopae collector<sup>-1</sup> day<sup>-1</sup>), standard error of the mean, and index of dispersion (ID=variance/mean).

To generate estimates of annual larval supply at different sampling intervals, the daily supply data were subsampled at intervals of 2, 3, 4, 5, and 7 days because these intervals were the simulated sampling intervals used in Hettler et al. (1997). Subsampled time series were generated beginning with each possible start date such that there were 2 subsampled data sets at a 2-day interval beginning on either day 1 or day 2 of the original daily data, 3 data sets at a 3-day interval beginning on day 1, day 2, or day 3 of the original daily data, and so on. Mean daily settlement was used as the proxy for annual larval supply. For comparisons of interannual variability and sampling interval, we calculated the coefficient of variation (CV) of the annual means for the 11 years of data for each sampling interval.

Observed and subsampled estimates of annual larval supply were compared with data on fishery landings with correlation analysis. Data on fishery landings (in kilograms) and effort (catch per trip) were obtained from the North Carolina Division of Marine Fisheries

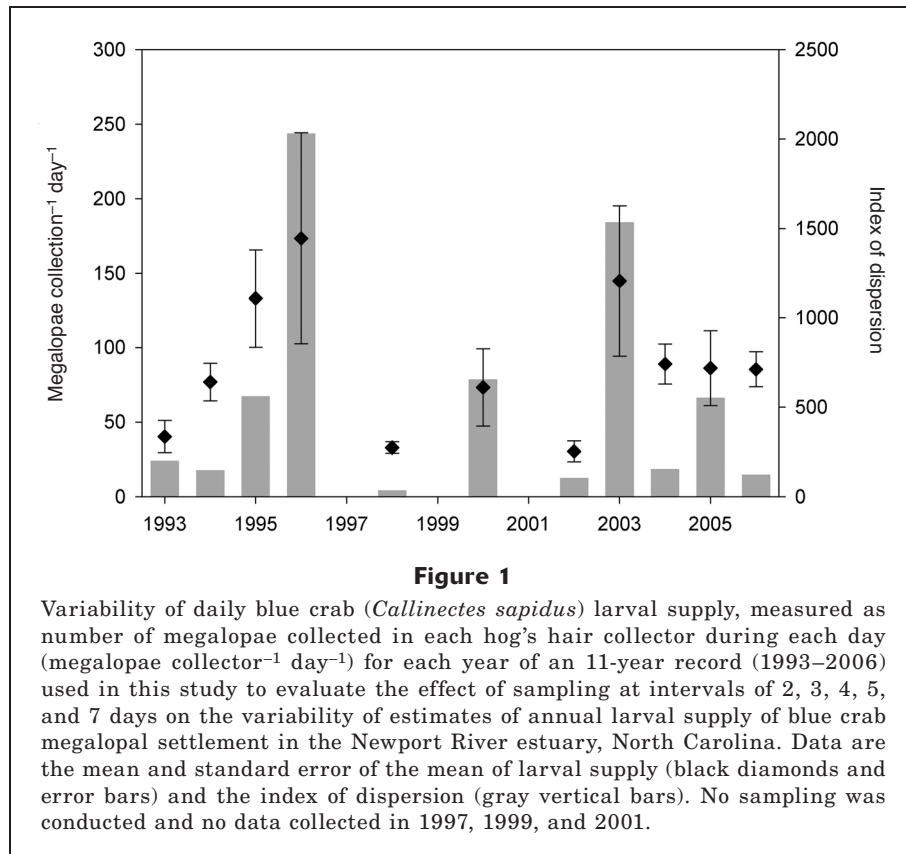
for hard, soft, and peeler crabs landed statewide in the crab pot fishery. Catch per unit of effort (CPUE) was compared with mean annual larval supply derived from daily sampling at a lag of 2 years, the approximate age at which crabs enter the fishery (Forward et al., 2004). Confidence intervals were calculated as in Wing et al. (1995). CPUE data were not available for comparison with 2005 and 2006 larval supply at the time of this analysis. One outlier (1994 larval supply and 1996 landings) was removed before analysis because of extremely high CPUE relative to larval supply. The remaining estimates of mean annual larval supply were significantly correlated with CPUE (coefficient of correlation [ $r$ ]=0.88,  $P$ =0.003). These estimates represent a useful sample data set derived from observed data for testing the effect of sampling interval on recruit–stock relationships. Please note that this analysis is not intended to be a recruit–stock analysis for the blue crab fishery in North Carolina. For such an analysis, see Ogburn et al. (2012).

Subsampling the data on daily larval supply generated more than one estimate of supply for each year, yielding many possible combinations of annual estimates. For example, there were 2 possible abundance estimates for each year at a 2-day sampling interval, depending on the start date of sampling (e.g., sampling start date of 1 September or 2 September). With 2 possible values for each of the 8 years of data, there were 2<sup>8</sup> or 256 possible data combinations. The number of possible combinations increased dramatically with sampling interval (Table 1). For the subsampled estimates of larval supply, significant correlations between lagged supply and CPUE were considered to be those correlations that exceeded the 95% confidence interval of the comparison between data from daily sampling and CPUE ( $r$ >0.69). Analyses were conducted in ActiveState Perl, vers. 5.10.0 (The Perl Foundation, Walnut Creek, CA<sup>1</sup>).

## Results

The variability of estimates of annual larval supply was higher in years dominated by a single large pulse in larval supply and increased as sampling interval increased. For the sample period of September to November, both the ID (calculated from daily data; Fig. 1) and the variation in estimates of annual larval supply at the 2-day sampling interval (Fig. 2) were lowest in 1998 (ID=34; 10.8–11.1 megalopae collector<sup>-1</sup> day<sup>-1</sup>) and highest at the 7-day interval in 1996 (ID=2027; 29–167 megalopae collector<sup>-1</sup> day<sup>-1</sup>). The mean CV of the 7-day sampling interval was more than 2.5 times the mean CV of the 2-day interval (Table 2). Extending the sampling period to June–November (2004–06 only)

<sup>1</sup> Mention of trade names is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.



resulted in a 20% decrease in the CV (from a mean of 0.27 to 0.22) at the 7-day interval (Table 2). The CV was typically small in years, such as 1998 (CV=0.34 at the 7-day interval), when supply was spread over many days (Fig. 3A) and large in years, such as 2000 (CV=0.99 at the 7-day interval), in which supply was dominated by a single pulse (Fig. 3B).

Subsampled estimates of annual larval supply were rarely correlated with CPUE of the crab fishery at sampling intervals >2 days. At a 2-day sampling interval, larval supply was significantly correlated with landings for 64.5% of possible comparisons (Table 1). There were no possible combinations of estimates of larval supply that were correlated with CPUE at the 3-day sampling interval. A small proportion of combinations (<15%) were significantly correlated with CPUE for longer sampling intervals, primarily because of an increase in the range of correlation coefficients with an increasing number of comparisons even as the mode decreased (Table 1).

## Discussion

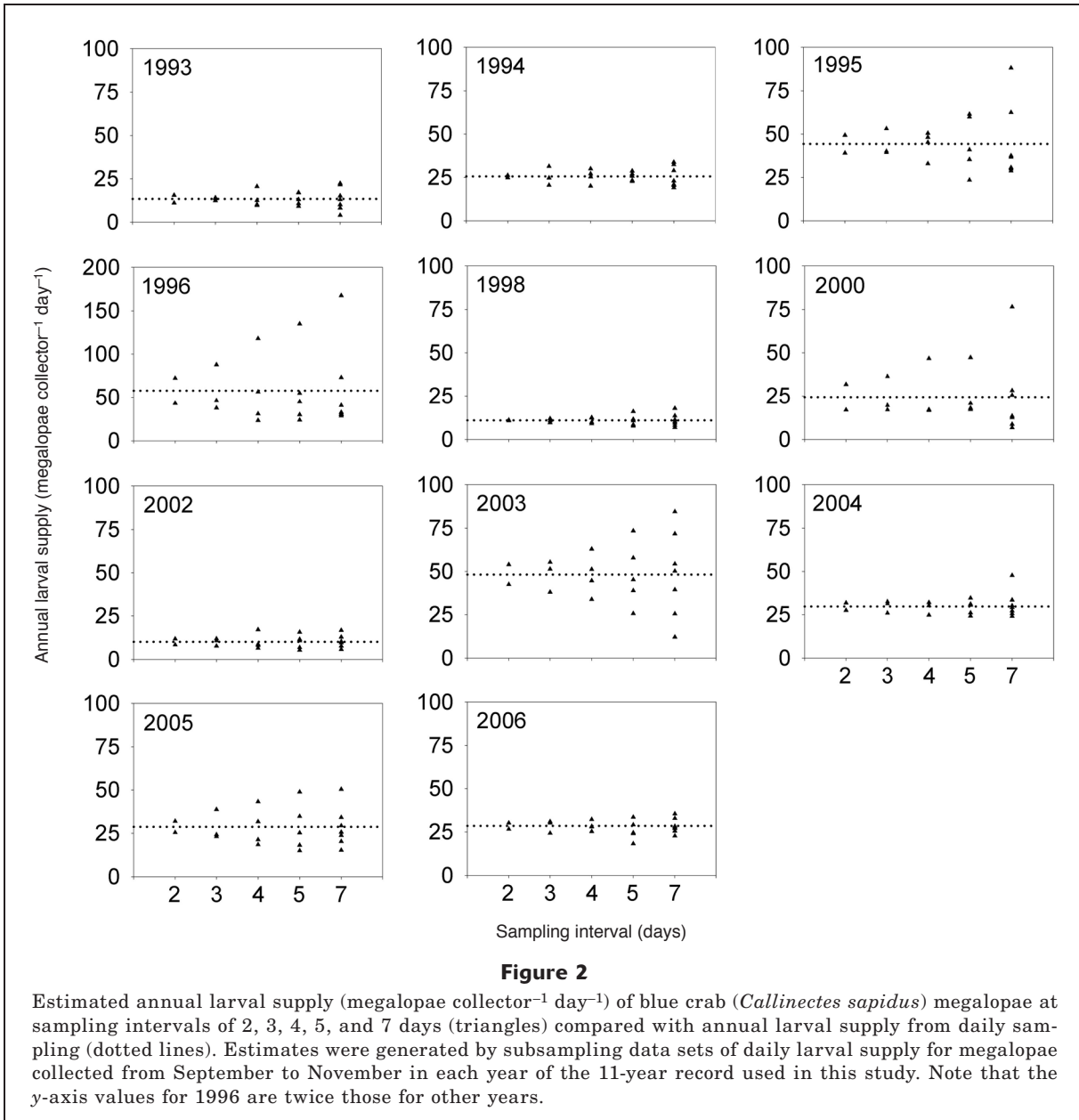
Monitoring the supply of larval shellfishes and finfishes is a costly and time-consuming endeavor. Researchers often use sampling intervals of a week or more to reduce the cost and effort associated with data collection (e.g., Warlen, 1994), but it is important to fully understand the

**Table 1**

Sampling intervals, numbers of possible comparisons between annual estimates of blue crab (*Callinectes sapidus*) larval supply and catch per unit of effort in the North Carolina blue crab fishery ( $N$ ), percentages of comparisons with significant correlations (type-1 error [ $\alpha$ ]=0.05 coefficient of correlation [ $r$ ]>0.69), modes of correlation coefficients, and differences between the lowest and highest values of correlation coefficients in this study to evaluate the effect of sampling at intervals of 1–7 days on the variability of estimates of annual larval supply derived from an 11-year record of blue crab megalopal settlement.

Interval	$N$	% significant	Mode	Difference
1	1	100	0.88	—
2	256	64.5	0.68	0.30
3	6561	0	0.59	0.41
4	65,536	2.3	0.56	0.87
5	390,625	7.4	0.52	1.08
6	1,679,616	7.9	0.54	1.10
7	5,764,801	13.8	0.5	1.22

consequences of choosing a particular sampling interval. Sampling at intervals longer in duration than the wind or tide-driven processes that underlie fluctuations in larval supply could result in high variability in esti-



mates of annual supply. Hettler et al. (1997) suggested that a weekly (7-day) sampling interval was sufficient for estimating the annual supply of larval Atlantic menhaden in the Newport River estuary, North Carolina, but their analysis did not account for interannual variability in the pattern of larval supply because it was based on a single year of data. We detected large differences in the variability of daily larval supply among years, suggesting that a single year of daily data collection is insufficient to characterize variability in larval supply. We recognize that our study is not directly comparable to that of Hettler et al. (1997) because of differences in sampling gear (settlement collectors vs. plankton nets) and potential differences in the temporal dynamics of larval supply among species. Regardless of these fac-

tors, if Hettler et al. (1997) conducted their study in a year of unusually episodic larval supply, they may have overestimated variability in annual estimates of supply at each sampling interval. More important, if their study was conducted in a year in which larval supply was relatively constant over time, they may have dramatically underestimated variability.

The increase of the sampling interval from daily to every other day (2-day interval) decreased the chances of detecting a significant correlation between annual blue crab larval supply and CPUE in the North Carolina pot fishery to only 65% when a significant correlation ( $r=0.88$ ) was observed for daily data. Significant correlations were never detected at a 3-day sampling interval but were detected at longer intervals, only

**Table 2**

Coefficients of variation for the September–November and June–November data sets on nightly blue crab (*Callinectes sapidus*) megalopa settlement. Data presented are for subsampling of the data sets of daily larval supply at intervals of 2, 3, 4, 5, and 7 days. Means and standard deviations (in parentheses) are included separately for the 2004–06 period for comparison between the September–November and June–November sampling periods; data were collected in the longer period from June to November only in 2004–06.

Sept.–Nov.	Sampling interval				
	2 days	3 days	4 days	5 days	7 days
1993	0.23	0.06	0.38	0.27	0.50
1994	0.04	0.21	0.16	0.10	0.24
1995	0.16	0.17	0.18	0.37	0.49
1996	0.35	0.45	0.75	0.77	0.88
1998	0.02	0.11	0.16	0.30	0.34
2000	0.43	0.43	0.61	0.54	0.99
2002	0.24	0.21	0.47	0.41	0.37
2003	0.17	0.19	0.25	0.38	0.52
2004	0.10	0.12	0.11	0.14	0.26
2005	0.16	0.30	0.39	0.48	0.40
2006	0.09	0.13	0.10	0.22	0.15
1993–2006 Mean	0.18 (0.13)	0.22 (0.13)	0.32 (0.22)	0.36 (0.19)	0.47 (0.26)
2004–06 Mean	0.12 (0.04)	0.18 (0.10)	0.20 (0.16)	0.28 (0.18)	0.27 (0.13)
June–Nov.	2 days	3 days	4 days	5 days	7 days
2004	0.03	0.14	0.04	0.05	0.20
2005	0.06	0.28	0.14	0.43	0.31
2006	0.04	0.01	0.06	0.06	0.14
2004–06 Mean	0.04 (0.02)	0.14 (0.14)	0.08 (0.06)	0.18 (0.22)	0.22 (0.09)

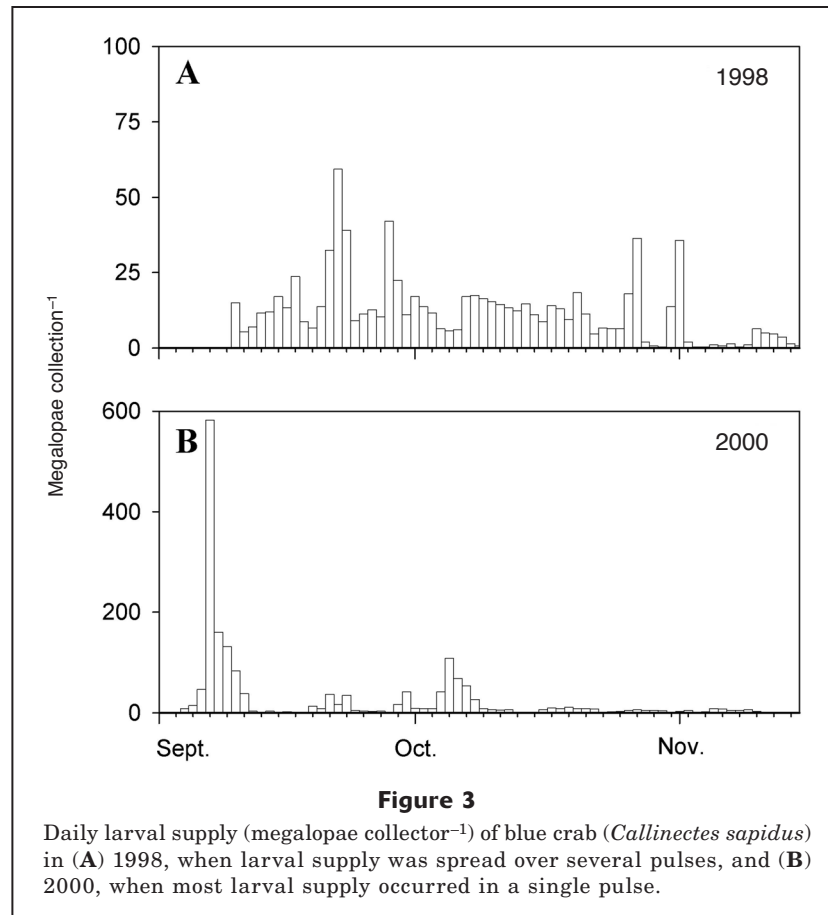
by chance as the range of correlation coefficients increased with an increasing number of possible data combinations (Table 1). This increase in the likelihood of detecting a significant recruit–stock relationship by chance at longer sampling intervals (nearly 14% at the 7-day interval) represents an additional concern for the design of larval-supply studies. Determining the level of variation that is appropriate for a particular sampling effort is not the subject of this article. However, if we set the maximum acceptable CV at 0.25, equivalent to 25% of the actual annual mean based on daily sampling, the 2-day sampling interval performed within this threshold in 9 of 11 years, but the 7-day interval provided a sufficient estimate of larval supply in only 2 of 11 years (Table 1). Assuming that extending the sampling period from 3 to 6 months would reduce the CV by 20% in all years, as observed in this study for 2004–06, we predicted there would have been no change in the number of years for which the CV exceeded 0.25. Therefore, scientists or managers must consider carefully the goals of multiyear larval surveys before they establish the appropriate sampling interval. For instance, weekly surveys probably are sufficient for estimation of seasonal trends in the supply, size, or age of larvae (Hettler et al., 1997). If the goal of a survey is to determine annual year-class strength, however,

weekly sampling can result in widely ranging estimates of annual larval supply, and sampling daily or every other day may be necessary. It should be noted that we assumed that larvae do not remain on collectors for more than one day. Longer sampling intervals may be appropriate if larvae are retained on or in collectors for the entire sampling interval and if there are no density-dependent effects of additional larvae.

## Conclusions

Determination of the acceptable level of variability in estimates of larval supply is the responsibility of the scientist or manager who is to conduct a particular survey. An increase in the sampling interval may reduce costs but can result in highly inaccurate estimates of abundance in some years. The episodic nature of blue crab larval supply appears to be consistent with other marine species (e.g., Warlen, 1994; Shanks and Roegner, 2007). Therefore, the results of this study are likely to apply across a wide range of marine species. If recruit–stock relationships are the goal of a larval-supply survey, we recommend that supply be sampled daily or every other day to maximize the likelihood of detection of significant relationships.





## Acknowledgments

We thank H. Diaz, R. Irvine, J. Lax, R. Mitchell, A. Schick, M. Smith, J. Thompson, and J. Venezia for assistance with larval sampling. Technical assistance was provided by J. Priddy. This study was supported under North Carolina Sea Grants 2004-1772-01, -33, and -43, National Science Foundation Grants no. OCE-9819355 and OCE-0095092, and U. S. Department of Commerce, NOAA, National Marine Fisheries Service, under PL 88-309, Project 2-296-R, NOAA Living Marine Resources Cooperative Science Center NA05OAR4811017.

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