

Temperature effects on the vertical distribution of lobster postlarvae (*Homarus americanus*)

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Abstract

In situ observations of postlarvae of the American lobster (*Homarus americanus*) were used to quantify behavioral depth regulation with respect to intrinsic and extrinsic factors. Postlarvae spent 65% of the time near the surface (0–0.5-m depth), which is less than expected based on previous plankton net surveys. The proportion of time spent at the surface decreased over the season (~0.80 to ~0.57) and was correlated with increasing depth of the 12°C isotherm. Postlarvae remained in waters above 12°C, suggesting that it may serve as a minimum temperature threshold. The seasonal trend was removed, and the residuals were used to examine the daily variation about the seasonal trend. These daily variations in the residuals were correlated with the depth of the thermocline. Residual data were also used to examine vertical distribution as a function of time of day. The proportion of time spent near the surface was lowest at midday and greatest in the morning and late afternoon. This shift in vertical distribution was not correlated with light intensity, indicating that it may be part of an endogenous rhythm. When the data were parsed with respect to light intensity, and developmental stage, an ontogenetic shift was observed from positive to negative phototaxis.

Larval settlement often drives the abundance of benthic populations (Gaines and Roughgarden 1985), and we are increasingly aware of the importance of larval behavior in the successful transport, delivery, and settlement of planktonic larvae to the benthos (Sponaugle et al. 2002). Larvae frequently encounter a nonhomogeneous environment, and their behavioral response to changing environmental conditions may have a profound effect on settlement. Unfortunately, our understanding of the interplay between larval behavior and hydrography in the period leading to settlement is often hindered by the inherent difficulty of sampling a planktonic phase that is hyper-dispersed and patchily distributed with unpredictable vertical distribution. The vertical distribution of larvae is particularly important as it may influence net transport, depth of settlement, and larval abundance as estimated from plankton samples.

The postlarva of the American lobster (*Homarus ameri-*

canus) presents an excellent example in which a life stage has been quantified extensively by using surface sampling techniques, yet the vertical distribution in response to changing environmental conditions in situ has not been well defined. Although the first three larval instars are broadly distributed in the water column, the postlarval stage is believed to be primarily neustonic and has been sampled at the surface in most studies quantifying distribution and abundance (for review, see Ennis 1995). Such abundance estimates have been used to estimate postlarval production, its relationship to settlement (Incze and Wahle 1991; Incze et al. 1997, 2000b), and ultimately landings in the lobster fishery (Fogarty and Idoine 1986; Miller 1997). The accuracy of abundance estimates derived from plankton samples relies on knowing what proportion of the population resides in the neuston. Most estimates from plankton net samples place 78–96% of the postlarval population in the top 0–0.8 m of the water column (Harding et al. 1982, 1987; Hudon et al. 1986) and rely on the tacit assumption that the postlarvae remain in the neuston for the duration of that stage. If significant intrastage behavioral or environmental changes occur, estimates of distribution and abundance based on neuston samples could be in error. Furthermore, the vertical swimming behavior of the postlarvae with respect to environmental variables may contribute to spatial and vertical patterns of settlement observed in the field.

Observations in the field and laboratory suggest that the vertical distribution of postlarvae is not constant, and that the proportion of the population represented in surface samples may vary with intrinsic and extrinsic factors. Strong thermoclines may serve as a thermal barrier to vertical movements, with postlarvae remaining above the thermocline. The most convincing evidence of this comes from laboratory studies by Boudreau et al. (1991, 1992), who demonstrated in an artificial water column that the proportion of postlarvae at the surface increased with shoaling thermocline depth and increasing thermocline gradient (although their thermocline gradient was much steeper than are thermoclines

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typically encountered in the field). In addition to the thermal structure of the water column, the actual temperature may also play a role in vertical distribution as growth and development of postlarvae are highly temperature dependent, with an optimal growth rate in the 15–17°C range and a sharp increase in mortality at temperatures below 12°C (Mackenzie 1988). Thus, there is a strong incentive for postlarvae to remain in warm surface waters. Light intensity has also been implicated as a controlling factor in the field, where more postlarvae are captured at the surface on cloudy days than in bright sun (Hudon et al. 1986), and anecdotal reports indicate the abundance of postlarvae in surface waters is lowest at noon and highest near sunrise and sunset (Harding et al. pers. comm.). However, both laboratory and field studies have reported no difference in the vertical distribution between day and night (Ennis 1975; Harding et al. 1987; Boudreau et al. 1992). An ontogenetic shift in phototaxis from positive to negative observed in laboratory studies (Hadley 1908; Botero and Atema 1982) provides an intrinsic mechanism for deeper vertical distribution with age.

Light and thermal structure likely interact with larval behavior and ontogeny to drive changes in the proportion of the population residing in surface waters, but we have yet to account for these factors in estimates of total abundance derived from plankton samples. Lobster postlarvae are naturally low in abundance (typically <20 in 1,000 m³) (Incze et al. 1997), making it difficult to quantify vertical distribution using plankton net samples (Fogarty 1983; Harding et al. 1987), and temporal changes in a surface abundance may simply be owing to advection of organisms to and from the sampling site (Incze et al. 2000a). Although laboratory studies have provided detailed accounts of larval swimming behavior and vertical distribution, these results have not been validated in situ by using natural populations of postlarvae.

The objective of the present study was to quantify the behavioral response of postlarvae to changing environmental conditions in situ. Specifically, I tested the hypotheses that thermocline depth and magnitude, temperature, light intensity, and the age of postlarvae exert control over postlarval vertical distribution. To circumvent problems associated with plankton net sampling, I observed individuals in situ to quantify the vertical distribution of postlarvae. Behavioral observations of larvae in situ have been used in studies of fish (Leis and Carson-Ewart 1997, 2001), ascidians (Bingham and Young 1991; Stoner 1992; Young 1986), and crabs (Shanks 1985). Previous observations of lobster postlarvae in situ have provided valuable information on behavior, settlement, and swimming direction and speed (Ennis 1975; Cobb et al. 1983, 1989b), suggesting that this method might be useful for quantifying the vertical distribution of postlarvae.

Methods

Experiments were conducted in the coastal waters of the Gulf of Maine off Boothbay, Maine (~43°45.5'N, 69°38.0'W), at least 1 km from the nearest shore with a minimum water depth of 30 m. Lobster postlarvae were collected in the morning between 0700–1000 h local time (East-

ern standard time) by using a neuston net that sampled to a depth of 0.5 m. Neuston tows were limited to 10 min to minimize damage to postlarvae in the codend, and only individuals with both claws and no signs of damage were used in experiments. These individuals were presumed to be in good health, as they survived for several weeks when held in aquaria after capture (data not shown). Postlarvae were held individually in 500-ml clear polycarbonate containers in a water bath at ambient light intensity and sea surface temperature until their release (<5 h). Scuba divers released a postlarva at a depth of 0.5–1.0 m and followed it for 10 min with ~0.5-m separation between diver and postlarva. Ennis (1975) reported that laboratory-reared postlarvae released near the surface tend to stay near the surface, and that young postlarvae swam toward the surface when released near the bottom whereas older postlarvae remained near the bottom when released there. In the present study, postlarvae were released near the surface because they were captured near the surface. The focus of this study was temporal trends in vertical distribution, and postlarvae were only released at one depth to reduce the number of variables. A small conductivity, temperature, depth (CTD) probe (Minisonde 4a, Hydrolab Corporation) was mounted on a diver's forearm so that sensors could be maintained at the same depth as the postlarva to record a depth profile and environmental parameters (5-s sample interval). The postlarva was recaptured in the jar at the end of the trial and transported to the laboratory for determination of molt stage (methods of Sasaki 1984). Observations were limited to a maximum depth of 15 m, and trials were terminated when the postlarva descended below this depth. Observation of postlarvae in situ without prior capture as a control for handling effects was not feasible owing to the low abundance of postlarvae in the plankton. In more than 50 person-hours of underwater observation time during the trials, we encountered only one free-swimming postlarva not released by us.

Environmental parameters were recorded for each trial including: CTD cast 0–20 m (SBE-19, Sea Bird Electronics), light intensity from 0–10 m (Li-Cor, LI-188b with a LI-193SB spherical quantum sensor), wind velocity (Kestral 2000 wind meter), wind direction (handheld compass), and wave height (estimated). CTD casts were used to determine thermocline, halocline, and pycnocline depths and gradients, and the depth of isotherms from 11–18°C (in 1°C increments). Clines were identified by calculating the rate of change of the measurement with depth (data were averaged in 0.5-m bins). The depth bin with the greatest rate of change was considered the depth of the cline, and the rate of change was reported as the gradient of the cline. Light attenuation at 1- and 5-m depths was calculated relative to light intensity ~2 m above the sea surface. One hundred sixty-eight trials were completed between 19 July and 14 September 2002 (between 4 and 15 trials per day) between 0930 and 1600 h local time. Twenty-seven postlarvae were lost before the 10-min trial was completed, but the data were included in the analyses.

The total amount of time each postlarva spent above 0.5-m depth was used to determine the proportion of time at the surface. Proportion at the surface values over all trials had lower variance during the first minute of the trials, but var-

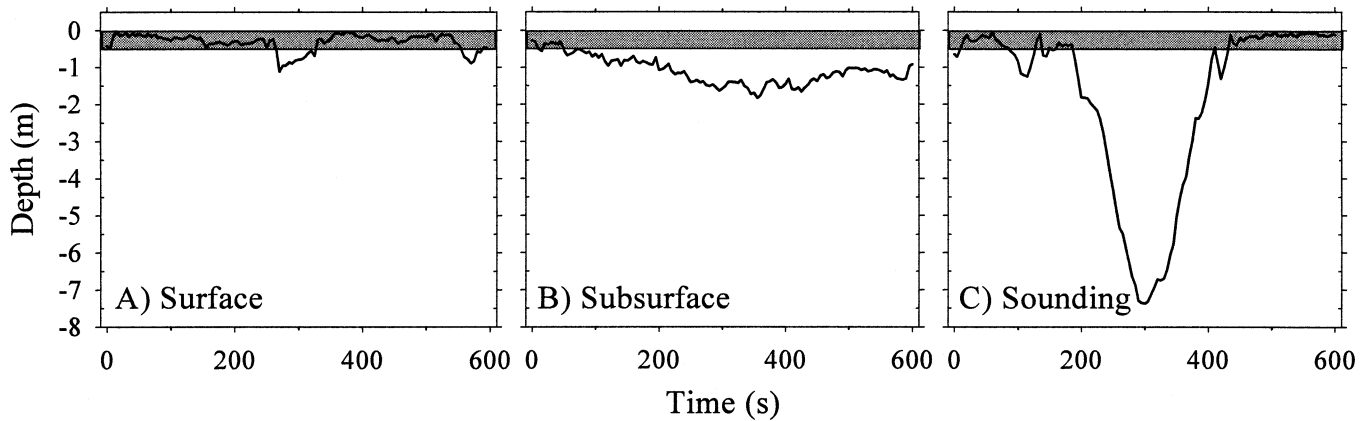


Fig. 1. Representative dive profiles of three lobster postlarvae illustrating the three qualitatively defined swimming behaviors: (A) surface, (B) subsurface, and (C) sounding. Swimming depth was recorded at 5-s intervals with a CTD mounted on a diver's forearm. The shaded area denotes the 0–0.5-m depth bin typically sampled by neuston nets.

iance increased by $\sim 15\%$ between the first and second minute and remained at that level or slightly higher for the last 9 min of the 10-min trial period. Low variance in the first minute was likely owing to the postlarvae being released at the same depth. Accordingly, the first 60 s of data were omitted from all analyses. If postlarvae were orienting toward a common depth, the variance would be expected to decrease over time, whereas a diverse range of swimming behaviors would likely result in increasing variance over time until all swimming depths were adequately represented in samples. Thus, minimal increases in variance during the last 9 min of the trial suggest that the trial duration was sufficient to quantify the vertical swimming behavior of the postlarvae.

Seasonal variation in the proportion at the surface was examined with correlation and regression analyses to determine relationships between daily averages of proportion at the surface and environmental variables. Tests for normality were conducted at the level at which the statistics were conducted (daily averages). The daily averages had a normal distribution, and therefore, the arcsin transformation typically used on proportion data would have had little effect on the data and was not used (Sokal and Rohlf 1995). Variation among days in the proportion at the surface was examined by subtracting the seasonal trend from the daily averages and analyzing the residuals with respect to environmental variables. Diel effects were also examined by using the detrended data, but the proportion at the surface was pooled from the entire season and averaged in ~ 2 -h bins. Day length decreased from 15 h to 12.5 h during the course of the study. To compensate for shifting sunrise and sunset, the time of day was converted into a proportion of daylight hours, with astronomical noon represented by a value of 0.5. Analysis of molt stage and light intensity effects was also conducted on proportion at surface values pooled over the whole season, but the seasonal trend was not removed because there was no seasonal trend in molt stage.

Results

Vertical swimming behavior was qualitatively categorized as surface, subsurface, or sounding (Fig. 1). "Surface" in-

dividuals spent the majority of time in the top 0.5 m of the water column. "Subsurface" postlarvae spent most of their time below 0.5 m but typically remained in the top 4.0 m. Individuals exhibiting "sounding" behavior descended below 4.0 m, usually followed by a return to shallower depth. In half of the observations of sounding behavior, the dive was initiated near the end of the trial and the return to the surface was not observed. On two occasions, individuals sounded to a depth in excess of our working depth, resulting in the termination of the trial. The swimming behavior of postlarvae resulted in a continuum of dive profiles between the categories depicted in Fig. 1, but most could be categorized as one of the three behavior types.

Postlarvae swam by using pleopods with claws either together and extended forward or held apart at $\sim 45^\circ$ to the body axis as reported in previous studies (Ennis 1975; Botero and Atema 1982; Cobb et al. 1983). Swimming was nearly continuous in all trials, although postlarvae spent up to a minute orienting immediately after release. Ascent and descent were typically active, involving a change in the angle of swimming, but descents were occasionally passive. In these cases the postlarva ceased swimming, spread its claws, arched its tail, and allowed itself to sink. Postlarvae actively avoided floating weed, seagrass, and debris by swimming either around or below the obstacles but never took refuge near them. The vertical position of postlarvae was unaffected by sea state, even when trials were conducted in seas exceeding 1 m. Breaking waves and occasional boat wakes tumbled postlarvae swimming very near the surface, but postlarvae quickly reoriented and usually continued swimming near the surface.

Postlarvae spent more time in the top 0.5 m than at any other depth (Fig. 2). The time spent in the 0–0.5-m depth bin accounted for 65% (± 36 SD) of the observation time when averaged over all 168 trials. Observations in the top 4.5 m accounted for 95% (± 16 SD) of the total time. The median swimming depth was 0.38 m, and the median maximum depth reached was 1.04 m. The maximum depth reached by an individual was 16.48 m, and this individual was recaptured while continuing to descend. During the 168 trials, 28 individuals exhibited sounding behavior, and the

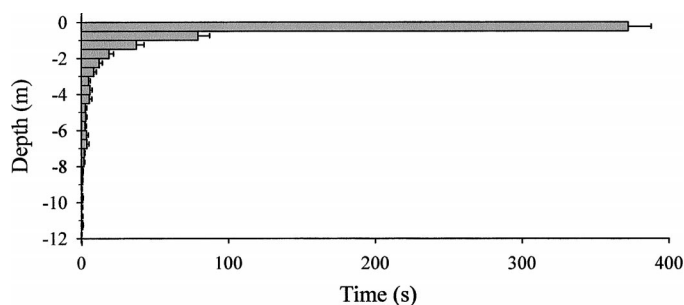


Fig. 2. Vertical distribution of postlarvae as a function of the average amount of time spent at in each 0.5-m depth bin over 168 trials (600-s trial^{-1}). Time spent in the surface 0–0.5-m bin accounts for 65% of the total observation time. Error bars denote ± 1 SE.

frequency of sounding over all trials was $1.08 \text{ dives indiv}^{-1} \text{ h}^{-1}$ (sensu Incze and Wahle 1991). The sample size was insufficient to determine whether diving frequency increased with molt stage.

Postlarvae spent the greatest amount of time in water temperatures between 15.5°C and 16.5°C and rarely descended into water below 12°C (Fig. 3). Time spent at each temperature (0.5°C intervals) was summed across all 168 trials, and the amount of time spent at each temperature reflects the combined effects of larval swimming behavior and the distribution of available temperatures. The dotted line in Fig. 3 illustrates the availability of temperatures as the proportion of trials in which each temperature was present (in the upper 20 m of the water column). It is noteworthy that the cumulative time spent at each temperature below 16°C decreased despite an increasing proportion of trials in which the temperature was available. This suggests that the decrease in time spent at lower temperatures was the result of larval swimming behavior rather than availability of the temperature. In contrast, time spent at temperatures above 17°C may have resulted from decreasing availability of the temperature in the trials.

Proportion of time at the surface decreased from $\sim 80\%$ to $\sim 57\%$ over the course of the season (based on regression values from Fig. 4). Each data point represents the daily average of the proportion at the surface including all trials from that day ($n = 4\text{--}17$). Data from one day (DOY 214) were identified as an outlier by using a Dixon test (Sokal and Rohlf 1995) and were not included in this analysis. A probable cause for this outlier was the sampling of only four postlarvae early in the day resulting in a high proportion of time spent at the surface. Subsequent analyses addressed the environmental factors driving the seasonal trend in proportion at the surface by using correlation analysis of the daily averages. Proportion at the surface increased significantly with decreasing isotherm depth for both the 11°C and 12°C isotherms (Table 1, Fig. 5). Similar relationships with the 13°C , 14°C , and 15°C isotherm depths were nearly significant ($0.05 < p < 0.10$). No other environmental variables were significantly correlated with the proportion at the surface. The 12°C isotherm was selected for regression analysis because the time spent at temperatures below 12°C was negligible (Fig. 3), suggesting that it may be a minimum temperature threshold for postlarvae. The 12°C isotherm de-

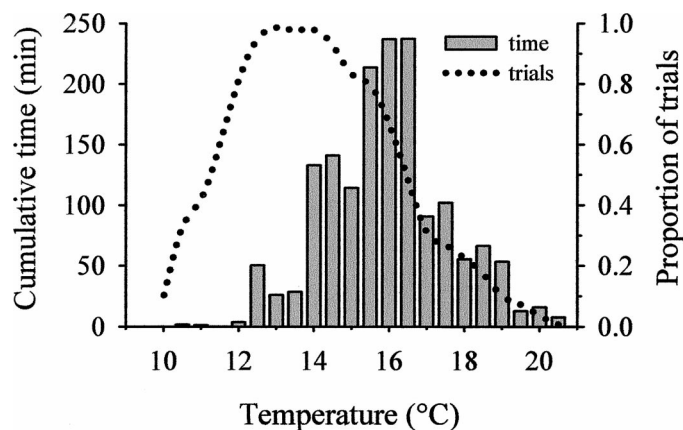


Fig. 3. The cumulative amount of time spent at each temperature in 0.5°C increments (bars) and the proportion of trials for which that temperature was present in the upper 20 m of the water column (dotted line). The cumulative amount of time at each temperature reflects postlarval behavior, and the proportion of trials represents the availability of each temperature.

scended linearly during our sampling period (Fig. 6), and late in the season, the deeper isotherm depth corresponded to lower proportion at the surface values.

The seasonal progression of the 12°C isotherm only explained 49% of the variation in the proportion at the surface. To determine the nature of daily variation about this seasonal trend, I de-trended the daily averages of proportion at the surface data by subtracting the regression in Fig. 5, and used correlation analysis to identify relationships between the residuals and environmental variables (Table 1). The depth of the thermocline and the pycnocline were the only two sig-

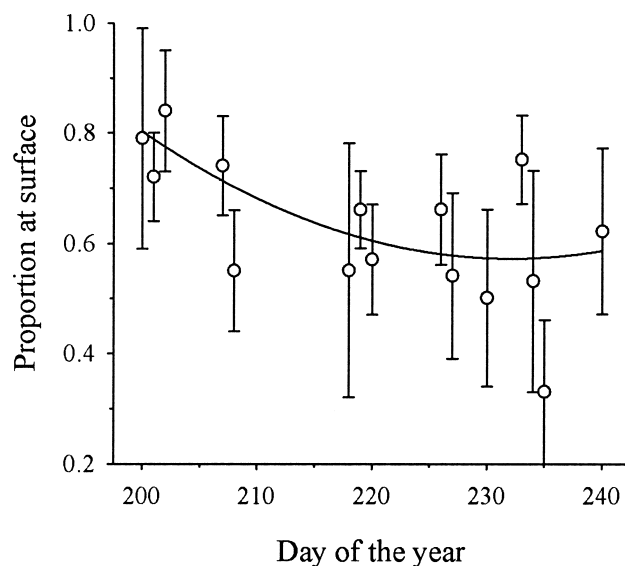


Fig. 4. Seasonal progression of mean proportion at the surface from 19 July–28 August 2002. Proportion at the surface represents the proportion of time each postlarva spent in the 0–0.5-m depth bin during a 10-min trial. Daily means include data from 4–15 trials, and error bars denote ± 1 SE. Data were fitted to the regression equation: $y = 0.0002x^2 - 0.104x + 12.642$ ($r^2 = 0.44$, $p = 0.032$, $df = 14$).

Table 1. Correlation analysis of proportion at the surface values and residuals versus environmental variables. Analysis was performed by using daily means of the proportion at the surface and environmental variables (left two columns). Proportion at the surface values were de-trended by subtracting the regression of 12°C isotherm depth versus proportion at the surface (Fig. 5). The residuals were reanalyzed with respect to environmental variables (right two columns).

Environmental variable	Proportion at the surface		Residuals	
	Coefficient	<i>p</i>	Coefficient	<i>p</i>
Surface temperature (0.5-m depth)	-0.470	0.090	0.224	0.441
Thermocline depth	0.189	0.518	0.595	0.025*
Thermocline gradient	0.302	0.293	-0.148	0.613
Halocline depth	0.301	0.295	0.501	0.068
Halocline gradient	0.140	0.634	0.165	0.573
Pycnocline depth	0.265	0.359	0.568	0.034*
Pycnocline gradient	-0.072	0.808	0.240	0.408
11°C isotherm depth	0.815	0.007**	-0.231	0.508
12°C isotherm depth	0.700	0.008**	0.201	0.490
13°C isotherm depth	0.497	0.071	0.315	0.273
14°C isotherm depth	0.479	0.083	0.227	0.435
15°C isotherm depth	0.522	0.067	0.301	0.318
16°C isotherm depth	0.212	0.531	0.002	0.996
17°C isotherm depth	-0.080	0.865	-0.333	0.465
Age (molt stage)	-0.375	0.168	-0.161	0.581
Wind velocity	-0.284	0.305	-0.259	0.371
Wind direction	-0.167	0.552	0.083	0.777
Wave height	-0.279	0.314	-0.464	0.095
Light intensity (in air)	0.027	0.925	0.301	0.295
Light intensity (0.5-m depth)	-0.180	0.521	0.170	0.561
Light attenuation (1.0-m depth)	-0.342	0.213	0.064	0.827

* $p < 0.05$; ** $p < 0.01$.

nificant correlations, and halocline depth was nearly significant ($p = 0.07$). In most trials the thermocline, halocline, and pycnocline all occurred at the same depth, so the correlation of all of these variables with the residuals was expected. The residuals increased with decreasing thermocline

depth (Fig. 7), and these daily variations in thermocline account for 44% of the variance about the seasonal trend. The thermocline was highly variable throughout and did not exhibit a significant linear trend (Fig. 8). There was no relationship between the proportion at the surface residuals and the magnitude of the temperature gradient. The minimum

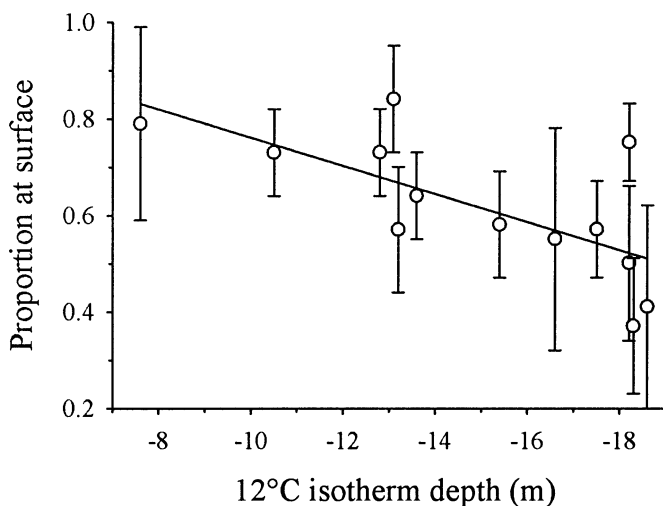


Fig. 5. Effect of 12°C isotherm depth on proportion at the surface. Isotherm depth was determined by using CTD data from casts conducted during each trial and daily mean proportion at the surface values (mean \pm 1 SE.) are as described in Fig. 4. Data were fitted to the regression equation: $y = 0.029x + 1.055$ ($r^2 = 0.49$, $p = 0.008$, $df = 12$).

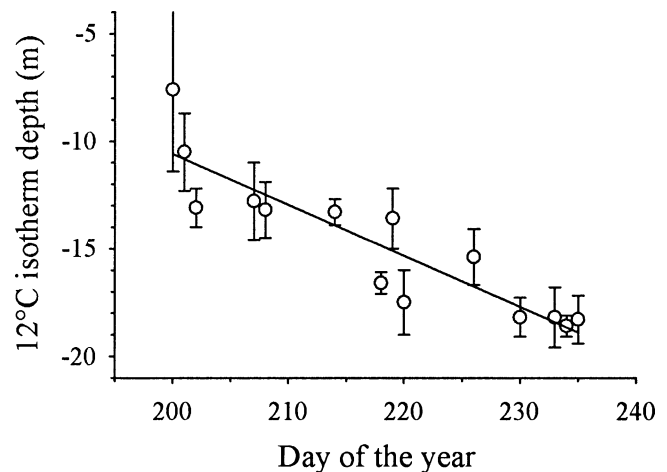


Fig. 6. Seasonal progression of the 12°C isotherm depth from 19 July–23 August 2002. Daily averages of isotherm depth (mean \pm 1 SD) calculated from CTD casts conducted during each trial. The 12°C isotherm descended below the depth of the CTD casts (20 m) after 23 August. Data were fitted to the regression equation: $y = -0.240x + 36.83$ ($r^2 = 0.81$, $p < 0.001$, $df = 13$).

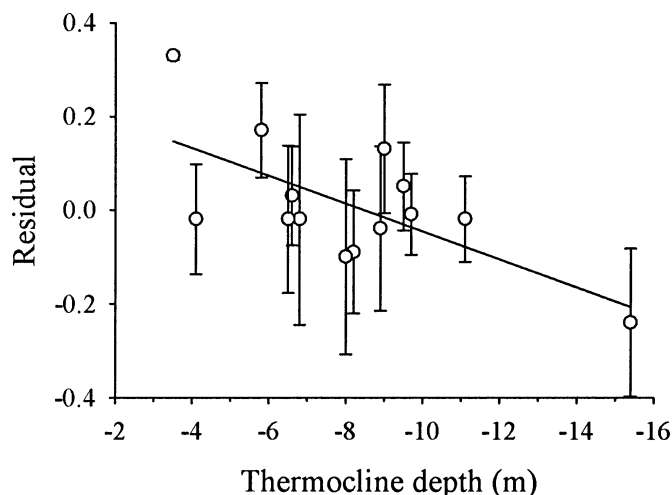


Fig. 7. Effect of thermocline depth on proportion at the surface residuals. Daily average proportion at the surface values were de-trended by subtracting the regression of 12°C isotherm depth and proportion at the surface (Fig. 5), and the residual reflects daily variation about the seasonal trend. Thermocline depth was determined from CTD casts conducted with trials. Data are daily means ± 1 SE and were fit to the regression: $y = 0.0300x + 0.2518$ ($r^2 = 0.44$, $p = 0.008$, $df = 13$).

and maximum thermocline gradients were $0.16^\circ\text{C m}^{-1}$ and $2.73^\circ\text{C m}^{-1}$, respectively and the average was 1.20 C m^{-1} (based on temperature change over 0.5-m increments).

Time of day also played a role in proportion at the surface with the lowest proportion occurring midday and the highest in the morning and late afternoon (Fig. 9). Logistically, we were unable to maintain a strict regime of sampling times, but analysis of diel effects was possible by pooling the proportion at the surface data for all trials and de-trending them with respect to 12°C isotherm depth (to remove seasonal trend). Because of early afternoon cloud cover (probably resulting from the development of a sea breeze), light levels did not exhibit a characteristic peak at midday in our samples. Hourly average light intensity (at 0.5-m depth) was $1,150\text{--}1,300 \mu\text{mol s}^{-1} \text{m}^{-2}$ from 0900–1300 h, dropped to $950\text{--}1,050 \mu\text{mol s}^{-1} \text{m}^{-2}$ between 1300 and 1500 h, and returned to $1,250 \mu\text{mol s}^{-1} \text{m}^{-2}$ for the 1500–1600 h period. There was no significant relationship between hourly averages of light intensity and the proportion at the surface residuals. There was no correlation between hourly averages of molt stage and the proportion at the surface residuals; therefore, it is unlikely that ontogenetic changes in phototaxis influenced the analysis of diel effects.

Proportion at the surface values varied with light intensity when averaged by the molt stage of the larvae. The proportion at the surface in low light ($<800 \mu\text{mol s}^{-1} \text{m}^{-2}$) conditions increased with molt stage progression, and the proportion at the surface in bright conditions ($>800 \mu\text{mol s}^{-1} \text{m}^{-2}$) decreased as the molt stage progressed (Fig. 10). Pairwise comparisons (*t*-test) for each molt stage indicated that D1' postlarvae (early molt stage) spent significantly greater proportion of time at the surface in bright light than in low light ($p = 0.017$), whereas the proportion at the surface was significantly lower in bright conditions for both D1''' ($p =$

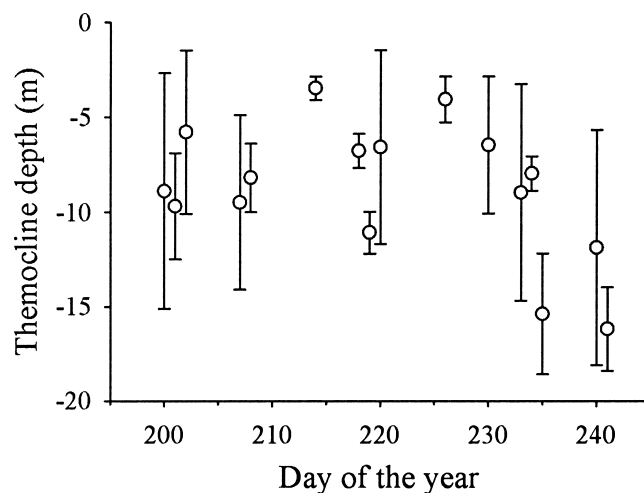


Fig. 8. Seasonal progression of thermocline depth. Thermocline depths were determined from CTD casts taken during each trial. The thermocline deepened late in the season, but no significant relationship was found. Data are daily means ± 1 SD.

0.004) and D2–3 ($p = 0.006$) molt stages (late molt stages). There was no significant difference in the D1'' molt stage ($p = 0.275$), indicating that it may be a transitional molt stage between positive and negative phototaxis. Light intensity of $800 \mu\text{mol s}^{-1} \text{m}^{-2}$ (at 0.5-m depth) represented a natural break in the light data that primarily reflected cloud cover. Molt stages A and B were absent from my samples, and only one C and three D0 molt stages were collected, which was insufficient for analysis. The molt stage composition of postlarvae in my collections did not vary predictably over the course of the season, therefore it was not necessary to de-trend the proportion at the surface data with respect to

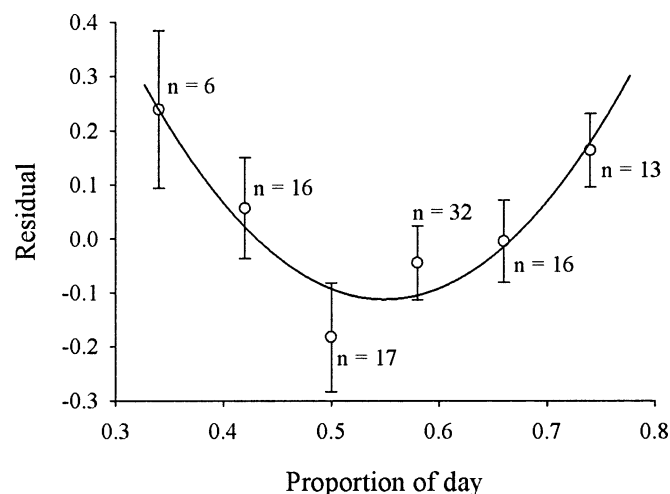


Fig. 9. Effect of time of day on proportion at the surface residuals. Residuals (de-trended with respect to isotherm effects; Fig. 5) for all 168 trials were pooled and averaged (mean ± 1 SE) by the proportion of day in approximately 2-h bins. Trials were conducted between 0900 and 1600 h local time, but proportion of day was used to account for changes in day length during the study. Data were fitted to the regression equation: $y = 7.99x^2 - 8.79x + 2.30$ ($r^2 = 0.88$, $p = 0.040$, $df = 3$).

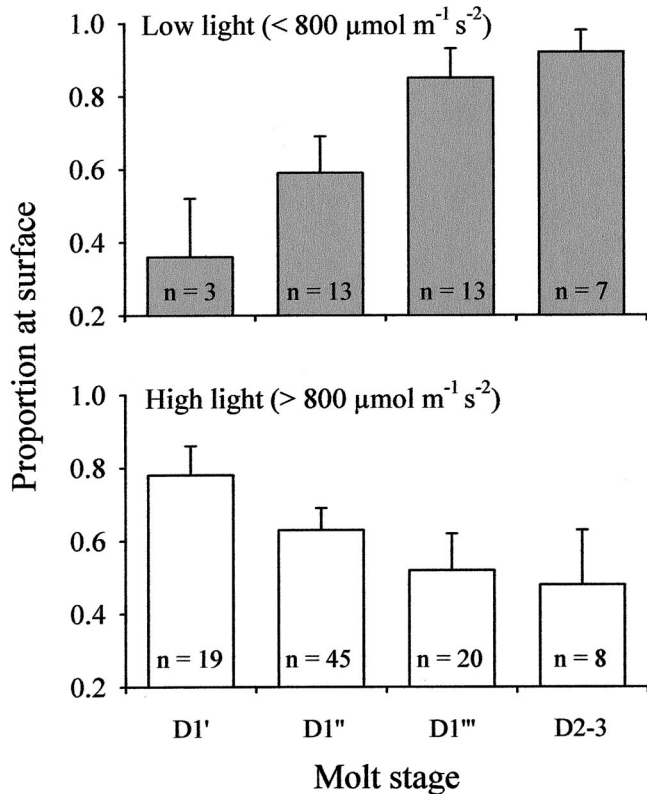


Fig. 10. Effect of light intensity on proportion at the surface (means \pm 1 SE) as a function of postlarval development (molt stage). Molt stage of postlarvae increases from left to right. Light intensity was measured at 0.5-m depth for each trial and $800 \mu\text{mol s}^{-1} \text{m}^{-2}$ represented a natural break in the data owing to the presence or absence of cloud cover. The number of trials included in each average is indicated within the bar.

the seasonal effects of the 12°C isotherm before conducting this analysis. This also means the shift in phototaxis with molt stage did not affect the seasonal trend in proportion at the surface.

Discussion

Vertical distribution—Postlarvae spent less time at the surface than was expected based on previous studies of vertical distribution. The average proportion of time at the surface of 0.65 observed in the present study (Fig. 2) suggests that, on average, 65% of the population resides in the top 0.5 m. Previous estimates of abundance report 78–96% of the population represented in surface samples (Harding et al. 1982, 1987; Hudon et al. 1986), indicating that total abundance has been chronically underestimated. Also, the proportion of postlarvae at the surface is not constant and decreases from 0.80 to 0.57 as the postlarval season progresses. However, the results presented in the present study support the efficacy of surface sampling for postlarvae as they spent more time at the surface than any other depth.

Observation of behavior in situ raises the question of whether observer presence influences the behavior of the organism. Although I did not attempt to quantify observer

influence, there are several qualitative observations that suggest observer influence was minimal. Lobster postlarvae use a tail-flip escape behavior but rarely exhibited this behavior during trials. Orientation with respect to the diver was not consistent, and on several occasions, postlarvae were lost when they turned and swam directly into the diver. At the end of each trial, I was able to approach the postlarva and recapture it in the 500-ml sample jar without the postlarva exhibiting an escape response until the last moment. Previous in situ observations of postlarval behavior both by divers and from boats have also reported that the postlarvae appeared uninfluenced by observer presence (Ennis 1975; Cobb et al. 1983, 1989b). It should be noted that the behaviors reported in this study were consistent with previous field and laboratory observations suggesting that behavior was not fundamentally altered by the presence of a diver.

Although the potential for observer influence cannot be discounted, the vertical distribution reported in the present study probably represents an improvement on studies that have used nets to quantify the vertical distribution of postlarvae. In addition to the challenge of sampling a low abundance, patchily distributed organism, previous studies have typically missed critical portions of the water column in their sampling efforts. Hudon et al. (1986) reported 78–90% of postlarvae in the 0–0.8-m depth range, but did not sample below 2.4 m. Harding et al. (1987) sampled at 0–1 m and at 5 m, thereby missing the entire range between 1 and 5 m. These gaps in sampling likely result in an underestimate of postlarvae below the surface and a corresponding overestimate of the proportion of postlarvae at the surface. The fine-scale resolution of vertical swimming behavior achieved by using direct observation fills the critical gaps and provides a better estimate of the proportion of postlarvae represented in surface samples.

Temperature—Temperature was the primary correlate for the vertical distribution of postlarvae. The proportion at the surface decreased with increasing depth of the 12°C isotherm, which appeared to be driving the seasonal progression of the proportion at the surface. The 12°C isotherm was considerably deeper than was the typical swimming depth of the postlarvae, suggesting that a more proximate cue was responsible for their shallow vertical distribution. Although they only approached statistical significance ($0.05 < p < 0.10$), the relationships between proportion at the surface and the 13°C , 14°C , and 15°C isotherms suggest that postlarvae actually responded to temperatures below 16°C . These isotherms were found much closer to the depth range of the postlarvae and may provide the proximate cue driving vertical distribution. The decrease in the cumulative amount of time spent at each depth below 16°C (Fig. 3) is consistent with the hypothesis that postlarvae actively avoid lower temperatures. Colder temperatures were close to the surface early in the postlarval season, effectively compressing the vertical range and resulting in a higher proportion at the surface. Surface waters warmed later in the season, expanding the vertical range of the postlarvae, which resulted in a decrease in the proportion of time spent at the surface.

Daily fluctuation in thermocline depth was a secondary factor controlling vertical distribution. Trials were conducted

in coastal waters, where thermocline depth and temperature gradient fluctuate daily with factors such as cloud cover, wind stress, air temperature, internal waves, and tidal mixing. The depth of the thermocline was responsible for daily variation about the seasonal trend of proportion at the surface with a shallow thermocline, resulting in higher proportion at the surface. Thus, the vertical distribution of postlarvae varied predictably as a function of thermal structure through the coupled influence of the 12°C isotherm and the thermocline, and in both instances, the postlarvae remained above the thermal structure.

Behavioral regulation of depth often changes ontogenetically in decapod crustacean larvae, with the final planktonic instar exhibiting precise depth regulation (Sulkin 1984; Anger 2001). *H. americanus* exhibits a change in vertical distribution with early larval stages distributed over the upper 40 m of the water column (Harding et al. 1987) and the postlarval stage restricted to the top 5 m (presented in the present study). The change in vertical distribution is accompanied by an ontogenetic shift in thermal tolerance, which may provide an intrinsic mechanism for regulating vertical distribution. MacKenzie (1988) reported increasing stage-specific mortality with developmental stage in larvae reared at 10°C with mortality of 10%, 39%, 74%, and 81% for stages I, II, III, and postlarva, respectively. Postlarval mortality increased from 18% to 81% as temperature was reduced from 12°C to 10°C, and postlarvae in 10°C water rarely succeeded in molting to the next instar. Thus, laboratory experiments suggest that 12°C may be a minimum temperature at which postlarvae remain viable, providing a strong selective pressure to remain above the 12°C isotherm. In the present study, the amount of time spent at each temperature dropped nearly to zero at temperatures below 12°C, with only 2% of the postlarvae descending to temperatures of 12°C and below (Fig. 3). These observations are consistent with the hypothesis that 12°C serves as a thermal threshold for postlarval behavior. MacKenzie (1988) also found that postlarvae have a maximum growth rate (based on dry weight) between 15°C and 17°C. The histogram of time spent at each temperature had a mode at 16°C, and postlarvae spent ~56% of their time between 15°C and 17°C, indicating that postlarvae spent the majority of time at the optimal temperature for growth.

Boudreau et al. (1991, 1992) proposed that thermocline strength and depth were the primary factors influencing vertical distribution of postlarvae. In laboratory studies, they found that postlarvae stayed above the thermocline and that settlement was greatly reduced in the presence of a strong thermocline. However, they found significant effects only when the temperature gradient of the thermocline was ~10°C m⁻¹ or greater (interpreted graphically based on temperature change over 0.5 m), and a gradient of ~4.5°C m⁻¹ had no significant effect of the vertical distribution of postlarvae. Therefore, it is not surprising that there was no effect of the strength of the thermal gradient in the present study, as all of the trials had a gradient <2.7°C m⁻¹. Despite weaker thermoclines in situ, the depth of the thermocline was correlated to daily variations in the proportion at the surface. The relationship was not evident until the data were detrended with respect to the seasonal progression of the 12°C

isotherm and only explained 44% of the daily variation about the seasonal trend (Fig. 7). The importance of thermocline in maintaining vertical distribution was probably diminished by the substantially weaker thermoclines found in situ. In contrast to studies by Boudreau et al. (1991, 1992), I found that a specific temperature (12°C) was a better predictor of vertical distribution in situ than the thermocline structure.

Diel effects—Proportion at the surface also varied during the course of the day, with the most time spent at the surface in the morning and late afternoon (Fig. 9). This is consistent with observations that postlarvae are least abundant in surface samples mid-day (Harding et al. 2000; E. R. Annis unpubl. data). The shift in vertical distribution over the course of the day was independent of light intensity, which suggests that the vertical migration may be part of an endogenous rhythm over which reactions to light intensity may be superimposed. The lack of correlation between light intensity and proportion at the surface in this analysis was probably owing to a similar distribution of molt stages in each of the hourly averages. It would be inappropriate to extrapolate these results beyond the limits of the data, but they do suggest that the most effective time to sample postlarvae at the surface is either before 1000 h or after 1400 h.

Light—Postlarvae exhibited a shift in phototaxis from positive to negative with increasing molt stage (Fig. 10), consistent with the shift in phototaxis reported by Hadley (1908) and Botero and Atema (1982). The shift in phototaxis may be an intrinsic mechanism driving settlement: as the molt stage progresses, increasingly negatively phototaxis makes postlarvae more likely to leave the surface waters for the dark crevices of the benthos (Botero and Atema 1982; Boudreau et al. 1990). This is supported by observations that when released at depth, early postlarval stages swim toward the surface, whereas late-stage postlarvae tend to stay near the bottom (Ennis 1975; Cobb et al. 1983). In the present study, postlarvae in molt stage D1'' appeared to be in transition between positive and negative phototaxis, with no significant difference in the proportion at the surface in high and low light conditions. The final molt stages (D1''' and D2–3) spent significantly more time below the surface in bright conditions, suggesting that postlarvae become behaviorally competent to settle during the D1'' molt stage or ~75% of the postlarval period (sensu Sasaki 1984). Previous laboratory studies predict that this transition occurs closer to molt stage C (Botero and Atema 1982; Cobb et al. 1989a) or approximately midway through the postlarval period (Herrick 1909). However, in plankton surveys of Buzzards Bay (Massachusetts) and Block Island Sound (Rhode Island), Cobb et al. (1989a) found abundant D0 or D1 molt stages, indicating that settlement does not occur until the later half of the postlarval stage. In the present study area, the molt stage D2–3 is often abundant (Incze et al. 1997, 2000a; Annis 2004), suggesting that settlement occurs late in the postlarval stage. Differences between studies may reflect problems extrapolating laboratory results to the field or simply regional variations in behavior (Cobb et al. 1989a; Incze et al. 1997). It is important to note that behavior is highly variable among individuals, and although the predom-

inate shift in phototaxis occurred during the D1" molt stage, individuals exhibited sounding behavior before achieving that developmental stage. The stages of the lobster life cycle are morphologically defined, and the shift in phototaxis during the postlarval stage illustrates that significant ontogenetic changes in behavior do not necessarily coincide with clearly defined morphological stages.

Implications for settlement—The thermally mediated behavior observed in the present study suggests that settlement may also be influenced by temperature. If 12°C serves as a minimum temperature threshold for postlarvae, successful settlement should be greatly influenced by the amount of time bottom temperature exceeds 12°C. Thus, settlement may vary not only as a function of postlarval production (Incze et al. 1997, 2000b) and available substrate (Botero and Atema 1982; Boudreau et al. 1990; Wahle and Steneck 1991), but also as a function of whether the bottom temperature reaches 12°C or the number of days that bottom temperature exceeds 12°C. Spatial patterns of settlement on the Maine coast show a distinct shift from low settlement in the east to high settlement in the west (Steneck and Wilson 2001). The bottom temperature at eastern sites (~10-m depth) is strongly influenced by the cold waters of the Eastern Maine Coastal Current, which is vertically well mixed and rarely exceeds 12°C (Brooks 1985; Townsend 1991). In contrast, western sites (Penobscot Bay and west) are characterized by warmer stratified waters in the summer and bottom temperatures exceeding 12°C (~10-m depth) (N. Pettigrew et al. unpubl. data). Vertical distribution of postlarvae was correlated with the 12°C isotherm, with postlarvae remaining well above the isotherm, suggesting that these regional settlement patterns may be driven in part by the bottom temperature or the depth of the 12°C isotherm.

In the vertical scale, settlement decreases with depth and is reduced to the limit of detection at 20 m (Wilson 1999), and the magnitude of settlement occurring below 20 m remains unknown. In the present study, the 12°C isotherm only descended below 20 m late in the summer season, and it follows that the number of days that bottom temperature exceeds 12°C decreases with depth. If postlarvae tend to stay above the 12°C isotherm, the likelihood of successful settlement would decrease with increasing depth. This is consistent with a positive relationship between average bottom temperature and abundance of settlers in sites between 5- and 20-m depth reported by Wilson (1999). Only two of the 168 (1.2%) postlarvae descended below the sampling depth of 15 m. If this is representative of the proportion of individuals sounding for bottom below 20 m, the equivalent dive frequency is 0.08 dives $\text{indiv}^{-1} \text{h}^{-1}$. This is an order of magnitude lower than the shallow sounding frequency of 1.08 $\text{indiv}^{-1} \text{h}^{-1}$ reported in the present study and the range of 0.7–2.9 $\text{indiv}^{-1} \text{h}^{-1}$ estimated by Incze and Wahle (1991). Forays to the bottom appear uncommon in deep water, and settlement on an areal basis should be much lower. There is more surface area available for settlement below 20 m, such that even low settlement rates could make a significant contribution to total settlement. However, much of the available area never exceeds 12°C, and elevated postlarval mortality

below 12°C (Mackenzie 1988) suggests that successful settlement in deep water is probably limited.

Correction factor for neuston samples—The validity of abundance estimates derived from neuston samples relies on knowing the proportion of the population represented in the sample. That proportion varies with environmental conditions, and the relationships described in the present study may be used to provide a proportion at the surface value specific to the environmental conditions at the time of collection. The effect of temperature drives seasonal trends in the proportion at the surface, and the regression equation provides the basis for calculating a correction factor (Fig. 5). The residual effect of daily variations in thermocline (Fig. 7) and the time of day (Fig. 9) can be estimated by using their respective regressions and added to the estimated proportion at the surface. This method will result in lower proportion at the surface and higher estimates of total postlarval abundance than those previously reported. Several factors may bias this estimate of proportion at the surface. The postlarvae used in this study were collected from surface waters and therefore may have been predisposed to swimming near the surface. They were released near the surface, which may also have contributed to a bias toward surface swimming, particularly in later molt stage individuals. A 10-min trial was sometimes insufficient to fully capture sounding behavior, resulting in an underestimate of the proportion of time spent below the surface. Thus, the proportion at the surface estimate presented in the present study should be viewed as a conservative estimate and may actually be lower.

The limitations of the proposed correction factor for total abundance must also be acknowledged. At present, they would most appropriately be applied to water masses with hydrography similar to central Maine coastal waters, as the vertical distribution may vary in areas with extreme temperatures or vertically mixed water columns. Further research is needed to determine the spatial extent of patterns reported in the present study before the correction factor can be applied as a general model for estimating abundance. Caution should be exercised in extrapolating beyond the bounds of the regressions as the minimum and maximum proportions at the surface have not been determined. The correction factor could also be confounded by large variations in molt stage composition, and the effect of proximity to shore on vertical distribution is unknown.

The vertical distribution of lobster postlarvae results from the interplay between intrinsic behavioral characteristics and extrinsic environmental conditions. Behavior was complex and highly variable among individuals, but variations in the proportion at the surface as a function of temperature, thermocline depth, and time of day were consistent with thermal tolerances, ontogenetic changes in behavior, and previous observations in the lab and field. The neustonic phase of the life cycle presents a convenient opportunity to quantify larval abundance in the period leading to settlement, but we must recognize that the population represented in neuston samples is not constant. Rather, it changes with hydrographic conditions and the ontogeny of the organism. Future work should expand the spatial extent to which these relationships

may be applied and examine the influence of proximity to shore and vertical mixing on the vertical distribution. Furthermore, the relationship between bottom temperature and settlement should be examined with respect to the thermal threshold reported in the present study as this relationship may contribute to the biogeography of settlement.

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