

VERTICAL DISTRIBUTION OF DECAPOD CRUSTACEAN LARVAE:
FIELD AND EXPERIMENTAL STUDIES

By

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VERTICAL DISTRIBUTION OF DECAPOD CRUSTACEAN LARVAE:
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Abstract

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We investigated the factors affecting vertical distributions of decapod crustacean larvae via both a field and a laboratory study. In our field study, we addressed vertical distribution in relation to predator avoidance and transport. We predicted that if behaviours aiming to maximize predator avoidance and seaward transport were present in decapod larvae, they would be evidenced by shallower vertical distributions and/or higher abundances on nighttime ebb tides. We collected larvae from discrete depths in Willapa Bay, Washington and related their abundance and vertical distribution to a suite of environmental variables. While vertical distributions were variable, *Neotrypaea californiensis* zoea were shallower at night, supporting our prediction for predator avoidance, and during one sampling, pinnotherid zoea were shallower on ebb tides, supporting our prediction for seaward transport. Abundances varied more predictably with tidal and diel phases than did vertical distributions, with both taxa most abundant on ebb tides, suggesting the presence of behaviours to enhance seaward transport. In a

mixed estuary, hydrostatic pressure (water height) would be a reliable indicator of tidal phase. The abundances of both taxa increased with water height and *N. californiensis* were shallower when the water column was deeper.

In our laboratory study, we investigated the impact of haloclines and a light/dark cycle on the vertical distribution of *N. californiensis*. Resource patches are often associated with physical discontinuities in the water column and the ability to cue to these discontinuities to locate prey could increase foraging success. We recorded the depth distributions of larvae in salinity stratified and vertically homogenous tanks using video cameras that panned the vertical extent of each tank hourly over periods of approximately 24 hours. Our results indicate that *N. californiensis* will aggregate in the presence of a physical thin layer and exhibit diel vertical migration, but that this response is plastic and possibly dependent on pre-conditioning. Our results add to the understanding of larval migration and will increase our ability to predict dispersal and recruitment dynamics. In addition, the aggregation of decapod larvae around thin layers has implications for the formation and/or erasure of thin layers.

KEYWORDS: Selective tidal stream transport; biological thin layers; decapod behaviour; vertical distribution; diel vertical migration; zooplankton behaviour

PROLOGUE

The vertical distribution of planktonic organisms can have population level consequences in terms of horizontal distribution, prey susceptibility, and food acquisition. We investigated the vertical distributions of the planktonic larvae of decapod crustaceans via both a field and a laboratory study. We have written up the two studies as separate publications and they will be presented here as separate chapters.

Chapter 1 describes the results of a field study conducted in Willapa Bay that addressed the vertical distribution and abundance of decapod larvae in relation to tidal and diel light phases and discussed these in terms of horizontal transport and predator avoidance strategies. We further related vertical distributions and abundances to potential cues of tidal phase, salinity, temperature, water height, and degree of stratification.

Chapter 2 describes a laboratory study in which we further investigated the effect of a light/dark cycle, as well as the effect of a halocline, on the vertical distribution of the larvae of the decapod, *Neotrypaea californiensis*. As resource patches are often associated with physical discontinuities in the water column, aggregation in these areas would have implications in terms of food acquisition and encounters with predators.

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CHAPTER 1:
VERTICAL DISTRIBUTION AND MIGRATION OF DECAPOD LARVAE IN
RELATION TO LIGHT AND TIDES IN WILLAPA BAY, WASHINGTON

ABSTRACT

Vertical migratory behaviour in plankton may result in both predator avoidance and horizontal transport. We predicted that if behaviours aiming to maximize predator avoidance and seaward transport were present in estuarine meroplankton such as decapod larvae, they would be evidenced by shallower vertical distributions and/or higher abundance on nighttime ebb tides. We collected larvae from discrete depths in a partially-mixed estuary, Willapa Bay, Washington and related their abundance and vertical distribution to a suite of environmental variables. While vertical distributions were variable, during our October 2006 sampling, pinnotherid first zoea were shallower on ebb tides and during our May 2007 sampling, *Neotrypaea californiensis* were shallower at night. Abundances varied more predictably with tidal and diel phases than did vertical distributions, with both taxa most abundant on ebb tides and specifically nighttime ebb tides for *N. californiensis*. Tidal patterns in abundance suggested the presence of behaviours to enhance seaward transport. The abundances of both taxa were found to increase with water height and the distribution of *N. californiensis* was shallower when the water column was deeper. Hydrostatic pressure varies with water height and would be a reliable indicator of tidal phase for either larval release or vertical migratory behaviour. Our results add to the understanding of larval vertical distribution and migration and will thus increase our ability to predict decapod dispersal and recruitment dynamics.

INTRODUCTION

The vertical distribution of a planktonic species can have population level consequences in terms of food acquisition, predation levels, metabolism, and reproduction (Hays 2003). Many species actively control their vertical distribution, often to maximize feeding or minimize predation risk. Because current speed and direction may vary with depth, the vertical distribution of a population also largely influences its horizontal distribution and is of particular importance to species with a benthic adult stage (Thorson 1950).

Planktonic larvae of many benthic estuarine species develop in coastal waters, returning to estuaries as postlarvae for settlement (Anger 2001; Bilton et al. 2002). Coastal development is considered favourable because estuaries are thought to have both a higher concentration of predators and to be more physiologically stressful environments as they experience large and rapid variation in temperature and salinity (Morgan 1995; Bilton et al. 2002).

Lower-estuarine invertebrates often possess behaviours that maximize the proportion of larvae reaching coastal waters (Christy and Stancyk 1982; Bilton et al. 2002; Paula et al. 2004). Tides are a predictably variable component of the physical environment that can indicate downstream or upstream water movement (Hill 1991). Seaward transport can be aided by exploiting tidal currents, either by releasing larvae at high water (Morgan and Christy 1995) or through synchronizing vertical migrations to coincide with specific tides, a behavioural mechanism called selective tidal-stream transport (STST) (Forward and Tankersley 2001). In STST, organisms are shallower during one tidal phase than

during the other tidal phase. Because water velocity in an estuary decreases nearer the bottom of the water column because of shear caused by a frictional surface, by altering their vertical position, organism can enhance unidirectional transport. Many examples of tidally-timed vertical migrations have been found amongst the larvae of decapod crustaceans (Cronin and Forward 1982; Zeng and Naylor 1996; Queiroga et al. 1997; Garrison 1999; see also Quieroga and Blanton 2005 for a review).

Decapods are also sensitive to the diel light cycle. In decapods, larval release occurs most frequently on nocturnal high tides (Morgan and Christy 1995; Paula et al. 2004) and larvae are also known to employ diel vertical migration (DVM) (Cronin and Forward 1982; Queiroga et al. 1997). These behaviours occur in a wide range of planktonic animals and are considered to be driven by predation (Bollens and Frost 1989; Bollens and Frost 1991; Morgan and Christy 1995; Hays 2003).

As swimming and orientation in decapod larvae are highly influenced by exogenous stimuli, vertical distribution and migratory behaviour may be influenced by various water column properties, including light, pressure, temperature, and salinity, and by interactions between these stimuli (Latz and Forward 1977; Sulkin et al. 1980). Light is known to cue vertical migrations (Forward et al. 1984) but unless tidal and diel cycles are synchronized, does not result in directional horizontal transport. Pressure is a ubiquitous cue for change in water height and many larval crustaceans are sensitive to changes in hydrostatic pressure (Wheeler and Epifanio 1978; Forward and Wellins 1989). Salinity, as an indicator of tidal phase, may also be an important cue for organisms seeking to

maximize unidirectional transport and many decapod larvae alter their behaviour with a salinity change and in response to salinity stratification (Lance 1962; Sulkin 1984). Forward (1989) demonstrated that the zoea of the crabs *Rhitropanopeus harrisi* and *Neopanope sayi* actively ascended in response to an increase in salinity. Decapod larvae have also been found to vary their swimming rates with temperature changes (Sulkin et al. 1980). As temperature may also vary with both depth and tidal phase it also represents a potential orientation cue to larvae. Of these four potential cues only hydrostatic pressure varies predictably with depth, making it the most reliable indicator of horizontal current direction (Sulkin 1984), particularly in situations of vertical mixing. Sulkin (1984) proposed hydrostatic pressure as the basis for larval depth control in decapods.

We asked whether decapod larvae in Willapa Bay, Washington, possessed behaviours that were synchronized to tidal and diel cycles. Willapa Bay is a strongly tidal, shallow estuary with a predominantly mixed water column during summer months. Vertical mixing may hinder an organisms' ability to vertically migrate as well as dampen cues given by environmental variables (Lochman 1995). The larval ghost shrimp, *Neotrypaea californiensis* and pea crabs, pinnotherids, are dominant decapod members of the mesozooplankton in Willapa Bay, Washington during summer months (Graham and Bollens, *in review*). We asked whether the patterns of abundance and vertical distribution of these taxa reflected STST and predator avoidance behaviours. We predicted that to enhance seaward transport larvae would have a shallower distribution and/or a higher abundance on ebb tides. To avoid visual predators, we predicted that

larvae would have a deeper distribution and/or reduced abundances during daylight. We further asked whether vertical distributions and abundances could be predicted by the various environmental variables, namely, salinity, temperature, degree of stratification and water height. Measures of degree of stratification and water height allowed us to look at the effect of vertical mixing and hydrostatic pressure, respectively, on the vertical distribution and abundances of these organisms.

MATERIAL AND METHODS

Study site. This study was conducted in Willapa Bay, Washington. It is characterized as a partially-mixed estuary with a strong tidal influence (Banas et al. 2004). Willapa Bay experiences mixed semi-diurnal tides with a mean tidal range of 2.7m (Banas et al. 2004). The bay covers an approximate area of 260 km² (Dumbauld et al. 1996). Approximately half of the bay's area (Andrews 1965; Hickey and Banas 2002) and volume (Banas et al. 2004) rests in the intertidal zone. We conducted our study from a single site located approximately 12 km from the mouth of the estuary, on the southern side of Willapa Channel, across from Toke Point, WA (46°41.525', -123°87.197' Fig. 1). Total water column depth varied between 6 and 10m during our samplings.

Field sampling. From an anchored boat, we collected replicate plankton samples from discrete depths within the water column every 3 hours over a tidal cycle during three sampling periods. Samples were collected using a 6.5 hp gas-powered, deck-mounted pump connected to a 6 cm diameter hose. Pump output was passed through a 500 µm

mesh net suspended in water. Plankton samples were preserved immediately in 10% buffered formalin. At the beginning and end of each sampling period, water column profiles for salinity and temperature were taken by CTD (Seabird SBE 25) casts. This sampling occurred on 16-18 May 2006, 9-10 October 2006 and 15-16 May 2007 over 26, 23, and 20-hour periods, respectively (Table 1.1). Samplings were timed to coincide with spring tides. In May 2006 and October 2006 replicate samples were taken from 3 depths: 1m above bottom, mid-water column (halfway between surface and bottom), and 1m below surface. In May 2007, a 0.5m above bottom (mab) sample was added. 157 samples were collected. In the laboratory, decapod zoea in the samples were counted and identified to the lowest possible taxonomic level based on Puls (2001).

Data analysis. We calculated weighted mean depths (WMD) of larvae after Frost and Bollens (1992) as normalized for depth by Queiroga (1998):

$$\text{Relative WMD} = \frac{\sum n_i \times d_i}{\sum n_i} \times \frac{1}{D_t}$$

where n was the abundance (number per cubic meter) of larvae at a depth, d , and D was the total depth of the water column. Dividing WMD by the total depth of the water column controlled for the variability in depth caused by tides and gave us a relative depth, where a value of 1 is the bottom and 0 is the surface (Queiroga 1998). Because the larval abundances from which relative WMD values were calculated varied widely, a separate weighting variable was calculated as by the square root of total abundance. This weighting variable caused WMD values resulting from higher abundances to have a higher effect on the outcome of our analyses.

To determine how the measured environmental variables affected the vertical distribution and abundance of zoea, the relative WMD and abundance ($\# \text{ m}^{-3}$) were analyzed in separate multiple linear regressions with water height, index of stratification (calculated by subtracting the salinity at the surface of the water column from that at the bottom), and depth specific values of salinity and temperature as predictors. Sampling date was included as a dummy variable. Where the data did not meet the assumptions of regression, Spearman's ranked correlations were calculated. All regression analyses were performed using SAS v. 9.1 at an alpha of 0.05.

The relationships of the vertical distribution of zoea to tidal phase and diel phase were investigated in two-way ANOVAs with sampling date used as a nesting factor.

Abundances were analyzed in a similar manner with depth included as a third factor. All abundances were $\log x+1$ transformed to meet the assumption of equality of variance.

Those data whose errors were not normalized were rank transformed, which equalized variances on all occasions. Mean ranks were analyzed in the original ANOVA models with interaction terms following the method proposed by Conover and Iman (1981). *P*-

values generated from this method were compared to the *P*-values calculated from the ANOVAs on untransformed log abundances as suggested by Iman et al. (1984) and significant values reported where they were in agreement. Results generated from the

rank transform approach were more conservative than those from the initial ANOVAs.

All ANOVAs were calculated using SPSS v.16 at an alpha of 0.05.

Environmental variables were also analyzed through ANOVAs to determine how they differed between groups. If they differed in a way that could confound regression and ANOVA results, the dependent variable was regressed on the environmental variable and the residuals analyzed in the ANOVA model to determine whether the previously observed pattern persisted.

To determine the contribution of various sampling dates to our results, each sampling date was also analyzed separately as above, when possible, and results reported where they differed from those of the combined dataset.

RESULTS

First zoea of *N. californiensis* and pinnotherids were collected during all three sampling periods (Table 1.2). Later stage pinnotherid zoea were also collected but not included in any analyses. No later stage *N. californiensis* zoea were found. *N. californiensis* collected in October 2006 were excluded from analyses due to low numbers of specimens.

Water column properties varied between samplings (Table 1.3). May 2007 had the widest range of values of salinity and temperature, clearly showing a tidal influence as well as some stratification. During the October 2006 sampling, temperature and salinity were homogenous over both depth and time.

Vertical distribution. While vertical distributions of both taxa were highly variable (Figures 1.2 and 1.3) we did detect relationships between relative WMD and water height, stratification, and both tidal and diel phase. The vertical distribution of *N. californiensis*, as calculated by relative WMD, was predicted by a combination of water height and stratification index ($F_{(3,15)} = 22.10$; $r^2 = 0.829$; $P < 0.001$). The dummy variable for sampling date indicated that this relationship was true for May 2007 only. In May 2006 we sampled relatively few individuals and the data retained in this analysis represented a narrow range of both salinity and stratification index values. Relative WMD became shallower when water height increased and when index of stratification decreased (Figure 1.4).

When both sampling dates were analyzed together, *N. californiensis* mean relative WMD was shallower at night ($F_{(1,14)} = 21.99$, $P < 0.001$; Figure 1.3b and 1.5a). However, the water heights sampled were also higher, and the level of stratification lower, during the night. To determine whether this relationship would confound the results of the regression and ANOVA, the residuals of relative WMD, after regression on water height and stratification index, were reanalyzed. The residuals retained the pattern of shallower mean relative WMD at night ($F_{(1,14)} = 7.39$, $P = 0.017$).

The relative WMD of pinnotherids was not predicted by any of the variables included in the regression model. When analyzed in a 2-way nested ANOVA with tidal and diel phase as main effects and sampling date as a nesting factor, pinnotherid mean relative WMD did not vary between main effects (Figure 1.5b) but did indicate an effect of

sampling date ($F_{(2,30)} = 4.01$, $P = 0.029$). When analyzed separately by sampling date, the mean relative WMD of pinnotherids in October was shallower during ebb tides than during flood tides ($F_{(1,13)} = 12.01$, $P = 0.005$; Figure 1.2b; Figure 1.5c).

Abundance. Abundances showed strong tidal and diel patterns during our sampled periods, increasing on ebb tides for both taxa, at night for *N. californiensis*, and during the day for pinnotherids (Table 1.4 and 1.5). The degree of vertical salinity stratification was not related to the depth-integrated water column abundance of either pinnotherid or *N. californiensis* zoea ($P = 0.149$ and 0.388 respectively).

The regression of *N. californiensis* abundance indicated a significant relationship with water height ($r^2 = 0.145$, $df = 95$, $F = 7.865$, $P < 0.001$; Figure 1.6). *N. californiensis* zoea were most abundant on ebb tides ($F_{(1,91)} = 16.10$, $P < 0.001$) and at night ($F_{(1,91)} = 13.18$, $P = 0.001$; Figure 1.7a). At night, water heights sampled were higher than those sampled during the day ($F_{(1,91)} = 35.44$, $P < 0.001$) but did not vary between tides. When analyzed in an ANOVA, residuals after regression on water height showed higher abundance specifically on night ebbs (Tide* Diel phase, $F_{(1,92)} = 6.80$, $P = 0.011$).

Pinnotherid abundance increased with water height (Spearman's $r^2 = 0.370$, $df = 156$, $P < 0.001$) and salinity (Spearman's $r^2 = 0.281$, $df = 156$, $P < 0.001$; Figure 1.8). Pinnotherid zoea were more abundant on daytime ebb tides than on daytime flood tides (Tide*Diel phase, GLM on ranks, $F_{(1,33)} = 9.28$, $P = 0.003$; Figure 1.9a) but results differed between sampling dates (GLM on ranks, $F_{(2,135)} = 3.71$, $P = 0.027$; Table 1.5). During the May

2006 sampling, pinnotherids were most abundant at night ($F_{(1,47)} = 5.12$, $P = 0.028$) while during the October 2006 sampling, abundances were greater during the day (GLM on ranks, $F_{(1,39)} = 10.03$, $P = 0.003$; Figure 1.9b) and gave a weak indication of increased abundance at midwater during ebb tides (Tide*Depth, GLM on ranks, $F_{(2,39)} = 3.17$, $P = 0.053$; Figure 1.9c). The ANOVA on water height indicated that daytime samples overall were taken when the water level was higher ($F_{(1,44)} = 8.53$, $P = 0.005$). To determine whether this relationship would confound the results of the regression and ANOVA, the residuals of abundance, after regression on water height, were reanalyzed in an ANOVA. The same, though stronger, pattern was revealed in the residuals. There were significant interactions between tide and depth (GLM on ranks, $F_{(2,39)} = 3.70$, $P = 0.034$) and between diel phase and tide (GLM on ranks, $F_{(1,39)} = 6.15$, $P = 0.018$), with abundance being higher specifically on daytime ebb tides.

DISCUSSION

For both *N. californiensis* and pinnotherid zoea, our sampling revealed clear patterns in abundance, and to a lesser extent in vertical distribution, associated with tidal and diel phases and water height that indicate that these taxa possess behaviours that promote export out of the estuary and reduce susceptibility to visual predators. Vertical distributions of *N. californiensis* and pinnotherids were highly variable. Where they did differ between tidal and diel phases, however, it was in the predicted manner. Variation in abundance with tidal phase was much more consistent than variation in mean depth, with both *N. californiensis* and pinnotherids being more abundant on ebb tides.

Predator avoidance. Our results showed that *N. californiensis* were shallower at night. This shallower nighttime distribution was attributed mostly to one sampling which occurred over two days and a single night, and we therefore hesitate to infer any greater behavioural pattern from this result alone. In a laboratory experiment, however, first zoea of *N. californiensis* did migrate upward during periods of darkness and descend in response to light (see chapter 2) which lends evidence in support of the existence of DVM behaviour. As is commonly observed for decapod larvae (reviewed by Queiroga and Blanton 2005), *N. californiensis* were more abundant at night. This pattern has been observed previously where *N. californiensis* were most abundant specifically on night ebbs (Pimentel 1983). Higher nocturnal abundances and DVM are generally considered to be predator avoidance mechanisms (eg. Bollens and Frost 1989; Bollens and Frost 1991; Hays 2003).

The abundances of pinnotherid zoea also varied between light and dark phases, but the diel phase of highest abundance differed between sampling dates. That we were only able to identify pinnotherids to family may have resulted in the inclusion of more specific taxa with different behavioural patterns. Indeed, in a Costa Rican estuary, the first zoea of two pinnotherid genera were found to have opposite diel patterns of abundance (Dittel and Epifanio 1990).

Transport. While vertical distributions were variable, in October, pinnotherid zoea were shallower during ebb tides, which is consistent with selective tidal stream transport (STST) promoting seaward transport. This pattern of migration was not affected by diel

phase. The depth of *N. californiensis* did not vary with tidal phase. Pimentel (1983) also found that first zoea of *N. californiensis* in the South Slough estuary, Oregon, were uniformly distributed across depths for both tidal phases. The results of a 24 hour sampling series conducted in Yaquina Bay, Oregon by McCrow (1972) showed increased densities of first zoea of *N. californiensis* occurring on the night ebb tide and peaking during the period immediately prior to highest high water. Peak abundances of first zoea are commonly sampled during periods of high water (Forward and Tankersley 2001), and may be the result of larval release and subsequent flushing leading to low abundances towards the end of ebb and beginning of flood tides. Field and laboratory studies have also shown that estuarine brachyurans tend to release their larvae at high water (reviewed by Forward 1987; Morgan and Christy 1995). McCrow (1972) suggested that *N. californiensis* larval release is synchronized to ebb tides but, to our knowledge, this hypothesis has never been tested. While tidally-synchronized larval release would be adaptive, particularly in a mixed estuary, we cannot discount the possibility of vertical migration occurring over a scale that we did not sample. Selective tidal stream transport (STST) can occur if larvae are distributed above the bottom boundary layer during one phase of the tide and within the boundary layer during the other phase (Forward and Tankersley 2001). DiBacco et al. (2001) demonstrated that first zoea of *Pachygrapsus crassipes* in San Diego Bay, California, USA, exploit the sediment-water interface during flood tides to maximize unidirectional transport. We cannot ascertain whether changes in abundance with tide are due to the presence of larvae below the bottom meter of the water column during flood tides or due to the release of individuals from eggs prior to the

ebbing tide. Nevertheless, the highly tidal pattern of abundance gives evidence of the presence of behaviours promoting export in both *N. californiensis* and pinnotherid zoea.

In our attempt to relate zoeal abundances and relative depths to salinity, temperature, water height, and degree of stratification, water height was consistently a significant predictor. Both *N. californiensis* and pinnotherid larvae were more abundant with increased water height and *N. californiensis* became shallower with increasing water height. Change in water height has been used as a proxy for change in pressure (Queiroga 1998). The lack of any relationship of abundance or vertical distribution with temperature or salinity in either taxon is interesting and may be due to the generally mixed condition of water column of the estuary (Lochman et al. 1995). Under these conditions, these factors would be unreliable indicators of tidal phase. Hydrostatic pressure, however, is a highly conservative indicator of tidal phase. In shallow estuaries, relative change in hydrostatic pressure would be large. Laboratory studies by Forward and colleagues (Forward and Buswell 1989; Forward and Wellins 1989; Forward et al. 1989) have shown that zoea of the estuarine brachyuran species *Rhithropanopeus larvae* and *Neopanope sayi* are able to perceive relatively small changes in absolute hydrostatic pressure and respond to rates of pressure change with active depth regulation.

While *N. californiensis* became shallower with increased water height, they also became shallower with decreased stratification. This suggests that shallower distributions are the result of increased vertical mixing. During our sampling periods, high water corresponded with increased water column homogeneity, barring us from making any

conclusions based on this result alone. To address this, we made a further prediction based on the DVM behaviour of *N. californiensis* (see next chapter). If the relationship of WMD to water height existed only at night, the observed shallower distribution observed was likely the result of behaviour. A passive change in WMD caused by vertical mixing would predict that the relationship would exist irregardless of diel phase. Indeed, when the data were reanalyzed by diel phase, *N. californiensis* WMD decreased with water height ($F_{(1,14)} = 7.39, P = 0.015$) only at night.

Pinnotherid zoea displayed vertical distributions predicted by the selective tidal stream export model during one of our samplings. Because we were unable to identify the sampled pinnotherids to species we cannot identify the range of its host species. However, despite the apparent possession of behaviours promoting export out of the estuary, later stage pinnotherid zoea were common in our samples. This might indicate that larvae reside in the estuary for a longer period prior to export, perhaps as the result of larval release further upstream. Increased estuarine residence time may favour the presence of tidal vertical migration behaviour.

We did not find any evidence for tidally-timed vertical migration in *N. californiensis*. The coupling of restricted adult range with tidally-timed larval release may be sufficient, particularly in a highly tidal estuary, to rapidly flush first zoea to the nearshore. Indeed, during our sampling we did not collect any individuals of a zoeal stage later than the first. Graham and Bollens (*in review*) sampled Willapa Bay bi-weekly for over two years and also never collected later developmental stages of *N. californiensis*. When sampling

Yaquina Bay, Oregon, and neighbouring coastal areas, McCrow (1972) found later zoeal stages only outside of the bay. The range of adult *N. californiensis* is generally restricted to areas near the estuary mouth (McCrow 1972; Bird 1982; Johnson and Gonor 1982) and it has been suggested that this is due to the greater degree of food sedimented and available to deposit feeders in these areas (Bird 1982). The need for rapid larval export may impose a further restriction on adult distribution within coastal bays and estuaries.

While there is a great deal of evidence for tidally synchronized larval release (Morgan and Christy 1995) the location of larval release is often too far from the mouth of the estuary to enable flushing of larvae on a single ebb tide. Tidally synchronized vertical migration has also been frequently documented (reviewed by Forward and Tankersley 2001; Queiroga and Blanton 2005) though our results lend only weak support for this behaviour in pinnotherid zoea. Tidal migration may have been occurring from below the lowest depth of sampling and contributed to the higher abundances of larvae we observed on ebbing tides.

Summary. Our results show that *N. californiensis* possesses behaviours to reduce susceptibility to visual predators. We also show that both *N. californiensis* and pinnotherids possess behaviours promoting seaward transport of the first zoeal stage, adding to earlier reports on larval export in decapod larvae. We found these behaviours to likely be linked to a potential cue, water height or hydrostatic pressure. Further study of physical processes, behaviours and cues governing vertical distribution of larvae will increase our ability to predict dispersal and recruitment dynamics.

TABLES

Table 1.1. Summary of sample collection details and timing from the three sampling dates.

Date and time range of samplings	Lunar phase (% illuminated)	Tidal range (m)	# samples	Avg. volume sampled (l)	Max. pump rate (l min ⁻¹)
2300 16 May - 0030 18 May 2006	93%	3.15	54	400	65
0930 9 Oct - 1800 10 Oct. 2006	90%	3.44	47	1380	484
1600 15 May - 1200 16 May 2007	2%	3.92	56	1815	454

TABLES (CONTINUED)

Table 1.2. Collection summary.

	<i>N. californiensis</i>		Pinnotheridae	
	# caught	Concentration (# m ⁻³)	# caught	Concentration (# m ⁻³)
16-18 May 2006	121	5.8	159	7.6
9-10 October 2006	17	0.3	464	6.4
15-16 May 2007	672	7.8	227	2.8

TABLES (CONTINUED)

Table 1.3. Summary of mean (\bar{x}), minimum and maximum values of temperature, salinity, and stratification which occurred during samplings.

	Temperature			\bar{x}	Salinity		Stratification Index		
	\bar{x}	min	max		min	max	\bar{x}	min	max
16-18 May 2006	14.1	12.4	16.0	27.5	25.9	28.3	0.3	0	0.9
9-10 October 2006	12.7	11.8	13.7	31.7	30.9	31.8	0.1	0	0.4
15-16 May 2007	11.9	9.5	14.6	28.7	23.3	31.8	1.1	0.2	2.7

TABLES (CONTINUED)

Table 1.4. *N. californiensis*. Summary of results of ANOVAs and regressions on *N. californiensis* abundance. Nonsignificant interactions terms are omitted. Significance is indicated by ‘*’ when $P < 0.05$ and ‘**’ when $P < 0.005$. Effects that were not analyzed are noted with ‘-’.

	Sampling Date		
	May 2006 and 2007	May 2006	May 2007
<i>ANOVA factors</i>			
Tide	**	**	*
Diel Phase		*	**
Depth			
Sampling Date		-	-
Tide*Diel Phase	*	**	
<i>Regression factors</i>			
Salinity			-
Temperature			-
Water height	*		-

TABLES (CONTINUED)

Table 1.5. **Pinnotheridae**. Summary of results of ANOVAs and regressions on pinnotherid abundance. Nonsignificant interactions terms are omitted. Significance is indicated by ‘*’ when $P < 0.05$ and ‘**’ when $P < 0.005$. Effects that were not analyzed are noted with ‘-’.

	Sampling Date			
	All	May 2006	October 2006	May 2007
<i>ANOVA factors</i>				
Tide	*			
Diel Phase		*	*	
Depth				
Sampling Date	*			
Tide*Diel Phase	**		*	**
Tide*Depth			*	
<i>Regression factors</i>				
Salinity	**	**		-
Temperature				-
Water height	**	*	**	-

FIGURE CAPTIONS

Fig. 1.1. Willapa Bay, Washington, USA. (★) Location of fixed station where all samples were collected.

Fig. 1.2. **Pinnotherids**. Proportion of first zoea caught at sampled depths over time. Shaded areas represent night. Tidal phase is indicated above the figure and abundance of larvae caught during that vertical pumping series is shown under each series. Total water column height varied over the sampled periods. This is represented by the blacked out areas at the base of the figures. Error bars represent ± 1 SE.

Fig. 1.3. *Neotrypaea californiensis*. Proportion of first zoea caught at sampled depths over time. Shaded areas represent night. Tidal phase is indicated above the figure and abundance of larvae caught during that vertical pumping series is shown under each series. Total water column height varied over the sampled periods. This is represented by the blacked out areas at the base of the figures. Error bars represent ± 1 SE.

Fig. 1.4. The relative WMD of *N. californiensis* collected on 16-18 May 2006 and 15-16 May 2007 in Willapa Bay, Washington, became shallower with increasing water height and decreasing index of stratification ($r^2=0.779$; $P < 0.001$). $N = 19$

Fig. 1.5. Mean relative WMD of first zoea by tidal and diel phase. *N. californiensis* zoea mean relative WMD was higher at night ($P = 0.005$) (A). When sampling events were analyzed together, mean relative WMD of pinnotherid zoea did not differ between tidal or diel phase (B), however, in October of 2006, zoea were shallower on ebb tides ($P = 0.005$) (C). Error bars represent ± 1 SE.

Fig. 1.6. The log (x+1) abundance m^{-3} of *N. californiensis* increased with water height above MLLW ($P < 0.001$) on 16-18 May 2006 and 15-16 May 2007 in Willapa Bay, WA. N = 96

Fig. 1.7. Mean log (x+1) *N. californiensis* abundances m^{-3} between tidal and diel phases. A) Mean *N. californiensis* abundance was higher at night ($P = 0.001$) and on ebb tides ($P < 0.001$). After regression on water height and temperature the residuals of *N. californiensis* abundance showed increased abundance specifically on ebb tides ($P = 0.011$). B) In the May 2006 sampling, *N. californiensis* were most abundant on nighttime ebb tides ($P = 0.018$). Error bars represent ± 1 SE.

Fig. 1.8. Pinnotherid abundance m^{-3} increased with water height (Spearman's $P < 0.001$) and salinity (Spearman's $P < 0.001$). N = 157

Fig. 1.9. Mean log (x+1) pinnotherid abundance m^{-3} between tidal and diel phases and depths. A) When analyzing the entire data set, pinnotherid zoea were more abundant on

daytime ebb tides than during daytime flood tides ($P = 0.003$). Log pinnotherid abundances from the October 2006 sampling showed a pattern of B) increased abundance on daytime ebbs ($P = 0.018$) and C) at midwater on ebb tides ($P = 0.034$). Error bars represent $\pm 1SE$.

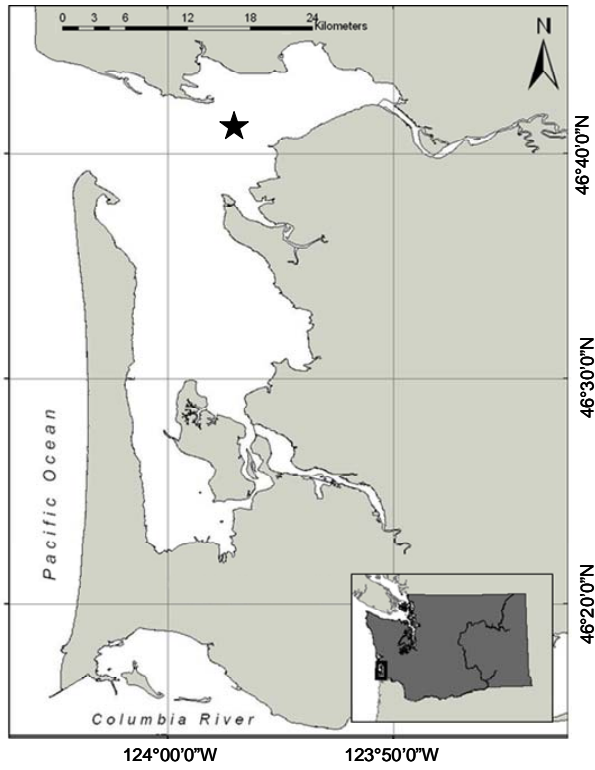
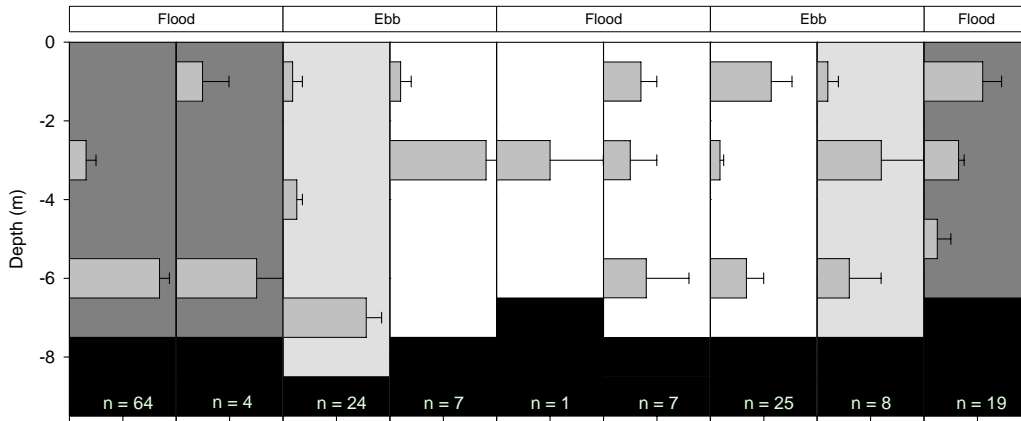
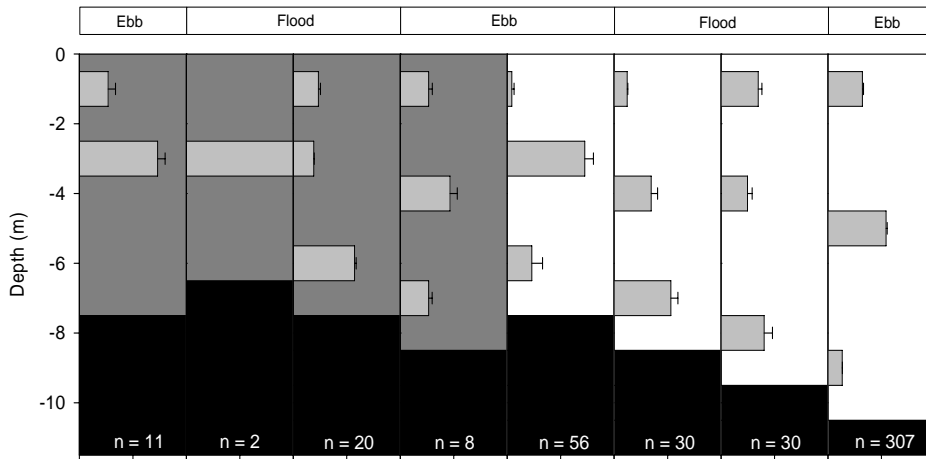


Fig. 1.1.

a) Pinnotherids, May 16-18th, 2006



b) Pinnotherids, October 9-10th, 2006



c) Pinnotherids, May 15-16, 2007

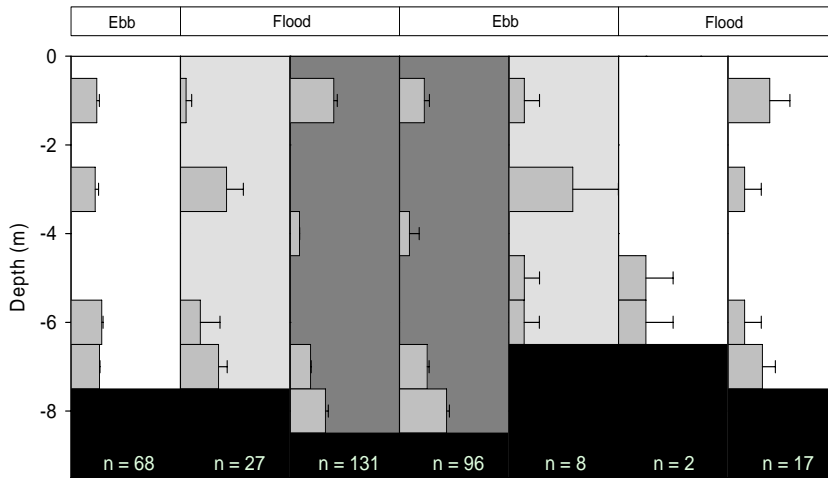
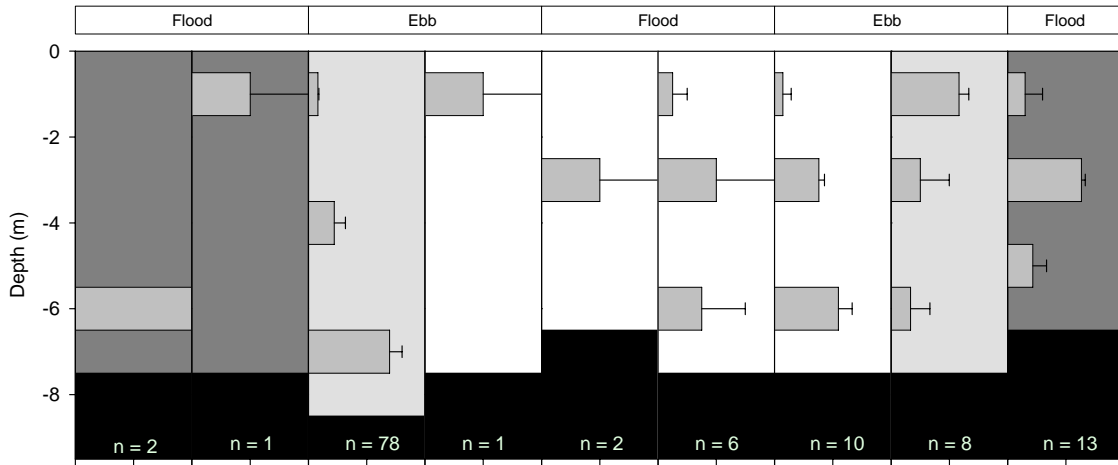


Fig. 1.2.

a) *Neotrypaea californiensis*, May 16-18, 2006



b) *Neotrypaea californiensis*, May 15-16, 2007

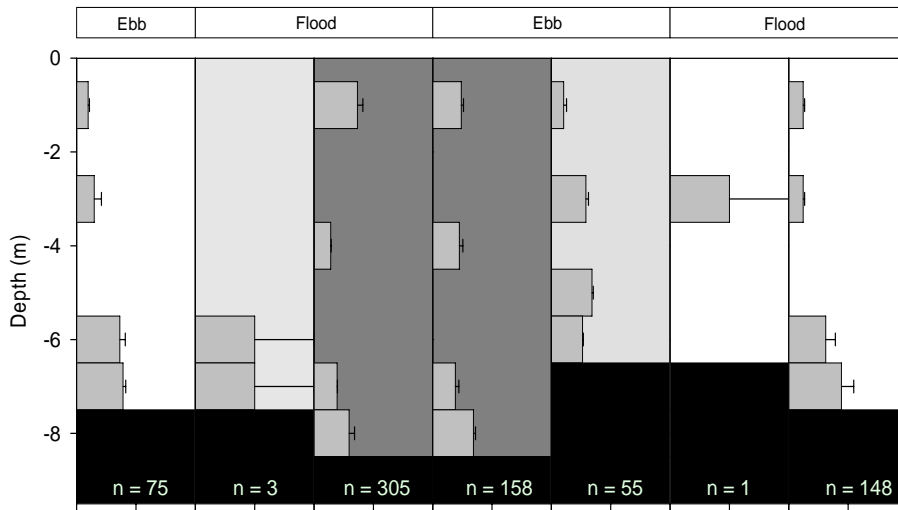


Fig. 1.3.

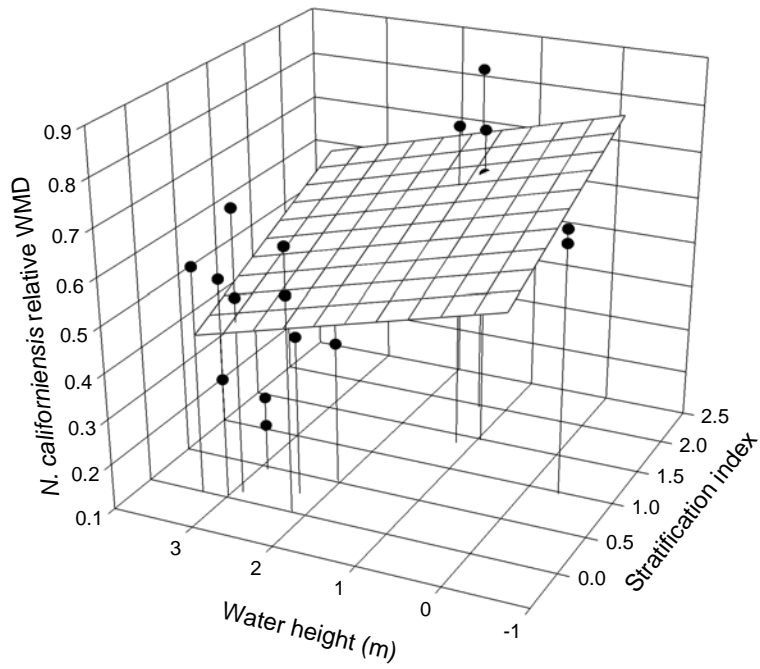


Fig. 1.4.

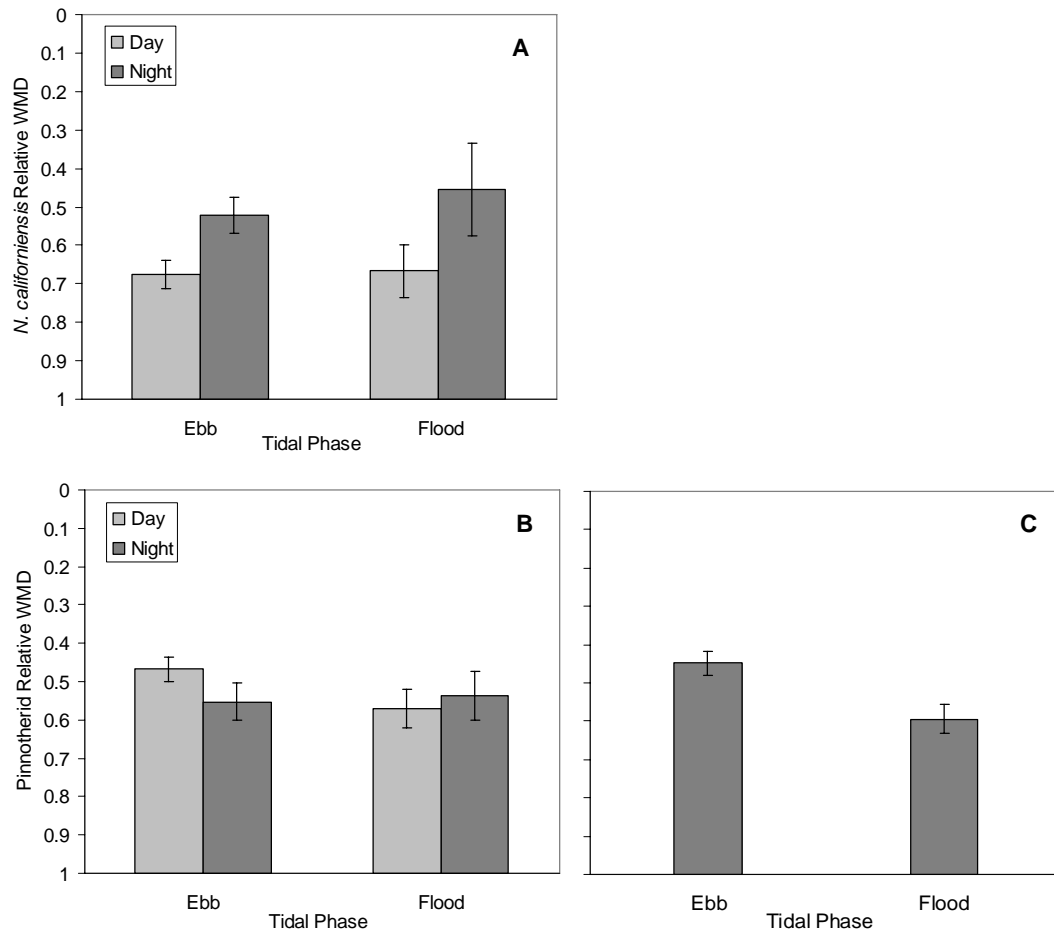


Fig. 1.5.

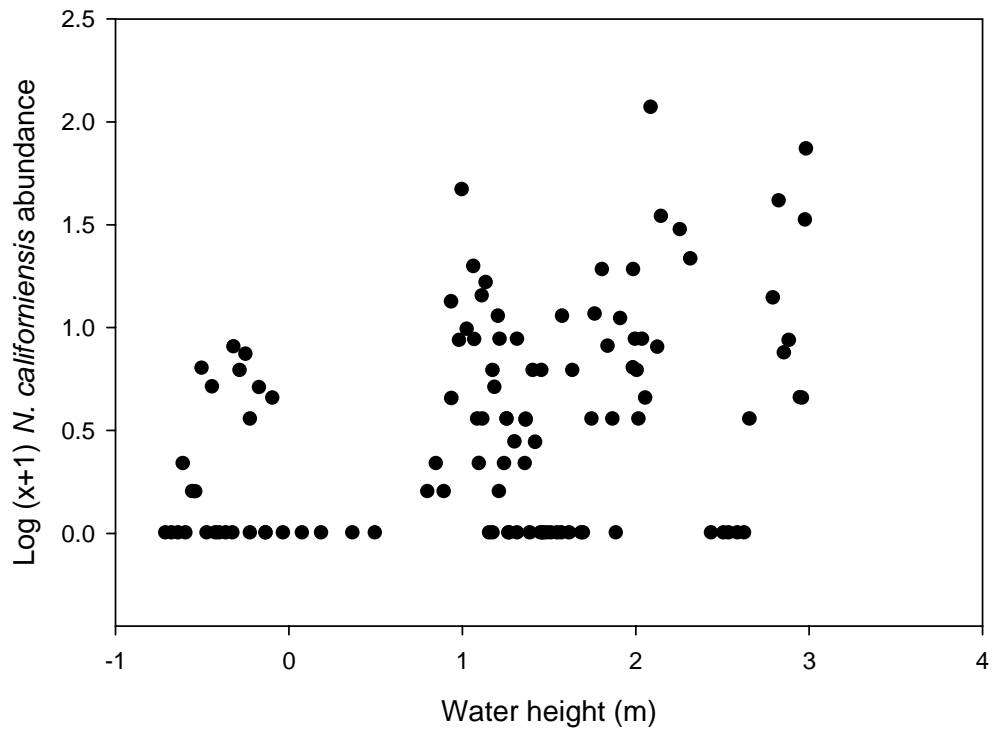


Fig. 1.6.

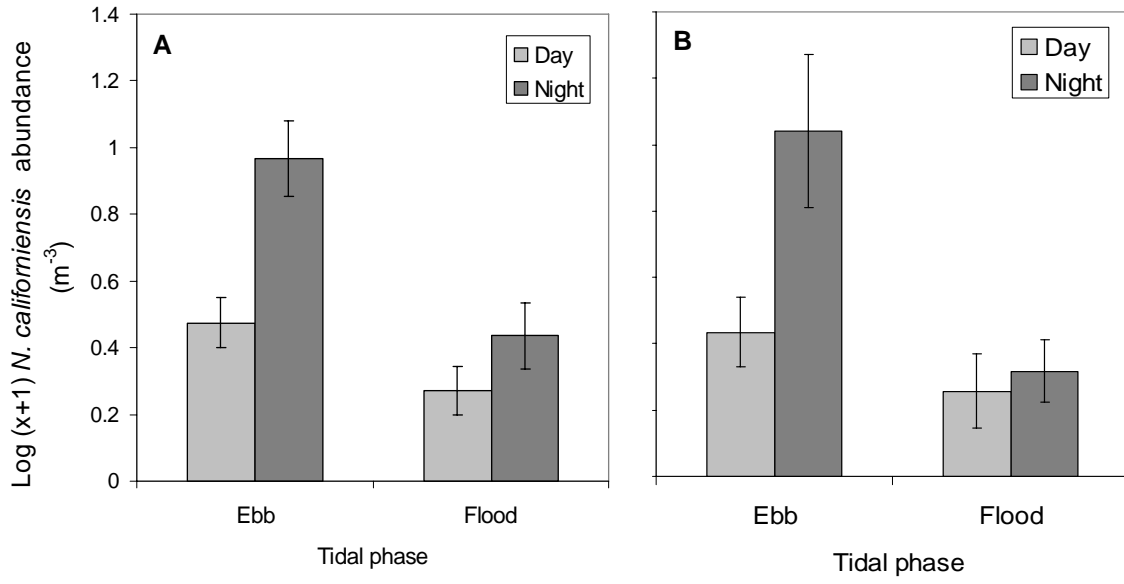


Fig. 1.7

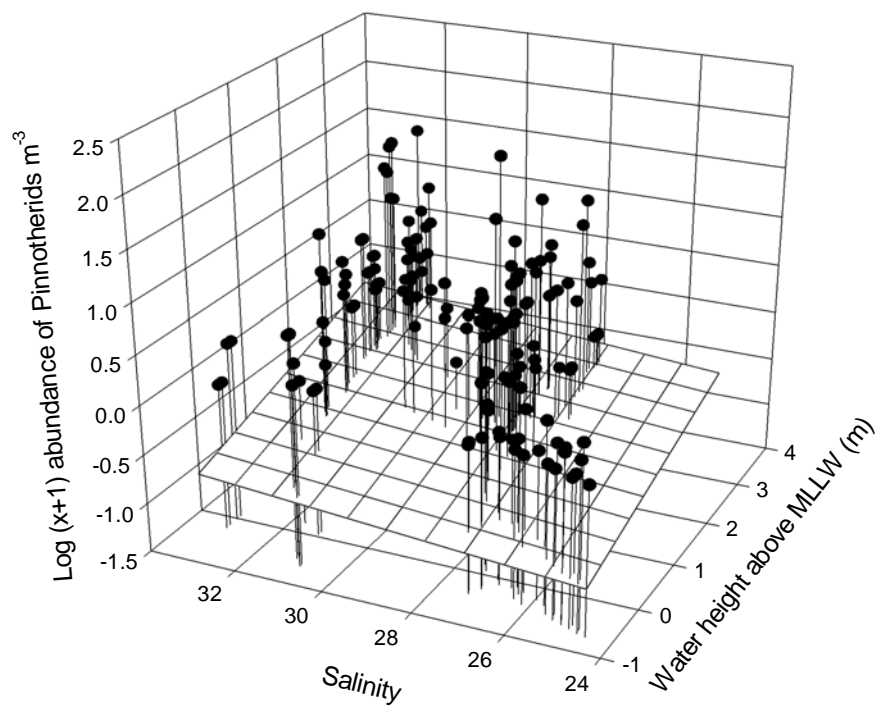


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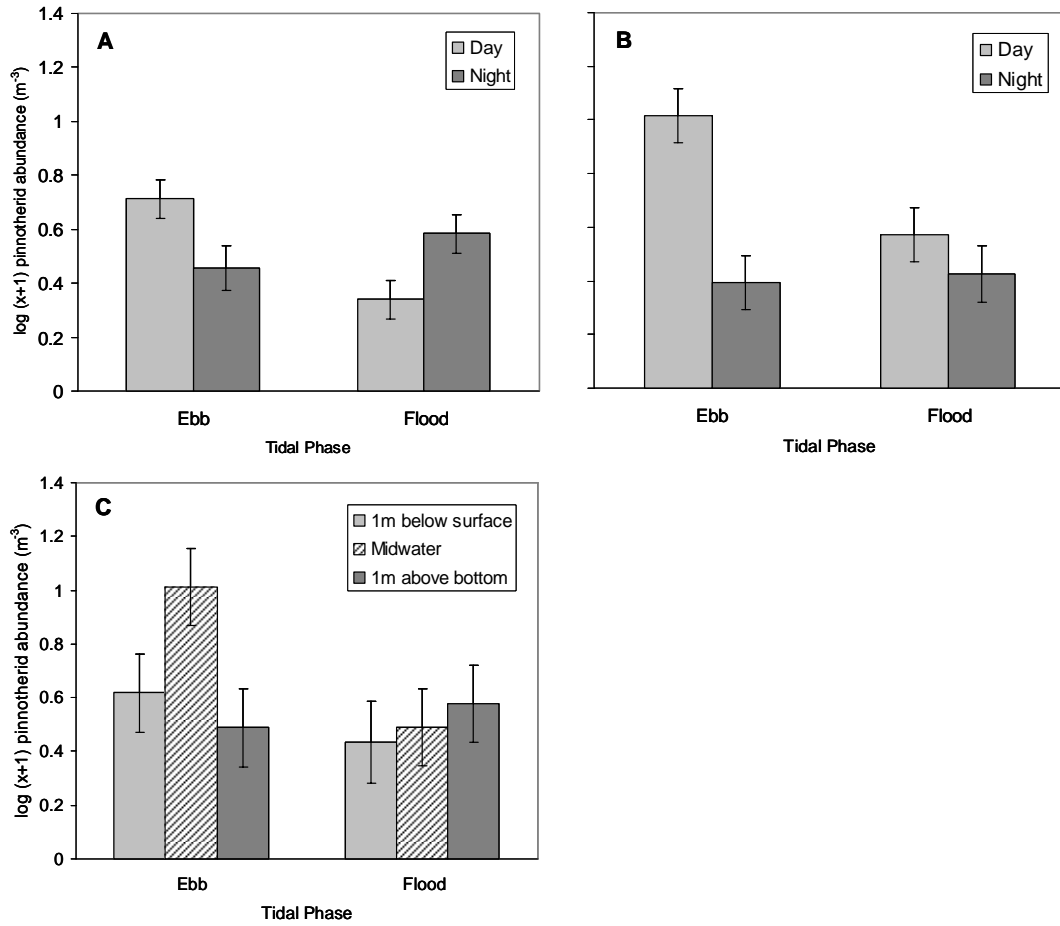


Fig. 1.9.

CHAPTER 2:

BIOLOGICAL THIN LAYER FORMATION IN RESPONSE TO PHYSICAL THIN
LAYERS: INTERACTIONS BETWEEN THE LARVAL DECAPOD, *NEOTRYPAEA*
CALIFORNIENSIS, SALINITY AND LIGHT

ABSTRACT

Resource heterogeneity is a major driver of zooplankton behaviour. As resource patches are often associated with physical discontinuities in the water column, the ability to cue to these discontinuities in order to locate prey patches could increase zooplankton foraging success. The objective of this study was to determine the effect of an artificial physical thin layer (halocline/pycnocline) on the vertical distribution of the first zoea of the decapod, *Neotrypaea californiensis* and how this distribution was affected by a light/dark cycle. We recorded the depth distributions of larvae in salinity stratified and vertically homogenous 2-m tall tanks using video cameras that panned the vertical extent of each tank hourly over periods of approximately 24 hours. There was a greater degree of crowding in stratified treatments and during darkness. Our results indicate that *N. californiensis* will aggregate in the presence of a physical thin layer and exhibit diel vertical migration (DVM), but that this response is plastic and possibly dependent on pre-conditioning. In the field, the aggregation of decapod larvae around thin layers may play a role in the formation, maintenance and/or erasure of thin layers as well as make these layers important foraging sites for higher trophic levels.

INTRODUCTION

As the average concentration of food in the ocean is often too low to support the growth observed in zooplankton species, the ability to exploit patchily distributed resources is linked to zooplankton survival (Bainbridge 1953; Mullin and Brooks 1976; Daro 1988; Tiselius 1992). Resource patches can occur in the form of biological thin layers. These thin layers of phytoplankton are often found at density discontinuities in the water column (Nielsen et al. 1990; Bjornsen and Nielsen 1991; Tiselius et al. 1994; Hanson and Donaghay 1998; Deksheniaks et al. 2001; Rines et al. 2002; McManus et al. 2008; Peterson and Peterson 2008). Biological thin layers range from tens of centimeters to several meters in depth (Hanson and Donaghay 1998; Deksheniaks et al. 2001; Rines et al. 2002), can persist for several days (Nielsen et al. 1990; Rines et al. 2002), and span several kilometers horizontally (Rines et al. 2002). Field observations demonstrate that planktonic grazers aggregate at these thin layers (Nielsen et al. 1990; Castro et al. 1991; Tiselius et al. 1994; Holliday et al. 1998; Gallagher et al. 2004). For several groups of copepods these aggregations are considered to have a behavioural cause (Leising and Franks 2002; Gallagher et al. 2004; Woodson et al. 2007).

Several experimental studies have also shown that zooplankton form aggregations or otherwise respond to thin layers of food (Bochdansky and Bollens 2004; Clay et al. 2004; Ignoffo et al. 2005), while other studies show that these aggregations can occur in the absence of food, which suggests that these some species use physical cues to maintain a preferred position (Lance 1962; Tiselius 1992; Lougee et al. 2002; Woodson et al. 2007). For instance, Tiselius (1992) observed that individual *Acartia tonsa* (Copepoda) increase

the frequency of behaviours associated with feeding when they encountered a halocline that did not contain food particles. The estuarine copepod, *Eurytemora affinis*, also responded to the presence of a salinity/density gradient with increased proportional residence time within the layer and altered swimming behaviour (Woodson et al. 2007).

Many zooplankton groups exhibit diel vertical migration (DVM), which is generally considered a predator avoidance behaviour (eg. Bollens and Frost 1989; Bollens and Frost 1991; Hays 2003). By aggregating at thin layers, zooplankton may be increasing their risk of predation as residence in a patch will only decrease an individual's risk of predation if predators do not also aggregate at that patch. Higher trophic levels can aggregate at oceanographic discontinuities (Healey et al. 1990; Genin 2004) and there is some evidence that this also occurs at thin layers. Larval herring, *Clupea harengus* and *Clupea pallasii*, have been found to aggregate in the area of experimentally created thin layers (Batty 1994; Clay et al. 2004). Ctenophores have also been observed aggregating at interfaces (thin layers) in the field (Harder 1968). Indeed, Saiz et al. (1993) found that the copepod, *Acartia tonsa*, reduced the amount of time spent in food patches when in the presence of a predator. Many questions remain as to how responses to thin layers themselves interact with predator avoidance behaviours. If zooplankton encounter a thin layer but not food, will they maintain their position within that layer? Will zooplankton disperse from a thin layer during light to avoid detection by visual predators?

In the present study, we investigated the effect of the presence of physical thin layers (salinity/density stratification) and the light/dark cycle on the vertical distribution of the

first zoea of the decapod, *Neotrypaea californiensis*, in a series of laboratory experiments. To date, most behavioural research related to thin layers has focused on copepod species (see Leising and Franks 2002; Lougee et al. 2002; Saiz et al. 2003; Bochdansky and Bollens 2004; Woodson et al. 2005; Woodson et al. 2007), with only a few exceptions (Batty 1994; Lougee et al. 2002; Clay et al. 2004; Ignoffo et al. 2005). Decapod larvae are seasonally abundant members of the zooplankton community of coastal oceans (McConaugha 1992) and, as meroplankton, their response to thin layers can be assumed to be independent from the benefit of reproduction via aggregation with conspecifics. There is a large body of literature investigating the behaviour of decapod larvae in relation to environmental variables, some of which specifically address their response to salinity and density discontinuities (see Lance 1962; Roberts 1962; Sulkin and Van Heukelem 1982; O'Connor and Epifanio 1985). However, no previous studies, to our knowledge, have addressed the persistence of this response and its interaction with light. Our specific research questions were: How do larvae of *N. californiensis* respond to the presence of physical stratification? How is this response mitigated by light?

MATERIALS AND METHODS

We conducted three vertical distribution experiments on the first zoea of *N. californiensis*. Briefly, our experimental apparatus consisted of two 2-m high columnar Plexiglas tanks (200cm x 7.6cm x 5.1cm), each equipped with an overhead light source (65 watt GE Grow Bulb) and light diffuser, a panning monochrome video camera (Cohu) and an infrared light emitting diode (LED). As decapods are unable to perceive infrared light (Forward and Costlow 1974), the video camera was able to record shadow images

of the larvae as it panned without disrupting their behaviour. For further detail and application of this system see Speekman et al. (2000), Lougee et al. (2002), Clay et al. (2004), and Ignoffo et al (2005). All experiments were conducted at 19°C in a temperature and light-controlled room.

For each experiment, we stratified the water in the treatment tank at mid-depth by first filling the lower half of the tank with higher salinity water and then slowly pumping lower salinity water into the top half of the tank. The water in the control tank was vertically homogenous and the same salinity as the top layer of the stratified tank. The salinity of the water at the surface of the tank did not differ within an experiment but, as it was made to match the salinity at which the larvae were collected, did differ between experiments (Table 2.1). The seawater used in these experiments was made of Millipore filtered water and Instant Ocean sea salt.

N. californiensis first zoea included in each experiment were collected within 24 hours of the start of the experiment. Larvae were collected by oblique net tows from Willapa Bay, Washington, USA (46°34'46"N 123°56'32"W), and immediately diluted into seawater from the collection site. We observed the larvae immediately prior to the experiments, typically the day of or the day after collection, to ensure the inclusion of only healthy looking individuals. Equal numbers of larvae were placed into each tank from above.

Video cameras were programmed to record and begin panning every hour, with each pan taking 6 minutes. Lights were programmed to turn on and off according to the local

timing of sunrise and sunset. Due to technical difficulties, the duration of experiments varied (Table 2.1). At the end of each experiment, we measured temperature and salinity at 20cm increments using a hand-held YSI 85 salinity-temperature probe to ensure that stratification had persisted. Previous experiments have shown salinity stratification to persist in these tanks for several days (Lougee et al. 2002). Tapes of the camera pans were analyzed visually and the depth of each larva recorded to the nearest 5cm. Statistical analyses were performed using Minitab v.15 software with significance set at an alpha of 0.05.

To assess the extent of aggregation of the larvae, for each camera pan we calculated an index of mean crowding from the raw counts of larvae in each 5cm depth bin, after Lloyd (1967):

$$m^* = m + [(\sigma^2/m) - 1]$$

where m^* is the mean crowding, m is the mean number of larvae per bin, and σ^2 is the variance. To avoid biasing these indices, we omitted larvae from the top and bottom 5 cm bins because the presence of a surface (air or bottom) might cause a natural aggregation (Bochdansky and Bollens 2004). To assess the depth distribution of the larvae, we separated the tanks into three depth bins, surface (0-60cm), middle (60-120cm containing the halocline), and bottom (120-180cm) in order to compare the number of larvae in each bin.

Data analysis. Preliminary analysis indicated that the behaviour of *N. californiensis* differed between experiments ($F_{(4,92)} = 3.73$, $P = 0.007$; Figure 1). We therefore chose to

analyze the results of each experiment, both m^* and number of larvae pooled between depth bins, in separate ANOVAs using general linear models (GLMs).

In order to include light as a factor in our analysis while also including hour, we analyzed the effects of treatment and light in separate GLMs. The effects of treatment and hour as a repeated measure were analyzed in one GLM. We then analyzed the factors light and treatment together with hour as a continuous covariate in a separate GLM. Depth bin was included as a factor for the analyses comparing number of larvae in the top to the number of larvae in the middle of the tank. Where data did not meet the assumption of equality of variance it was square root transformed which normalized the variances in all cases. Data were only used from hours where paired treatment and control pans were available. Data on the number of larvae in the bottom portion of the tank were not included in the analysis to meet ANOVA's assumption of independence.

RESULTS

The salinity stratification created in our treatment tanks persisted through all experiments and the haloclines occurred over a depth of 40cm or less. In two of our experiments (1 and 3) there was a clear concentration of larvae around the center of the stratified (treatment) tanks, where the stratified layer was located (Figure 1). In experiment 1, most larvae initially positioned themselves toward the surface of the tanks. After the light was turned on, most individuals were in the area of the thin layer (Figure 1a). Although in both of these experiments the recordings of vertical distribution in the control tank ended prior to those of the stratified tanks, we can see that larvae continued

to congregate in the area of the thin layer for the remainder of the recording (35 hours). In experiment 3, larvae responded immediately to the thin layer but dispersed after 22 hours (Figure 1c). The homogenous (control) tanks for these experiments generally revealed a much more dispersed distribution, though in experiment 1 larvae distributed themselves at the bottom of the tank during darkness. Another difference between these two experiments is the period in which they are most highly aggregated at the thin layer. In experiment 1 larvae appeared to aggregate at the stratified layer during periods of light whereas in experiment 3 they aggregated during periods of darkness. Our second experiment differed markedly in that there was no detectable response to the stratification but instead, a strong response to the light/dark cycle in both stratified and homogenous tanks (Figure 1b).

To determine whether the presence of a halocline affected the degree of aggregation of *N. californiensis*, we compared m^* between stratified and control tanks and between periods of darkness and light. In our first experiment, there was a greater degree of crowding in our stratified tank ($F_{(1,23)} = 5.12, P = 0.033$; Figure 2a). The degree of crowding did not vary with the light/dark cycle ($F_{(1,43)} = 1.39, P = 0.245$). Though salinity measurements taken at the end of each experiment showed that our haloclines were maintained in each instance, during our second experiment, salinity stratification had no effect on m^* ($F_{(1,27)} = 2.18; P = 0.151$), though there was an effect of light ($F_{(1,51)} = 14.39, P < 0.001$; Figure 2b). In our third experiment, treatment again had a significant effect on m^* ($F_{(1,23)} = 8.13, P = 0.008$; Figure 2c) but light did not ($F_{(1,49)} = 1.06, P = 0.309$). In our third

experiment only, we saw a significant effect of time (where it was treated as a covariate) with crowding decreasing over time ($T = -2.84$, $P = 0.007$).

To determine whether *N. californiensis* was altering its vertical distribution in response to stratification or light, we compared the number of larvae in the top and middle depth bins. In our first experiment, when hour was included in the analysis as a repeated measure, there were more larvae in the top two bins of the stratified tank than in the top two bins of the homogenous tank ($F_{(1,72)} = 8.56$, $P = 0.010$). Our ANCOVA revealed an interaction between treatment, light level, and depth bin ($F_{(1,91)} = 24.53$, $P < 0.001$; Figure 3a). In the stratified tank, the number of individuals was larger near the surface during dark and larger in the middle of the tank during light. In the homogenous tank, the number of zoea in the two depth bins did not differ between light levels or depth bins.

Consistent with the results of our analysis of m^* , the vertical distribution of *N. californiensis* in experiment 2 was not significantly affected by the salinity stratification treatment. Our ANCOVA revealed a significant effect of light where the number of larvae increased in the top depth bin in both tanks when it was dark (Depth bin*Light, $F_{(1,103)} = 60.0$, $P < 0.001$; Figure 3b).

In our third experiment, the repeated measures ANOVA revealed an interaction between treatment and depth ($F_{(1,78)} = 15.07$, $P < 0.001$) where, in the stratified treatment, the mid-depth bin had more larvae than the top depth bin. In the homogenous tank, there were no differences in the number of larvae in the two depth bins. The ANCOVA showed that

this preference for the stratified layer varied with light level. During periods of light, the number of larvae in the top depth bin increased (Figure 3c; Treatment*Depth Bin*Light, $df = 107$, $F_{(1,99)} = 12.19$, $P = 0.001$). In the control tank, vertical distribution was unaffected by the light/dark cycle.

DISCUSSION

In two of our experiments, *N. californiensis* zoea aggregated in the area of the halocline. However the observation that individual *N. californiensis* crossed the thin layer in experiment 3 indicates that aggregations of *N. californiensis* were not due to the thin layer acting as a physical barrier. Physical thin layers are often associated with high concentrations of phytoplankton and zooplankton (Nielsen et al. 1990; Bjornsen and Nielsen 1991; Castro et al. 1991; Tiselius et al. 1994; Hanson and Donaghay 1998; Holliday et al. 1998; Gallagher et al. 2004) and residence within these layers would increase rate of encounter of *N. californiensis* with prey items. Thin layers also indicate the interface between two water masses and may, particularly for estuarine species like *N. californiensis*, be an important cue used in horizontal transport or position maintenance (Seuront 2006). As is common among estuarine decapods, *N. californiensis* possesses behaviours which promote export of first zoea from the estuary to coastal waters (Johnson and Gonor 1982; Pimental 1983). We would therefore predict that if *N. californiensis* were using physical thin layers as a cue for horizontal transport, they would position themselves above it, in the less saline, seaward flowing water. In experiment 2, larvae did position themselves above the thin layer, though the larvae in the two other

experiments aggregated within the layer, which in a stratified estuary would represent an area of no net horizontal movement.

Zooplankton have also been found to avoid thin layers (Bjornsen and Nielsen 1991; Alldredge et al. 2002). By avoiding biological thin layers, zooplankton may avoid exposure to toxic bloom-forming species, pathogens and predators. Such a potential interaction with predation-risk was observed by McManus et al. (2005) in Monterey Bay, California. An aggregation of zooplankton in the area of a biological thin layer dissipated during darkness and reformed at dawn. In our first experiment, we saw an aggregation at the surface during darkness and at the thin layer during light (Figure 1a) and in our third experiment aggregation at the thin layer persisted during both light and dark conditions (Figure 1e). We had predicted that *N. californiensis* would disperse from the layer during light as it may increase susceptibility to predation. The likelihood of aggregation at a thin layer during higher levels of ambient light may be affected by the starvation of the individuals, where the need to forage maximally during both day and night outweighs the increased risk of predation.

In our third experiment, larvae in the stratified tank positioned themselves in the area of the thin layer and maintained this position for 22 hours before dispersing to the top and the bottom of the tank (Figure 1e). It is interesting that they maintained this position for that length of time, over the light/dark cycle, without encountering food items. A predatory copepod, *Oithona nana*, also maintained its concentration about an artificial halocline under both light and dark conditions (Harder 1968).

While our results show that, in general, larvae were located in the area of the thin layer, there was some individual variability. Because we did not monitor an individual's position continuously, we cannot say whether the recording of individuals outside the thin layer represents certain individuals remaining outside the thin layer or whether it represents short-term forays from the thin layer. If thin layers are likely areas in which to find food, strategies of 'scanning' a thin layer for food would be beneficial. Because water above and below a physical thin layer is moving at different velocities than the water within that thin layer, temporarily altering their vertical position and re-descending or re-ascending to the thin layer would allow larvae to search for food in the horizontal dimension. Because thin layers often cover a horizontal area of several kms (Rines et al. 2002) this strategy would increase the likelihood of locating an area of thin layer containing a prey patch.

The lack of DVM behaviour in the control tanks (Figure 1b,f), especially in light of the results of our second experiment (discussed below), is puzzling. In our vertically homogenous tanks larvae were evenly dispersed throughout the top and middle depth bins, though an exception to this occurred in our third experiment during darkness, where larvae appeared to aggregate towards the bottom of the tank. Again, because we did not follow the movement of individual larvae, we cannot determine whether this dispersion was due to position maintenance or to unsynchronized vertical migration.

It is interesting that in our second experiment the larvae did not respond to the presence of the thin layer. Instead, the larvae in both treatment and control tanks responded to the light/dark cycle by positioning themselves towards the surface during darkness and dispersing and descending during light (Figure 1c-d). While light seemed to affect the distribution of larvae in our other experiments, its influence in both tanks in experiment 2 was far stronger than was seen in other tanks. The only differences between these experiments were the date of collection of the larvae and the salinities of the water used. The treatment tank had a bottom salinity that was 5 and 3 psu higher than that of experiments 1 and 3, respectively. However, the response was seen in both tanks and the salinity of the homogenous tank was 27, within the range used in the other experiments. We also did one preliminary experiment (data not shown) with salinities of 22-27 psu which also showed a response to light but not stratification. Differences in experimental design or treatment of larvae are unable to account for the difference in response, suggesting that these larvae, as a group, differed from those used in experiments 1 and 3. The larvae used in each experiment were collected on different occasions. While larvae included in each individual experiment likely shared similar histories, these histories would likely have differed between experiments. These cohorts may have differed in terms of age, (though all were first zoea), location of release, or past experience with predators and prey.

In summary, we found that the first zoea of *N. californiensis* altered their vertical distribution and aggregated in the presence of physical thin layers. Aggregations were maintained for up to 22 hours in the absence of prey. *N. californiensis* zoea also

responded to the light/dark cycle in a manner consistent with DVM. We tested 3 cohorts and each showed significant yet somewhat different responses, indicating that some shared within-cohort character determined their response. Behavioural plasticity is necessary to maximize foraging success while minimizing the risk of predation in a changing environment. In a study similar to ours, Lougee et al. (2002) also saw considerable within-species variability in vertical migration patterns in response to artificial thin layers. The different behavioural responses seen in this and our study illustrate the benefit of including field caught organisms in experiments. By not controlling the pre-conditioning of these larvae, we gained ecologically-relevant information on the range of potential responses to thin layers, from which we can form more directed questions aimed at understanding this plasticity and the vertical distributions measured in the field. Decapod aggregation in the area of natural thin layers may play a role in biological thin layer formation, maintenance, and/or erasure through their role as both predators and prey. Decapods could also provide a link in the transfer of the productivity of the thin layers to both higher trophic levels and, as migratory meroplankton, to new environments.

TABLES

Table 2.1. Summary of the duration and salinities of the paired stratified treatment tanks (T) and the homogenous control tanks (C), and the number and temporal order of the pans included in statistical analysis. If during the experiment any portion of the tank was obscured during the recording, that camera pan and its pair were excluded from analysis.

	Experiment 1		Experiment 2		Experiment 3	
	T	C	T	C	T	C
Duration	52	27	29	36	45	27
Pans (hours) analyzed	1-24, 27	1-24, 27	1-11, 13-29		1-27	1-27
Salinity	22-27	22	27-32	27	24-29	24
Number of larvae	25	25	30	30	19	19

FIGURE CAPTIONS

Figure 2.1. Vertical distribution of *N. californiensis* over time in salinity stratified and homogenous tanks. a-b) Experiment 1, c-d) Experiment 2, e-f) Experiment 3. Bars indicate the presence of larvae. Pale grey = 1 larvae, dark grey = 2-3 larvae, black = ≥ 4 larvae. Each column represents the distribution of individuals during one camera pan. To the right of each figure is a panel showing the measured salinities at the end of each experiment and indicating the position of the thin layer. The bars on top of each figure indicate periods of darkness (black) and light (white).

Figure 2.2. The effect of treatment and light on mean crowding (m^*) of *N. californiensis* larvae in experiments 1, 2, and 3. Each bar represents the mean number of larvae in a depth bin over the course of the experiment in one tank. Error bars represent +1 SD.

Figure 2.3. The effect of treatment and light on the number of *N. californiensis* larvae in the top (T) and middle (M) depth bins in experiments 1, 2, and 3 compared between lit (light bars) and dark (dark bars) conditions. Each bar represents the mean number of larvae in a depth bin over the course of the experiment in one tank. Error bars represent +1 SD.

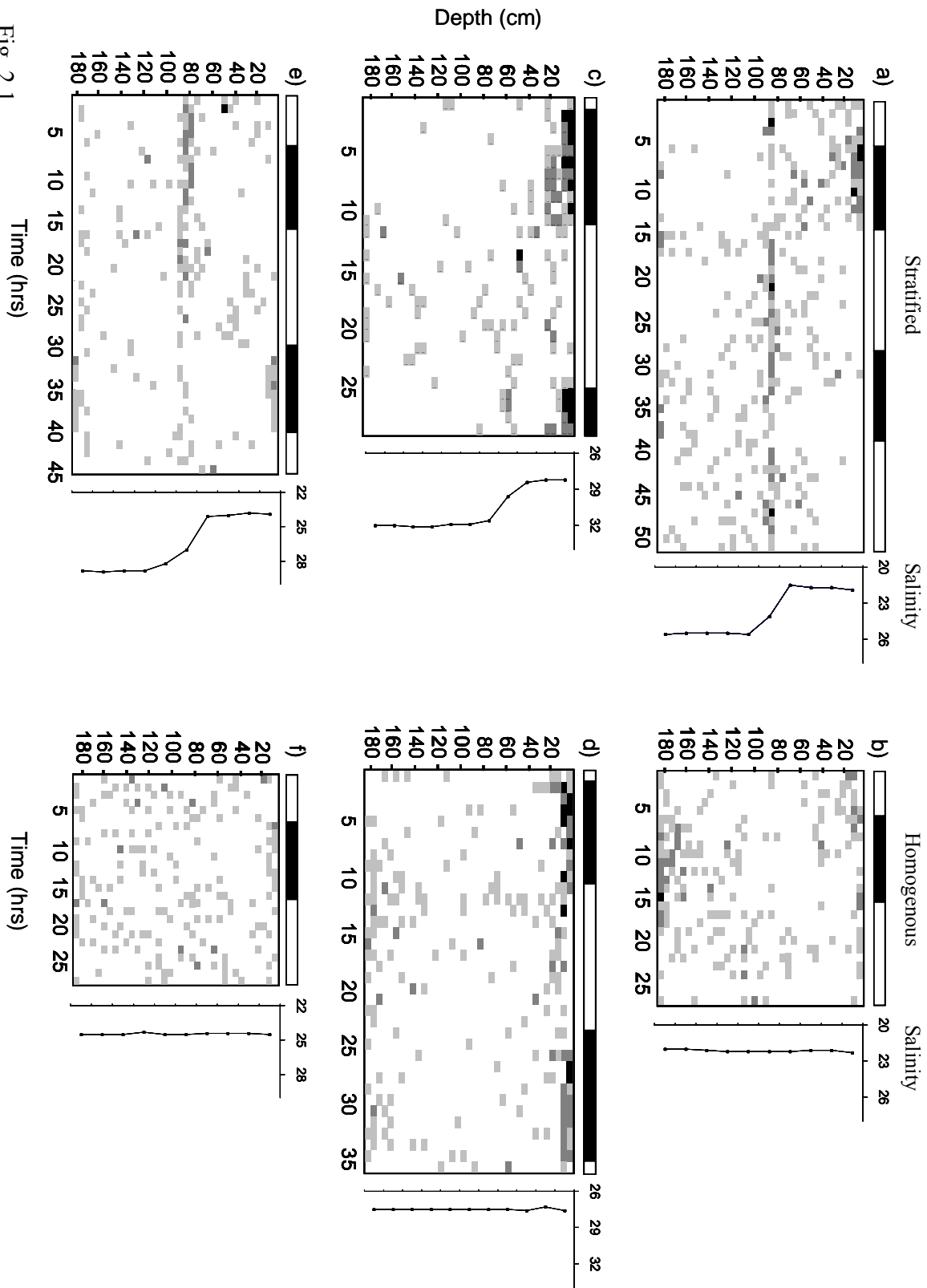


Fig. 2.1.

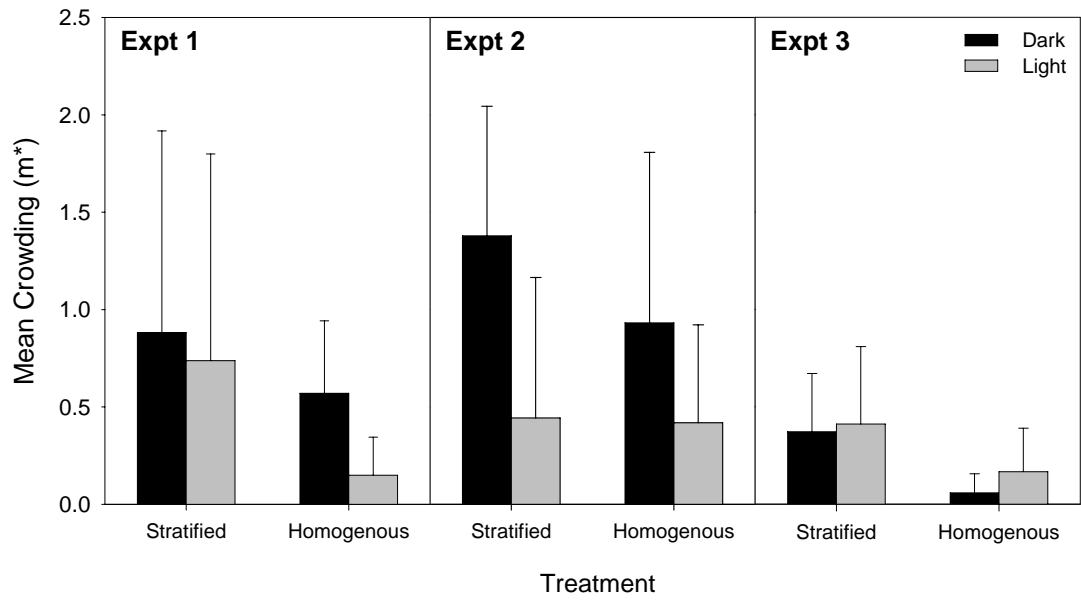


Figure 2.2.

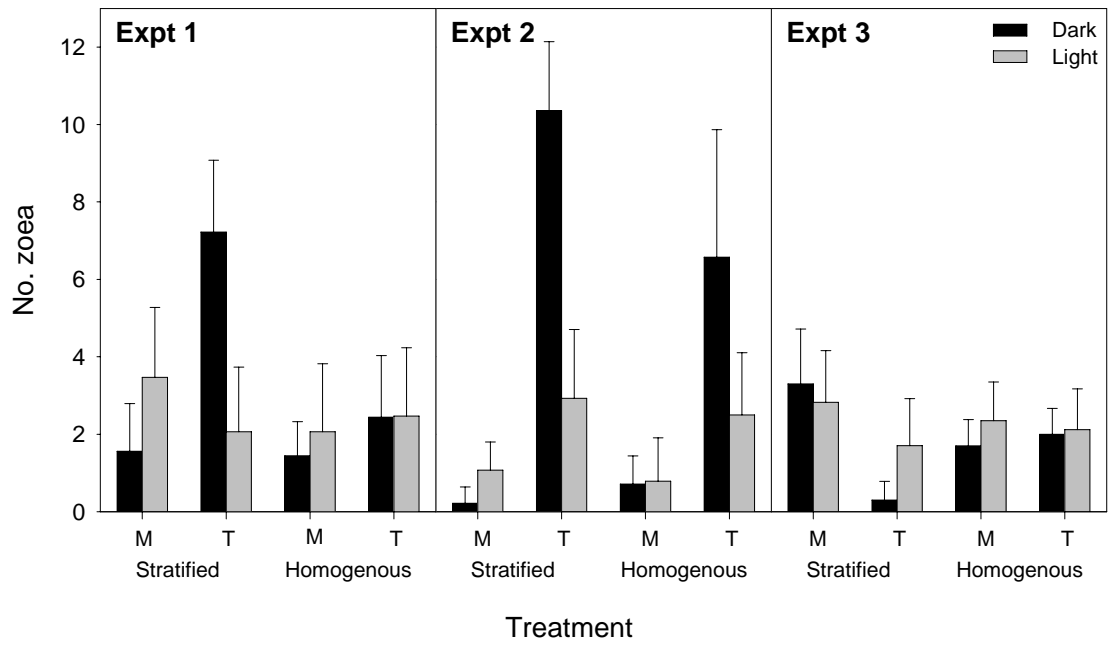


Figure 2.3.

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