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Reproductive periodicity and larval vertical migration behavior of European green crab *Carcinus maenas* in a non-native habitat

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ABSTRACT: The reproductive periodicity and vertical migration behavior of the European green crab *Carcinus maenas* in a non-native environment were characterized and the implications for invasion dynamics highlighted. Biweekly to monthly trap surveys in Pipestem Inlet, British Columbia, revealed ovigerous crabs from mid-February to June 2007. Concurrent meroplankton surveys confirmed green crab zoeae by mid-April, about 1 mo following peak abundance of ovigerous crabs (March 2007). All 4 zoeal stages of development were sampled between March and December 2007, but >95% were Stage I zoeae sampled between April and August. Vertical meroplankton surveys conducted in 2008 revealed diel-mediated vertical migratory (DVM) behavior; however the majority of sampled zoeae were aggregated near the pycnocline in surface outflowing waters. Negligible densities of post-Stage I zoeae (<1%) support the observation of zoeal export from Pipestem Inlet. Tidal-mediated vertical migratory behavior, characteristic in native European populations, was not observed in Pipestem Inlet. In contrast, low larval densities were sampled in surface (<1 m) and deep (>8 m) sampling strata. We hypothesize that low surface salinity (17–19 psu) and cold temperature (<12°C below the pycnocline) in deep strata constrained DVM. Our results highlight a need for better characterization of reproductive periodicity and zoeal migratory behavior in non-native habitats, which has implications for improving predictions of secondary spread and range expansion rates mediated via larval dispersal dynamics.

KEY WORDS: Invasive · Non-native · European green crab · *Carcinus maenas* · Larval abundance · Dispersal · Reproductive periodicity · Vertical migration behavior

INTRODUCTION

Native to intertidal and subtidal habitats of northwestern Europe, the European green crab *Carcinus maenas* has invaded Africa, Asia, Australia, the east coast of South America and both coasts of North America in the last century (Behrens Yamada 2001). On the west coast of North America, its primary introduction was to San Francisco Bay, California (Cohen et al. 1995, Grosholz & Ruiz 1995) followed by secondary northward spread to Oregon, Washington and the west coast of Vancouver Island, British Columbia via larval dispersal (Banas et al. 2009, Behrens Yamada & Kosro 2010).

Vertical migratory rhythms and their effects on larval dispersal have been well documented in marine invertebrate taxa, in particular for brachyuran larvae (zoeae), where they mediate larval dispersal within and between embayments and coastal habitats (reviewed by Queiroga & Blanton 2005). In general, larvae are strong vertical swimmers (Chia et al. 1984), but not able to counter mean horizontal currents (Queiroga & Blanton 2005). As such, vertical migratory rhythms represent an adaptive strategy to exploit vertical shear to mediate dispersal. Migratory rhythms exhibited by newly hatched green crab zoeae in their native range include tidal and diel syn-

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chronized behaviors that mediate net export from embayments to nearshore coastal waters, where larval development is completed before post-larval megalopae migrate back, prior to settlement (Queiroga & Blanton 2005). The advantages of development in coastal waters include reduced physiological stress (e.g. salinity) and predation pressure, while promoting enhanced dispersal and gene flow among populations (reviewed by Morgan 1995, but see Strathmann et al. 2002) as well as secondary range expansion in invaded habitats (Behrens Yamada & Kosro 2010).

Regional differences have been documented in behavioral and migratory patterns of green crab larvae sampled from disjunct native populations, which can affect dispersal and recruitment dynamics. For example, green crab larvae in Gullmarsfjord (Sweden) (Queiroga et al. 2002), a northern European microtidal estuary, did not exhibit the tidal-mediated vertical migratory behavior characteristic of southern European populations (Queiroga et al. 1997, Zeng & Naylor 1996a). Instead, larval transport in Gullmarsfjord was mediated by diel migrations synchronized with surface currents generated by sea and land breezes. Since circulation and the resultant transport was considerably more sluggish in Gullmarsfjord, all larval stages (I–IV) were sampled within the estuary and it is likely that some larvae completed development and recruited locally within the bay. In contrast, newly hatched zoeae (Stage I) from southern European populations are exported and dispersed in coastal waters while completing development, before the megalopae recruit to local or regional embayments.

The European green crab's potential for dispersal and the high habitat suitability of the British Columbian coastline has resulted in considerable concern for the potential ecological and economic impacts of this invader. The length and relative resilience of the planktonic larval phase in conjunction with larval behavior, which facilitates export into coastal waters, have contributed to the invasion success of green crab on every continent except Antarctica. A better understanding of larval green crab behavior in non-native habitats will result in a better characterization of larval dispersal, range expansion rates and the potential risk associated with this invader in non-native ecosystems (Therriault et al. 2008, Banas et al. 2009, Behrens Yamada & Kosro 2010). As such, the objectives of this study were to describe the vertical distribution and behavior of green crab zoeae spawned from a recently established, non-native population on Canada's west coast and to assess whether they exhibit behavior consistent with native populations, especially southern European populations, which have been identified as the putative source for established western North American populations (Darling et al. 2008, Tepolt et al. 2009). Specifically, we describe reproductive periodicity and vertical migratory behavior of newly hatched (Stage I) zoeae sampled in Pipestem Inlet, British Columbia.

MATERIALS AND METHODS

Study site

All field sampling was conducted in Pipestem Inlet (Fig. 1), a 10-km-long fjord-type estuary located in Barkley Sound on Vancouver Island's southwest coast that averages about 300 m in width, but ranges from >2 km at the mouth to about 100 m near the head. The depth of the inlet is shallowest near the entrance and at the head (<30 m) and deepest (ca. 70 m) about 3 km from the head. Pipestem Inlet was highly stratified during vertical meroplankton surveys (VMS) (Table 1). Despite semidiurnal tides that range from 2 to 4 m, circulation was dominated by freshwater runoff. Two-layered estuarine circulation, with low-density surface outflow and higher-density

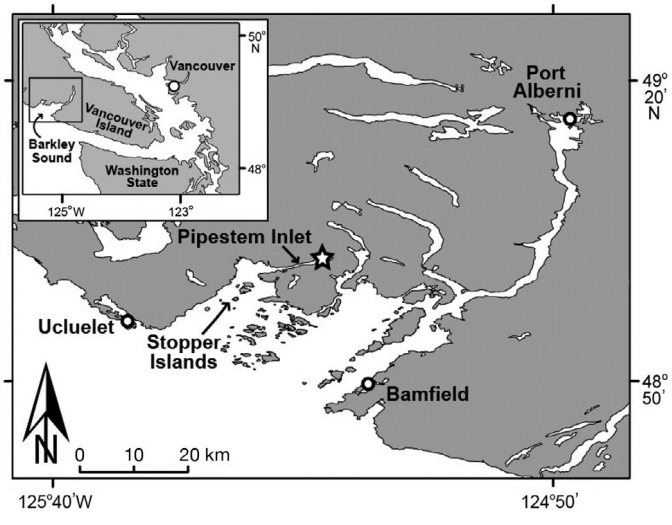

Fig. 1. C*arcinus maenas* adult, meroplankton and hydrographic surveys were conducted in Pipestem Inlet, a 10 km long fjord-type estuary in northwestern Barkley Sound on the southwest coast of Vancouver Island, British Columbia. Vertical meroplankton surveys were conducted at 1 station located at the head of Pipestem Inlet (☆). Tidal data were sourced from the Stopper Islands tide gauge

return flow below the pycnocline, was apparent from density and salinity profiles (Table 1) (Dyer 1997). The largest and most persistent source of freshwater was associated with stream runoff at the head of the Inlet and smaller intermittent streams along the Inlet's axis.

Sample collection

Adult green crab trapping and monthly meroplankton surveys (MMS) were conducted in Pipestem Inlet at 2 to 4 wk intervals from 26 January 2007 to 22 January 2008. Adult crab abundance was surveyed in intertidal and subtidal regions at the head of the inlet, the densest local green crab aggregation on Canada's west coast (Gillespie et al. 2007). Surveys for adult crabs employed 60 or 120 m ground lines deployed perpendicular to shore from intertidal to subtidal depths as deep as 20 m. Fukui traps (Model FT-100) baited with frozen herring were attached to ground lines at 10 m intervals, deployed during evening high tides and recovered after 1 full tidal cycle (ca. 12 to 15 h). All crabs were measured, sexed and ovigerous females noted.

The reproductive seasonality of green crabs in Pipestem Inlet could not be monitored by the presence of ovigerous crabs, as only 3 of 1182 (0.25%) trapped females were ovigerous. Instead, the relative abundance of trapped male and female crabs served as an indicator of reproductive activity. Since ovigerous green crabs do not forage (Behrens Yamada 2001), the proportion of trapped females will decrease relative to males as the proportion of ovigerous crabs in the population increases. Ovigerous green crabs have been reported to migrate to deeper waters during winter and spring, where water temperatures and salinities are more stable (Behrens Yamada 2001). This behavior could have an effect on sex ratios reported here, but since green crabs were not trapped deeper than about 12 m in our study, which sampled as deep as 20 m, the absence of ovigerous females is attributed to the lack of foraging behavior.

Green crab larval density was estimated opportunistically throughout Pipestem Inlet during monthly meroplankton surveys (MMS) to confirm adult spawning and reproductive periodicity. All samples were collected with a 75 cm diameter ring net (225 µm Nitex) towed at a speed of 1.5 to 2 knots and fitted with a flowmeter (General Oceanics®, Model 2030R) to estimate the relative volume of seawater filtered to standardize zoeal density (ind. 10^3 m^{-3}). Surface tows were conducted in the upper meter of the water column and oblique tows integrated larval abundance in the upper 10 m. While zooplankton samples were collected independent of tidal phase, diurnal samples were those collected at least 2 hr after dawn and 2 hr before dusk and nocturnal samples were collected at least 2 hr after dusk and 2 hr before dawn. Net depth was estimated as $Z_T = X\cos\theta$, where X is the amount of tow-line paid out and θ is the average angle of the tow-line estimated in minutes. Results were used to compare diurnal versus nocturnal larval density estimates for the surface (<1 m) and the upper 10 m portions of the water column; tidal effects were not considered in this part of the study.

Vertical distribution and migratory rhythms of green crab zoeae were assessed during 2 VMS conducted at the head of Pipestem Inlet between 3 and 6 July and 28 and 31 July 2008, hereafter referred to as Cruise 1 and 2, respectively. The same plankton net used in MMS was employed here, except for a closing mechanism that allowed discrete depths to be targeted. Each VMS cruise was divided into 9 sampling sessions, typically 4 to 6 h apart, for a total of 18 sessions that targeted combinations of tidal (ebb, flood) and diel (dawn/day, dusk/night) phases. Replicate samples per session were collected from each of 3 depth strata (N = 2 or 3 per stratum) comprising (1) surface (<1 m), (2) mid-depth (just above the pycnocline, typically 2 to 4 m deep), and (3) deep sampling strata (5 to 10 m below the pycnocline). Deeper strata were not targeted since preliminary samples collected at >15 m depths had negligible larval densities. Sampling depth relative to the pycnocline (i.e. mid-depth) was determined several times per day from CTD profiles (Seabird SBE 19plus) conducted prior to each sampling session.

Plankton nets were washed with filtered seawater and contents preserved in 5% buffered formalin. Samples were sorted and enumerated stereo-microscopically with Bogorov chambers. Larvae were identified and staged in accordance with Rice & Ingle (1975). A Folsom plankton splitter was used to quarter samples, which were processed until a minimum of 100 zoeae or the entire sample was counted. Total zoeal density was estimated for sample fractions not sorted. A LICOR (LI-1000) data logger and underwater quantum sensor (LI-1925A) was used to measure light profiles (PAR; 400–700 nm) during each sampling trip. Light intensity (µE s^{-1} m^{-2}) was measured every 0.5 m to a depth of 5 m at which intensity was effectively 0.

Statistical analysis

All statistical analyses were conducted with Systat (v.13.1). Larval density estimates from Cruises 1 and 2 were pooled to increase statistical power to test effects of sample depth, diel phase and tidal phase (3-way ANOVA) on the vertical distribution of zoeae. Mean zoeal density was significantly greater in Cruise 2, therefore vertical distribution of zoeae were standardized as the percentage sampled per depth strata per sampling session. Standardized estimates better characterized the relative vertical distribution and zoeal migration behavior than absolute density differences. Density data were log(x+1) transformed while percent data were arcsine transformed to meet ANOVA assumptions of normality and homogeneity of variance (Quinn & Keough 2002). All mutliple comparisons were conducted with Tukey's HSD test (hereafter, Tukey) (Quinn & Keough 2002)

The relative vertical distribution of zoeae was compared to pycnocline depth (P) and to a stratification index (S) (Tremblay & Sinclair 1990). Pycnocline depth corresponded to the depth interval where the change in density was maximal while S was calculated as:

$$S = \frac{\Delta\sigma_t}{\Delta z} \quad (1)$$

where Δz is the difference between 1 m and the greatest depth sampled and $\Delta\sigma_t$ is the difference in σ_t over the interval Δz. The vertical distribution of zoeae was examined using weighted mean depths (WMD) as described by Frost & Bollens (1992):

$$\mathrm{WMD} = \frac{(\sum n_i d_i)}{\sum d_i} \quad (2)$$

where n_i is the density of Stage I zoeae at depth d_i, the midpoint for each of 3 depth sampling intervals (see 'Sample collection' above). Replicate WMD estimates were used to test statistical differences between diel phases. The distribution of larvae with respect to pycnocline depth was done by comparing the WMD of larvae in the water column to the pycnocline depth (P). Cruise 1 and 2 samples were not grouped when estimating WMD since the average depth of the deep sampling layer was significantly deeper during Cruise 1 (12.2 ± 1.0 m; mean ± SE) than Cruise 2 (8.8 ± 0.6 m) (Student's t-test, $t_{2,8}$ = 3.130, p = 0.006).

Regression analysis was employed to assess the effect of water temperature, salinity and light intensity on zoeal distribution. Zoeal density estimates in surface sampling strata were regressed against ambient light from Cruise 2 only because zoeae actively avoided the surface layer during Cruise 1 (see 'Discussion') so that effectively no zoeae were observed in the surface layer. Regression analysis was not performed on deep strata since mean light intensities were negligible (0.31 ± 0.10 µE s^{-1} m^{-2}; mean ± SE) compared to surface strata due to intense phytoplankton blooms during both cruises. Mean zoeal densities were regressed against corresponding mean salinity and temperature values binned into 2 psu and 2°C intervals. Mean estimates of larval density within binned intervals were used to reduce effects of larval density outliers and to estimate sample variance.

RESULTS

Reproductive periodicity

The average size of crabs trapped in this study was 66.4 ± 11.8 mm (±SD) with carapace width ranging

Table 1. Seawater temperature, salinity and density values (mean ± SE) measured for each of 3 sampling strata during Cruise 1 (3–6 July 2008) and Cruise 2 (28–31 July 2008) of vertical meroplankton surveys (VMS). Sampled layers are (1) surface (<1 m), (2) mid-depth (near or above the pycnocline) and (3) deep strata (~10 m below the pycnocline). Mean temperature, salinity and density estimates are compared (ANOVA) between sampling layers for Cruises 1 and 2, while statistical comparisons of temperature, salinity and density within each layer (i.e. Cruise 1 vs. Cruise 2; results not shown) revealed no significant differences. **Bold** p-values are significant at p ≤ 005

Depth stratum		Temperature (°C)	Salinity (psu)	Density (kg m^{-3})
Cruise 1				
Surface (S)		19.07 ± 0.73	17.69 ± 2.24	11.77 ± 1.56
Mid-depth (M)		15.65 ± 0.58	28.48 ± 1.28	20.81 ± 2.16
Deep (D)		11.96 ± 1.25	29.31 ± 1.60	22.12 ± 1.47
	$F_{2,27}$	15.540	13.689	16.346
	p	**<0.001**	**<0.001**	**<0.001**
	Tukey	S > M > D	S < M = D	S < M = D
Cruise 2				
Surface (S)		17.65 ± 0.43	19.68 ± 1.61	13.64 ± 1.14
Mid-depth (M)		15.79 ± 0.30	30.25 ± 0.29	22.13 ± 0.28
Deep (D)		11.11 ± 0.14	31.42 ± 0.18	23.93 ± 0.16
	$F_{2,27}$	115.344	46.378	64.165
	p	**<0.001**	**<0.001**	**<0.001**
	Tukey	S > M > D	S < M = D	S < M = D

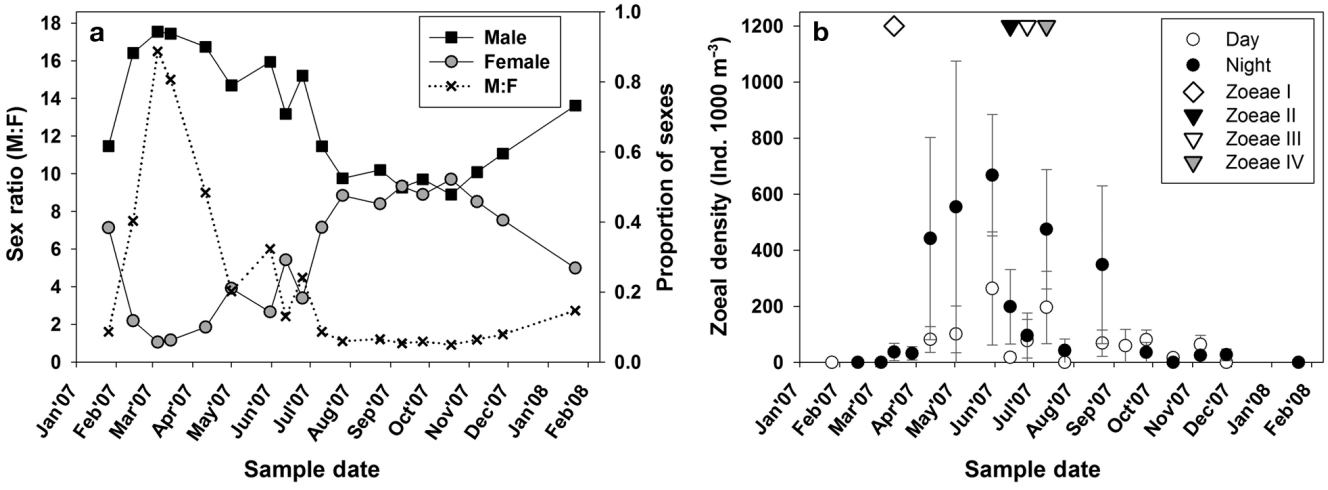

Fig. 2. Time series plots of (a) sex ratio and relative proportion of male and female green crabs *Carcinus maenas* trapped during monthly benthic trap surveys, and (b) combined mean zoeal density (Stages I–IV) in daytime vs. nighttime zooplankton samples from monthly meroplankton surveys (MMS). The majority of sampled zoeae (93.4%) were Stage I (see 'Results'). Symbols at the top of panel (b) indicate the initial appearance of Stage I, II, III, and IV zoeae, respectively. Error bars in (b): ±SE

from 34 to 95 mm, indicative of reproductive crabs (e.g. Behrens Yamada 2001). The relative abundance of adult male and female green crabs in biweekly to monthly surveys varied considerably over the course of a year. Sexes were equally abundant from mid-summer through mid-autumn (Fig. 2a), with male crabs becoming slightly more abundant in late November. The greatest discrepancy in the relative abundance of sexes occurred from mid-February to late June when males made up 85.4 ± 2.8% (±SE) of trapped crabs. This ratio decreased through July to roughly equivalent numbers of males and females by August.

All zoeal development stages (I–IV) were observed during MMS in Pipestem Inlet (Fig. 2b). Stage I zoeae appeared at relatively low densities in mid-March 2007, with highest densities observed from about mid-April to mid-August. Stage II, III and IV zoeae were initially sampled on 13 June, 26 June and 11 July 2007, respectively. The combined relative abundance of Stage I to IV zoeae was 93.4, 4.9, 0.9 and 0.8%, respectively, while only 3 megalopae were sampled. Stage I zoeae were significantly more abundant in nighttime (134.6 ± 70.9 ind. 10^3 m^{-3}; ±SE) than daytime (14.7 ± 4.8 ind. 10^3 m^{-3}) surface plankton tows (Student's *t*-test, $t_{2,47}$ = 1.998, p = 0.026). Mean zoeal density in the upper 10 m of the water column was greater, but not significantly different, in nighttime (163.0 ± 50.1 ind. 10^3 m^{-3}) vs. daytime oblique tows (81.6 ± 30.9 ind. 10^3 m^{-3}; Student's *t*-test, $t_{2,65}$ = 0.903, p = 0.372).

Vertical zoeal distribution

Depth-discrete plankton samples were collected during VMS when the water column at the head of Pipestem Inlet was highly stratified (Table 1). Mean pycnocline depth during Cruise 1 (2.66 ± 0.27 m; ±SE) was deeper, but not significantly different from Cruise 2 (2.20 ± 0.26 m; Student's *t*-test, $t_{2,17}$ = 1.282, p = 0.217). Mean temperature and salinity within the mixed layer above the pycnocline was warmer and fresher during Cruise 1 than Cruise 2 (Table 1), while water below the pycnocline was significantly colder and saltier than the mixed surface layer (Table 1). The mid-depth sampling stratum, where the highest densities of zoeae were sampled (described below), was significantly warmer, but not significantly fresher than the water column below the pycnocline (Table 1).

As in MMS, zoeae sampled during VMS were predominantly Stage I; Stages I–IV comprised 99.86, 0.04, 0.08 and 0.02% of all zoeae sampled, respectively, with no megalopae sampled. Mean zoeal densities were consistently higher in Cruise 2 surface, mid-depth and deep sampling strata compared to Cruise 1, but significantly different only for mid-depth samples (Table 2).

The relative (%) vertical distribution of Stage I zoeae in the surface, mid-depth and deep strata was similar in Cruises 1 and 2 (Fig. 3). The mean percentage and density of zoeae was always highest in mid-depth samples (i.e. near the pycnocline) compared to surface or deep samples, which were comparable

Table 2. *Carcinus maenas* Stage I zoeal density (ind. 10^3 m^{-3}) and relative percent (%) distributions sampled in 3 strata: surface layer (<1 m), mid-depth (near or above the pycnocline), and deep (~10 m below the pycnocline). Mean ± SE density estimates are provided for Cruise 1 (3–6 July 2008) and Cruise 2 (28–31 July 2008) of vertical meroplankton surveys (VMS). Sampling depth specific statistical comparisons between Cruises 1 and 2 zoeal density estimates and relative percent (%) are provided (Student's *t*-test). **Bold** p-values are significant at $p \leq 0.05$

Depth stratum	Cruise 1 N	Cruise 1 Density	Cruise 1 %	Cruise 2 N	Cruise 2 Density	Cruise 2 %	Depth-specific comparisons Density	Depth-specific comparisons %
Surface	14	26.6 ±19.2	0.32 ±0.23	9	399.7 ±254.8	1.99 ±1.06	$t_{2,21}$ = 1.838 p = 0.080	$t_{2,21}$ = 1.135 p = 0.274
Mid-depth	22	2883.7 ±634.0	34.1 ±3.83	27	6101.6 ±859.3	28.65 ±4.02	$t_{2,47}$ = 2.895 **p = 0.006**	$t_{2,47}$ = 0.961 p = 0.171
Deep	9	698.8 ±201.0	16.15 ±4.36	9	1834.8 ±570.1	9.98 ±3.03	$t_{2,16}$ = 1.879 p = 0.079	$t_{2,16}$ = 1.870 p = 0.076
	$F_{2,42}$ p Tukey	22.701 **<0.001** M > D > S	43.784 **<0.001** M > D > S		16.538 **<0.001** M > S = D	15.234 **<0.001** M > S = D		

except that zoeae were effectively absent from the surface layer during Cruise 1 (Table 2, Fig. 3a).

A significant difference in mean vertical distribution of Stage I zoeae was observed among sampling strata, while diel and tidal phases had no overall effect (Table 3). A significant interaction between depth and diel phase indicated that the vertical distribution of zoeae differed in daytime versus nighttime samples. Mean zoeal density in surface and mid-depth sample layers was significantly higher during nighttime, while the deep sampling stratum had significantly higher larval densities during daytime (Table 3). These patterns are consistent with DVM behavior and consistent with observed diel changes in zoeal WMD, which were significantly shallower in nighttime samples for both cruises (Fig. 3). Nighttime WMD for Cruise 1 and 2 were about 1.9 m and 1.5 m shallower than respective daytime distributions. However, depth-integrated (cumulative) mean zoeal density did not differ between diel phases (night vs. day). This reveals that most larvae in Pipestem Inlet were not migrating below the upper portion of the water column sampled in this study and that the DVM behavior exhibited by Stage I zoeae was restricted to the upper water column and concentrated near the pycnocline (Fig. 3, Table 3).

There was a significant logarithmic relationship between the density of Stage I zoeae and ambient light levels in the surface sample layer (Fig. 4a). Light intensity in this layer ranged from 0 (night) to 775 µE s^{-1} m^{-2}. The exponential shape of this curve reveals that Stage I zoeae are sensitive to small increases at low light intensities; a small increase from 0 to 50 µE

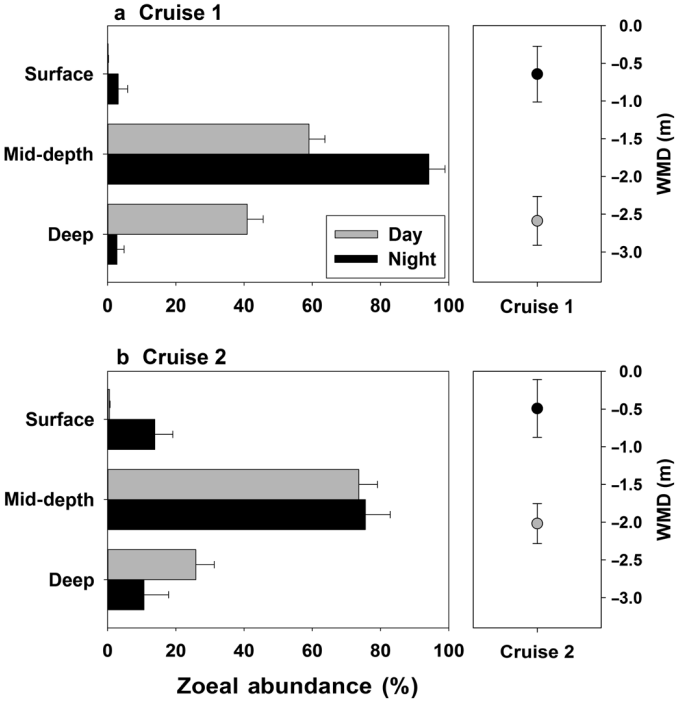

Fig. 3. Relative (%) vertical distributions and weighted mean depth (WMD) of *Carcinus maenas* Stage I zoeae sampled during vertical meroplankton surveys (VMS) conducted (a) 3–6 July 2008 (Cruise 1) and (b) 28–31 July 2008 (Cruise 2) at daytime (grey) and nighttime (black). Sample depth layers: (1) surface (<1 m), (2) mid-depth (near or above the pycnocline), and (3) deep (Cruise 1, 12.2 m; Cruise 2, 8.8 m). Nighttime WMD are significantly shallower than daytime estimates during both cruises (Student's *t*-test; Cruise 1, $t_{2,7}$ = 2.454, p = 0.044; Cruise 2, $t_{2,7}$ = 2.454, p = 0.013). Cruise 1 and 2 data was not collated to estimate WMD because the 'deep' strata was significantly shallower during Cruise 1 (see 'Materials and methods'), which affects WMD estimates. Error bars: ±SE

Table 3. Three-way ANOVA comparing effects of sampling depth, diel phase and tidal phase on the relative (%) vertical distribution of *Carcinus maenas* Stage I zoeae. Sampled layers are surface layer (<1 m), mid-depth (near or above the pycnocline), and deep (~10 m below the pycnocline); diel phases include daytime (d) vs. nighttime (n) samples; tidal phases include high vs. low tidal (see 'Materials and methods' for details). Cruise 1 and 2 data were combined and arcsine transformed prior to analysis. **Bold** p-values are significant at p ≤ 0.05

Source	df	MS	*F*	p	Tukey
Depth (Z)	2	324.21	29.81	**<0.001**	M > D **(p < 0.001)**; M > S (**p < 0.001**); S ≈ D (p < 0.106)
Diel phase (D)	1	0.014	0.01	0.622	
Tidal phase (T)	1	5.607	0.52	0.932	
Z × D	2	38.971	3.58	**<0.001**	S (d < n; $F_{1,21}$ = 15.81, **p < 0.001**); M (d < n; $F_{1,45}$ = 5.20, **p = 0.027**); D (d > n; $F_{1,16}$ = 20.14, **p < 0.001**)
Z × T	2	5.354	0.49	0.924	
D × T	1	1.445	0.13	0.947	
Z × D × T	2	4.331	0.4	0.953	
Total	76	10.875			

s^{-1} m^{-2} resulted in a considerable reduction in mean zoeal density in the surface layer and density remained low at higher intensities. Zoeal density at mid-depth (near the pycnocline) was correlated to light intensity during Cruise 1 (Fig. 4b), but not during Cruise 2 (Fig. 4c). Light intensities at depth were lower than expected and likely due to an intense phytoplankton bloom during both VMS cruises. Daytime light intensity at the mid-depth (ca. 2 m) and deep sampling strata averaged about 20.7% (44.6 ± 10.6 µE s^{-1} m^{-2}; ±SE) and 0.2% (0.31 ± 0.10 µE s^{-1} m^{-2}) of mean surface light intensity, respectively.

The lowest mean zoeal densities corresponded to salinity minima in the surface layer (Fig. 5a), especially during Cruise 1, that were significantly fresher by ca. 2 psu (Table 1). Mean zoeal density was significantly and positively correlated with salinity over the 15 to 32 psu range observed in this study (Fig. 5a). This relationship predicts that mean zoeal density approximates zero in the vicinity of 17 psu (Fig. 5a). In contrast, mean zoeal densities were highest at intermediate seawater temperatures. The highest mean densities were observed at 15 to 17°C, while minima occurred at extreme *in situ* observations (8–10°C and 20–22°C) (Fig. 5b). The relative density of Stage I zoeae were highly constrained by these *in situ* temperature and salinity ranges during VMS (Fig. 6).

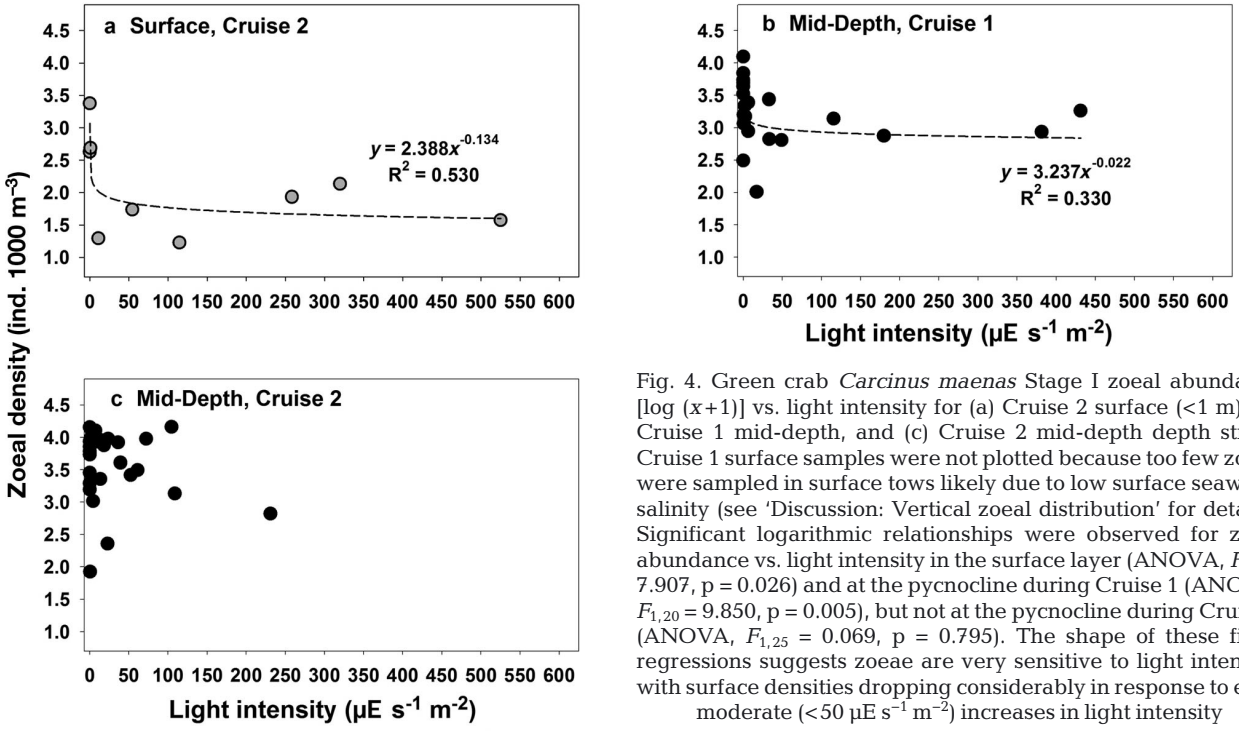

Fig. 4. Green crab *Carcinus maenas* Stage I zoeal abundance [log (*x*+1)] vs. light intensity for (a) Cruise 2 surface (<1 m), (b) Cruise 1 mid-depth, and (c) Cruise 2 mid-depth depth strata. Cruise 1 surface samples were not plotted because too few zoeae were sampled in surface tows likely due to low surface seawater salinity (see 'Discussion: Vertical zoeal distribution' for details). Significant logarithmic relationships were observed for zoeal abundance vs. light intensity in the surface layer (ANOVA, $F_{1,8}$ = 7.907, p = 0.026) and at the pycnocline during Cruise 1 (ANOVA, $F_{1,20}$ = 9.850, p = 0.005), but not at the pycnocline during Cruise 2 (ANOVA, $F_{1,25}$ = 0.069, p = 0.795). The shape of these fitted regressions suggests zoeae are very sensitive to light intensity, with surface densities dropping considerably in response to even moderate (<50 µE s^{-1} m^{-2}) increases in light intensity

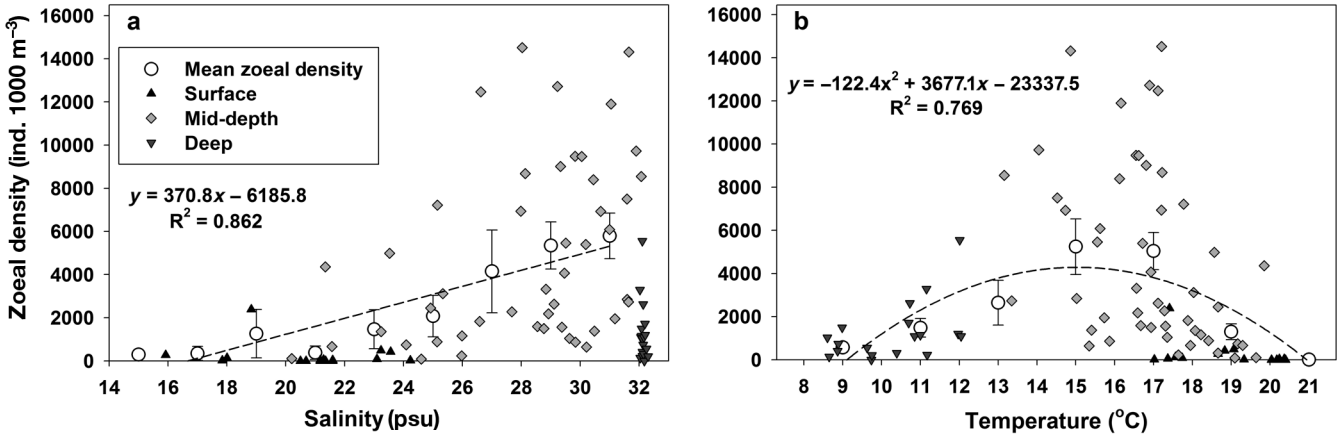

Fig. 5. Green crab *Carcinus maenas* Stage I zoeal density plotted against seawater (a) salinity and (b) temperature. Filled symbols represent zoeal density estimates at corresponding salinity or temperature from individual plankton tows. Open symbols represent mean zoeal density estimated from plankton tows divided into salinity (range 14 to 32 psu, in intervals of 2 psu) and temperature (range 8 to 22°C, in intervals of 2°C). Zoeal density is plotted at the sampling interval midpoint. Dashed lines and equations represent best fit regressions between mean zoeal density vs. (a) salinity (ANOVA, $F_{2,7}$ = 43.75, p < 0.001) and (b) temperature (ANOVA, $F_{2,10}$ = 6.47, p < 0.016). Error bars: ± SE

DISCUSSION

Reproductive periodicity

Green crabs in Pipestem Inlet were reproductive from late winter-early spring through summer, based on the relative density of ovigerous crabs and presence of newly hatched zoeae in plankton samples. The initial appearance of zoeae lagged the start of the brood season by about 2 mo, which corresponds with laboratory observations. Eggs extruded from ovigerous crabs held *in vivo* at 12°C hatched after ca. 2 mo (Behrens Yamada 2001), while *in situ* zoeal density in our study became negligible about 2 mo after ovigerous crab density became negligible. This brood cycle periodicity is consistent with populations in Willapa Bay, WA (USA), located about 300 km south of Pipestem Inlet (Banas et al. 2009). By comparison, Stage I zoeae were observed over a 2 mo period only (June–August) in Nova Scotia (Cameron & Metaxas 2005) compared to the 4 mo period in this study.

Variability in the reproductive periodicity among green crab populations has been attributed to variation in environmental conditions (Klassen & Locke 2007). Seawater is warm enough in *C. maenas*' native European range to support protracted or multiple spawning events each year (Klassen & Locke 2007). Populations in the English Channel and Wadden Sea breed throughout the year, though ovigerous females are most abundant in late winter and spring, while newly hatched zoeae peak during spring and

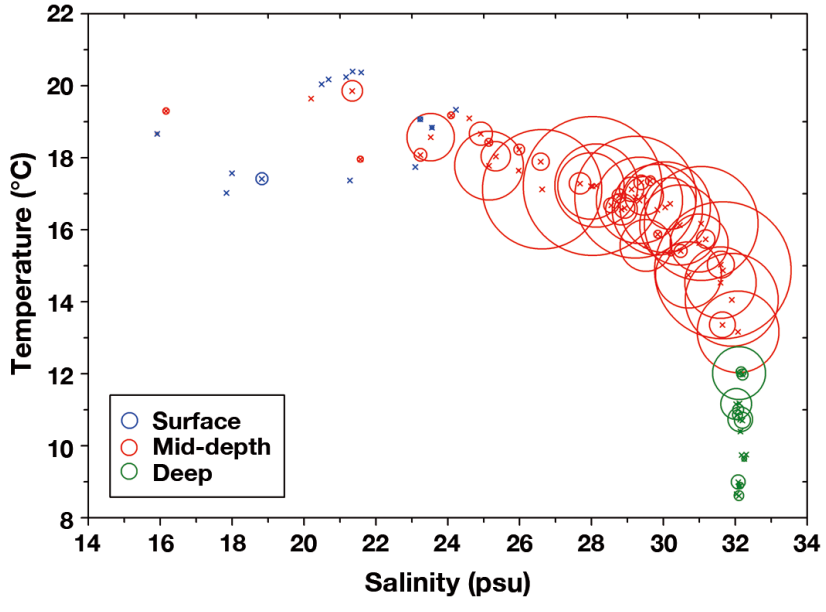

Fig. 6. Relative density of Stage I green crab *Carcinus maenas* zoeae sampled from surface (<1 m), mid-depth (near or above the pycnocline) and deep (~10 m below the pycnocline) sampling strata. Each 'x' represents 1 zooplankton sample plotted against mean sample strata salinity and temperature. The size of the circles represents relative zoeal density; 'x' markers without corresponding circles indicate negligible or no zoeae sampled. The majority of zoeae (79.8%) were sampled between 13 and 19°C and 26 and 32 psu

early summer (see Queiroga et al. 1994, Baeta et al. 2005). In warmer coastal waters off southern Portugal, ovigerous females develop about 2 mo earlier (January-Febuary; Almaça 1982, Queiroga 1993). This seasonality is very similar to reproductive cycles reported in western North America (Cohen et al. 1995, Banas et al. 2009, this study) where annual seawater temperatures rarely drop below about 10°C, similar to native European habitats. Colder spring and winter temperatures (0–2°C) along the east coast of North America coincides with a later and shorter breeding season (3–4 mo) for non-native populations established in Maine, Nova Scotia, Prince Edward Island, and Gulf of St. Lawrence (Berrill 1982, Cameron & Metaxas 2005, Klassen & Locke 2007, Audet et al. 2008).

Climate trends and projections for coastal surface waters off Vancouver Island are about 1.5°C warmer over the next 50 yr with spring and fall threshold temperatures likely to occur earlier and later, respectively (Christian & Foreman 2013). These conditions will be comparable to present-day southern locations along the west coast of the USA where non-native green crab populations experience protracted and biannual spawning (Behrens Yamada et al. 2005, Banas et al. 2009, Behrens Yamada & Kosro 2010). Longer reproductive seasons and enhanced larval production associated with warmer coastal surface waters will enhance production, development and survival of green crab larvae released from populations at present-day northern range limits (deRivera et al. 2007). This will also likely enhance northward range expansion of *C. maenas* given projected latitudinal warming and stronger nearshore coastal currents associated with increased precipitation over watersheds emptying into coastal waters (IPCC 2007, Walther et al. 2009).

Vertical zoeal distribution

Vertical distribution and migratory rhythms of brachyuran larvae mediate dispersal between embayments and nearshore coastal habitats (reviewed by Queiroga & Blanton 2005). The negligible number of post-Stage I zoeae sampled in this study is indicative of net larval export from Pipestem Inlet, which has also been documented in embayments throughout *C. maenas'* native range (Queiroga et al. 1994, 1997, 2002, Zeng & Naylor 1996a). At their southern native range limit, zoeae hatched during nocturnal high tides are exported from embayments via tidally timed migrations between surface and bottom layers during ebb and flood tides, respectively (Queiroga et al. 1994, 1997, Zeng & Naylor 1996a). This behavior, termed selective tidal stream transport (STST), effectively mediates net export of newly hatched zoeae into nearshore coastal waters before development of post-Stage I zoeae, generally <10 d after hatching (reviewed in deRivera et al. 2007).

The export of zoeae from the head of Pipestem Inlet is not attributable to STST, as migratory rhythms in this study were not synchronized to tidal phase. Zoeae did exhibit diel migrations between shallow sample strata at night and deeper layers during the day, but this behavior could not mediate export as diel and tidal cycles were not synchronized within the inlet. Instead, most zoeae were sampled in surface and mid-depth strata and likely exported in the surface outflow of the inlet's 2-layer estuarine circulation (Doe 1952). This export was enhanced during VMS due to above average rainfall (and runoff) reflected in lower mean surface salinities during our study (17.7–19.7 psu, July 2007) compared to other years (28.6 psu July 2009, 27.6 psu July 2011; authors' unpubl. data). Prominent zoeal aggregations near the pycnocline have been observed in other crab species, e.g. the obligate estuarine mud crab *Rhithropanopeus harrisii* that migrated above and below the pycnocline during ebb and flood tidal phase respectively, effectively minimizing net transport (Cronin & Forward 1986).

The migratory rhythm exhibited by Pipestem Inlet zoeae was most similar to observations from Gullmarsfjord (Sweden), an estuary at the northern extent of *C. maenas*' native European distribution (Queiroga et al. 2002). As in Pipestem Inlet, tidally synchronized behavior was not observed. Instead, net export was mediated by rhythmic diel migrations into surface layers after sunset, in conjunction with nocturnal land (offshore) breezes (Queiroga et al. 2002). In addition, migratory rhythm of Gullmarsfjord zoeae was not endogenously controlled (inherited) (Queiroga et al. 2002) as shown in southern European populations (Zeng & Naylor 1996b). Instead, vertical migratory behavior in Gullmarsfjord zoeae represented an exogenous response to light intensity with the highest larval densities in surface waters at night and deeper layers during the day.

Rhythmic migratory behavior in our study appears exogenously controlled, at least in part, as the vertical distribution of zoeae statistically changed in response to changes in salinity. Zoeae exhibited limited nocturnal migrations into surface sampling strata during VMS except during Cruise 1 when low surface salinities (17.7 psu), due to prolonged rain-

fall, resulted in effectively no zoeae (~0.3%) in the surface strata. Surface salinities only 2 psu higher during Cruise 2 (19.7 psu) revealed a significantly greater proportion of zoeae in the surface layer. Unfortunately, surface salinities during VMS never exceeded 20 psu so it is not possible to determine whether a greater proportion would migrate into the surface stratum at salinities more typical for the inlet (see July 2009, 2011 data above). Decapod larvae have been observed to respond to and avoid even small changes in salinity (Tankersley et al. 1995, Queiroga & Blanton 2005). Green crab megalopae avoided the surface layer when salinity was >2 psu lower than the near bottom salinity (Queiroga 1998), while newly hatched hermit crab zoeae (*Pagarus longicarpus*) aggregated near the pycnocline instead of migrating into the surface layer when salinity was 5 psu lower (Roberts 1971). Mean salinity differences between surface and pycnocline strata in this study were 10.6 and 10.8 psu in Cruise 1 and 2, respectively. Additional field and laboratory studies (e.g. Zeng & Naylor 1996b), are needed to better assess exogenous versus endogenous control of the behavioral responses observed in Pipestem Inlet zoeae.

The avoidance of low salinity strata is likely due to zoeal physiological limits. *C. maenas* zoeae reared at 15 psu (Anger et al. 1998) or 14 and 17 psu (Bravo et al. 2007) were not able to complete Stage I development, while zoeae reared at 20 psu either did not complete development (Bravo et al. 2007) or development was significantly delayed with higher mortality compared to zoeae cultured at 25 or 32 psu (Anger et al. 1998). Even limited exposure can have significant effects; Anger et al. (1998) observed lower growth, development and survival rates in newly hatched zoeae exposed to 20 psu for short periods of time (24 to 72 h) with greater effects at longer exposures to reduced salinities.

Green crab zoeae also avoided the cold, deep water in Pipestem Inlet. The vast majority of zoeae sampled in VMS remained in surface or mid-depth strata (i.e. above the pycnocline) despite previous studies that sampled zoeae as deep as 30 to 45 m (Queiroga 1996). CTD profiles revealed that a 3 m layer below the pycnocline (thermocline) averaged 11.9 and 11.1°C during Cruises 1 and 2 respectively; 3.7 to 4.7°C colder than the mid-depth strata and 6.6 to 7.1°C colder than the surface layer. The avoidance of colder, deep strata is consistent with optimal temperatures for zoeal development and survivorship, typically 12.5 to 20°C (reviewed by deRivera et al. 2007).

In summary, *C. maenas* zoeae hatched in Pipestem Inlet did not exhibit the vertical migratory rhythms observed in most native European populations, though it did share a diel periodicity with Gullmarsfjord zoeae (Queiroga et al. 2002). Since larval dispersal represents a major vector in the secondary spread (range expansion) of non-native green crab, a better description of planktonic behavior in these environments, instead of simply inferring these behaviors from native populations, can have important implications when modeling larval dispersal potential, range expansion rates and associated ecological risk (Therriault et al. 2008). Invasive organisms often are capable of modifying ecologically important physiological, morphological, behavioral, and life-history characteristics in response to environmental cues (phenotypic plasticity), which provides an adaptive mechanism to enhance the ability of an invasive species to exploit non-native habitats (reviewed by Smith 2009). Banas et al. (2009) employed larval behavioral scenarios based on native European studies in conjunction with local circulation modeling to characterize potential dispersal and retention of green crab zoeae in Willapa Bay, USA and showed that modeled behaviors were secondary to the bay's circulation in determining larval retention. Results from this study suggest that behavioral scenarios inferred from native ranges may not be representative of non-native larvae, especially those in western North America. Variations in zoeal behavior relative to physical processes can have significant impacts on larval dispersal (Queiroga & Blanton 2005) and invasion dynamics.

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