A. Metaxas · C. M. Young

Behaviour of echinoid larvae around sharp haloclines: effects of the salinity gradient and dietary conditioning

Abstract We examined larval response to a range of sharp haloclines and determined the effect of dietary conditioning on that response in the sea urchins *Echinometra lucunter* and *Arbacia punctulata*. We reared larvae in the laboratory under a high or low concentration of either single (*Isochrysis galbana*) or mixed (*Isochrysis galbana*, *Dunaliella tertiolecta*, *Thalassiosira weissflogii*) microalgal species. For both species of sea urchins, rate of larval development was faster and age-specific larval length and width were greater in high-ration than low-ration diets. We examined the distribution of two- and four-arm larvae of *E. lucunter* from each diet treatment and of four-arm larvae of *A. punctulata* from the high-ration diets in cylinders with experimentally constructed haloclines. In three of the halocline treatments, the salinity of the bottom layer was 33‰ and that of the top layer was 21, 24 or 27‰ (21/33, 24/33 and 27/33) and in a fourth one, the salinities of the bottom and top layer were 30 and 21‰, respectively (21/30). The position of larvae in the cylinders varied with the steepness of the halocline and with dietary conditioning for both sea urchin species and all developmental stages tested. Significantly more larvae crossed the haloclines into water of 24 and 27‰ salinity than into water of 21‰ salinity. We observed an effect of diet on the position of larvae in the cylinders, and that effect varied among halocline treatments for both species. The proportion of larvae of *E. lucunter* that crossed the halocline was greater in low- than high-ration diets in the 24/33 and 27/33 treatments. Position of four-arm larvae in the cylinders also varied with food quality in high-ration diets: for *E. lucunter* in the 24/33 treatments, and for *A. punctulata* in the 21/30 treatments, more larvae from the single- than from the mixed-species diets were present above the halocline. Salinity in the adult habitat during most of the active reproductive period ranged from 15 to 40‰. We showed that larvae can respond to gradients in salinity, and therefore can remain within a water mass of higher salinity overlying the adult habitat. However, survival of poorly fed larvae may be increased if they are introduced into a new water mass and carried away from a nutritionally poor environment.

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A. Metaxas[1] · C.M. Young
Department of Larval Ecology,
Harbor Branch Oceanographic Institution,
5600 U.S. 1 North, Fort Pierce,
Florida 34946, USA

Present address:
[1](✉) MS 34, Woods Hole Oceanographic Institution,
Woods Hole, Massachusetts 02543, USA

Introduction

Although it is well established that larval supply can have a pronounced effect on adult distribution of marine invertebrates (e.g. Gaines et al. 1985; Minchinton and Scheibling 1991), little is known about the factors that affect larval survival (Rumrill 1990). Larvae may remain in the plankton for periods ranging from a few hours (e.g. ascidian tadpoles) to months (e.g. echinoderm plutei and bipinnaria). While in the plankton, larvae may be subject to food limitation, predation, and dispersal over large distances (for review see Young and Chia 1987)

Dispersal during the planktonic larval phase has received increased attention recently, and both water-circulation and larval behaviour have emerged as important factors affecting dispersal (e.g. Banse 1986; Young and Chia 1987; Ebert and Russell 1988; Tremblay and Sinclair 1992; DeVries et al. 1994). Since larval swimming speeds are lower than most ocean current speeds (for review see Chia et al. 1984), larval horizontal dispersal is mainly affected by water circulation. Larvae are carried by currents away from parental habitats into the open ocean (e.g. Emlet 1986; Pedrotti and Fenaux 1992) where they may experience great mortality (Rumrill 1990). However, currents also can

deliver large numbers of larvae back to the adult habitat, resulting in great pulses of recruitment (e.g. Shanks and Wright 1987; Farrell et al. 1991). Although larvae probably cannot control their horizontal position directly, they may be able to regulate their vertical position in the water column, particularly in enclosed systems with predictable circulation, such as estuaries or fjords (for review see Young 1995). Most of the evidence is for larvae with strong swimming abilities, such as crab and lobster zoea and megalopea (e.g. Scarratt and Raine 1967; Cronin and Forward 1982) and ascidian tadpoles (Vázquez and Young 1996; Young and Svane in preparation). Through well-timed vertical migration, these larvae can remain in or return to the water mass overlying the adult habitat.

During dispersal, larvae of some invertebrates tend to aggregate at density discontinuities in the water column such as pycnoclines (e.g. Tremblay and Sinclair 1990; Raby et al. 1994), turbidity fronts and tidal bores (Pineda 1991). Increased phytoplankton concentrations also have been recorded at pycnoclines; the chlorophyll maximum layer is often located near or at the pycnocline (for reviews see Harrison et al. 1983; Weeks et al. 1993). Aggregation of zooplankton and ichthyoplankton at density discontinuities (e.g. Banse 1964; Kingsford 1990; Davis et al. 1992) could be a behavioural response to the increased food concentration there (but see Tremblay and Sinclair 1990) but also could result from hydrodynamics. It has not been established whether larvae, particularly weak swimmers, have the ability to control their position with respect to such discontinuities. Some studies have suggested that larvae of certain taxa cannot cross pycnoclines (e.g. Banse 1964; Harder 1968), whereas others have shown that larvae respond to thermoclines (e.g. McConnaughey and Sulkin 1984; Pennington and Emlet 1986; Boudreau et al. 1991) and haloclines (e.g. Harder 1968; Roberts 1971; Mann et al. 1991). Although echinoderms have been the focus of extensive research on larval ecology, the behavioural response of their larval stages to density discontinuities remains largely unknown.

Passive dispersal of larvae across haloclines can be affected by dietary conditioning in a number of ways. Poorly fed echinoderm larvae may develop longer arms to increase the length of their ciliary bands, and therefore enhance feeding (Strathmann 1987; Hart and Strathmann 1994). Diet will determine larval carbon, protein and lipid content (e.g. Pechenik 1987; Thompson and Harrison 1992), and therefore can affect larval density. Larval shape and density may in turn influence buoyancy and sinking rate. Dietary conditioning also may affect some aspect of larval physiology that controls ciliary activity, and therefore larval swimming performance. Alternatively, dietary conditioning may operate directly on a behavioural response of larvae to density discontinuities.

In this study, we examined the response of sea urchin larvae to sharp haloclines in the laboratory by exposing larvae reared under different diets to a range of salinity gradients. In order to assess larval nutritional condition, we measured larval rates of development and larval shape under the different dietary treatments. We discuss possible implications of a response to haloclines to larval distribution in the field.

Materials and methods

Fertilization

We collected adults of *Echinometra lucunter* and *Arbacia punctulata* from the low intertidal and shallow subtidal zones of the rock jetty on the south side of Fort Pierce Inlet, Fort Pierce, Florida (27°44′12″N; 80°32′20″W) on 21 June and 27 April 1995, respectively. Spawning was induced in the laboratory within 1 to 2 h after collection by injecting ~2 ml of 0.55 *M* KCl through the peristomial membrane. Fertilization was achieved by mixing sperm and eggs of three pairs of *E. lucunter* and two pairs of *A. punctulata*. Egg size was 121.4 to 123.6 mm for *E. lucunter* ($n = 25$) and it was 56.2 μm for *A. punctulata* ($n = 24$). Fertilization success was 98.5 to 98.8% for *E. lucunter* and it was 96.2% for *A. punctulata*.

Larval rearing and dietary conditioning

Following successful fertilization, we combined the zygotes from all urchin pairs of each species and transferred them to 12 culture jars (2 or 4 litres in volume) in densities of 1 zygote ml^{-1} of seawater, 33‰ in salinity. We reared larvae under four different microalgal diets: a high ration of either single (*Isochrysis galbana*) or mixed (*Isochrysis galbana*, *Dunaliella tertiolecta*, *Thalassiosira weissflogii*) microalgal species, hereafter referred to as single-high and mixed-high diets, respectively; and a low ration of either single or mixed microalgal species, hereafter referred to as single-low and mixed-low diets, respectively (Table 1). The composition of these diets was based on previous studies by Fenaux et al. (1988) and George and Young (in preparation). Because of microalgal species' differences in cell size, we standardized food concentration between the single- and mixed-species diets to total microalgal biovolume rather than total cell number. In the mixed-species diets, each species was added to one-third of total biovolume in the particular ration. We cultured the microalgae in nutrient-enriched filtered seawater prepared as in Harrison et al. (1980) and modified as in Thompson et al. (1991), at 20 to 23 °C under natural light.

We used three replicate culture vessels for each of the four diet treatments. The larval cultures were kept in a temperature-controlled room (23 to 24 °C) with fluorescent lighting (~50 μE $m^{-2}s^{-1}$) under a 12 h light:12 h dark cycle. We changed the water in the culture jars and added exponentially growing microalgae every 2 d.

Larval development

We determined rate of larval development by recording the number of larvae in each developmental stage for a total of 20 to 50 larvae removed from one to all three of the jars from each diet treatment on the following days: for *Echinometra lucunter*, 2, 5, 8, 10, 15, 18, 23 and 31 d after fertilization; for *Arbacia punctulata*, 2, 6, 15, 20, 26, 32 and 46 d after fertilization. We also measured larval length from the anterior end of the post-oral rod to the posterior end of the somatic rod and larval width at the posterior end of the post-oral rod in a total of 20 to 50 larvae from each diet treatment on the following days: for *E. lucunter*, 2, 5, 8, 10, 15, 23 and 31 d after fertilization; for *A. punctulata*, 2, 15, 32 and 46 d after fertilization.

Larval response to haloclines – experimental design

We established sharp haloclines in plexiglass cylinders (22 cm height and 7 cm diameter) which were graduated in 1-cm segments and

Table 1 Microalgal species composition, density and biovolume in the four diet treatments provided to each echinoderm larval stage. For biovolume calculations, cells of *Isochrysis galbana* and *Thalassiosira weissflogii* were considered spherical with radii of 2.53 and 5.06 μm, respectively. Cells of *Dunaliella tertiolecta* were considered cylindrical with radius = 5.06 μm and height = 10.12 μm

Larval stage	Diet	Biovolume (μm^3 ml^{-1})	Species	Density (cells ml^{-1})
Two-arm	High food	34 000		
	single species		*I. galbana*	500
	mixed species		*I. galbana*	170
			D. tertiolecta	56
			T. weissflogii	21
	Low food	10 200		
	single species		*I. galbana*	150
	mixed species		*I. galbana*	50
			D. tertiolecta	17
			T. weissflogii	6
Four-arm	High food	68 000–136 000		
	single species		*I. galbana*	1000–2000
	mixed species		*I. galbana*	333–667
			D. tertiolecta	112–223
			T. weissflogii	42–84
	Low food	17 000–34 000		
	single species		*I. galbana*	250–500
	mixed species		*I. galbana*	83–170
			D. tertiolecta	28–56
			T. weissflogii	11–21
Six- to eight-arm	High food	136 000–272 000		
	single species		*I. galbana*	2000–4000
	mixed species		*I. galbana*	667–1333
			D. tertiolecta	223–447
			T. weissflogii	84–167
	Low food	34 000–68 000		
	single species		*I. galbana*	500–1000
	mixed species		*I. galbana*	170–333
			D. tertiolecta	56–112
			T. weissflogii	21–42
≥ Eight-arm	High food	340 000		
	single species		*I. galbana*	5000
	mixed species		*I. galbana*	1700
			D. tertiolecta	560
			T. weissflogii	210
	Low food	68 000		
	single species		*I. galbana*	1000
	mixed species		*I. galbana*	333
			D. tertiolecta	112
			T. weissflogii	42

covered with a styrofoam cap. Two glass tubes (3 mm in diameter) penetrated through the middle of the cap: one tube reached to 3 cm above the bottom and the other tube reached to the bottom of the cylinder. We established haloclines by first filling the cylinder to 6 cm above the bottom with water of lower salinity. We then gravity-fed water of higher salinity through the glass tube to the bottom of the cylinder until the surface of the top layer was 16 cm above the bottom. We removed the longer glass tube slowly through the styrofoam cap so as not to disturb the halocline. We withdrew water samples with a Pasteur pipette at 0.5-cm intervals and we measured salinity in each sample with a temperature-compensated refractometer (American Optical). Generally, haloclines (i.e. the distance to the closest 0.5-cm interval from the bottom of the low-salinity layer to the top of the high-salinity layer) were 1 to 2 cm deep. We removed larvae from the culture jars and introduced them into the cylinder with a Pasteur pipette through the remaining glass tube to 3 cm above the bottom (introduction of all larvae took 1 to 2 min). After all larvae were introduced into a cylinder, we allowed them to swim for 30 min. Then we estimated visually the number of larvae in each 0.5-cm height interval starting from the bottom of the cylinder (recording the position of all larvae in a cylinder took < 2 min). Preliminary experiments showed that larvae reached a particular position in the cylinder within 10 to 15 min of introduction and usually maintained that position for up to 2 h. We did not observe differences in larval swimming behaviour between the two experimental salinities of bottom water of 30 and 33‰. We conducted the experiments under fluorescent light ($\sim$50 μE $m^{-2}s^{-1}$) in 23 to 24 °C, in a room with black walls that prevented light reflection onto the sides of the experimental vessels. To prevent variation in larval swimming behaviour resulting from a possible endogenous rhythm, we made all observations during the same time of day.

We established four halocline treatments. In three of the treatments, the salinity of the bottom layer was 33‰. The salinities of the top layer were 21, 24 and 27‰, in each treatment respectively (hereafter referred to as treatments 21/33, 24/33 and 27/33). In the fourth treatment, the salinity of the bottom layer was 30‰ and that of the top layer was 21‰ (hereafter referred to as treatment 21/30). We used this fourth treatment to determine whether the larval response in the 21/33 treatment was because of the absolute salinity of the top layer (in which case the response should be similar in the 21/30 and 21/33 treatments) or because of the 12‰ salinity difference between the two layers (in which case the response should be similar in the 21/30 and 24/33 treatments and different in the 21/33 treatment). For *Echinometra lucunter*, we also determined larval vertical distribution in the absence of a halocline, in cylinders filled with 33‰ seawater, but we did not include this treatment in the statistical analysis. For *Arbacia punctulata*, we did not use this treatment because preliminary experiments showed that there was no difference in larval vertical distribution between treatments with a 27/33 halocline and treatments with no halocline.

We conducted experiments with larvae in the two- and four-arm developmental stages for *Echinometra lucunter* (beginning 3 and

11 d after fertilization, respectively) and with larvae in the four-arm stage for *Arbacia punctulata* (beginning 16 d after fertilization). After the four-arm stage, most larvae did not swim but sank to the bottom of the cylinders. We used an orthogonal design with Diet (*E. lucunter*, four levels: mixed-high, mixed-low, single-high and single-low; *A. punctulata*, two levels: mixed-high and single-high) and Halocline (four levels: 21/30, 21/33, 24/33 and 27/33) as the two factors. (For *A. punctulata*, we could not obtain enough larvae for the experiment from the low-ration diets because of high mortality in these treatments.) We ran four replicates for each treatment combination over 4 d (a complete set of treatment combinations was run each day) using 50 to 100 larvae per replicate for the two-arm stage and 20 to 50 larvae for the four-arm stage. To simplify the analyses, we pooled a number of 0.5-cm intervals in the cylinder to create three levels of the factor Position: (1) at the halocline, (2) above the halocline and (3) below the halocline. To determine the number of larvae at the halocline, we established the depth of the centre of the halocline in each cylinder and estimated the total number of larvae present 1 cm above and 1 cm below the centre. We then estimated the total number of larvae at all 0.5-cm intervals above and all intervals below the halocline layer.

Statistical analyses

For each sampling date, we examined the independence of diet and the number of larvae at each developmental stage by two-way contingency tables using log-linear models with Diet (four levels) and Stage (*Echinometra lucunter*, four levels: 2-, 4-, 6- and 8-arm; *Arbacia punctulata*, six levels: 2-, 4-, 6-, 8-, 10- and 12-arm) as the main factors. We compared larval length and width among diet treatments and sampling dates by two-way ANOVA with Diet (four levels, see above) and Age (*E. lucunter*, six levels: 2, 5, 8, 10, 15 and 23 d after fertilization; *A. punctulata*, four levels: 2, 15, 32 and 46 d after fertilization) as fixed factors. Larval length and width were $\ln(x+1)$-transformed to successfully remove heterogeneity of variance as detected with Cochran's tests. A posteriori multiple comparisons within levels of the factor Age were done with Student–Newman–Keuls' (SNK) tests on the harmonic means of each level of the factor Diet. All tests were considered significant when $P < 0.05$.

For each sea urchin species for each developmental stage, we examined the independence of halocline treatment (four levels), diet treatment (*Echinometra lucunter*: four levels; *Arbacia punctulata*: two levels – see above) and position of larvae in the cylinders (three levels) by three-way contingency tables using a series of log-linear models (Fienberg 1970; Sokal and Rohlf 1981). Treatments are independent when a log-linear model fits the data well and the values of the *G*-statistic are low and therefore not significant. Firstly, we examined the fit to the data of the most complex model which contained the terms Diet, Halocline and Position, and the two-way interaction terms Diet × Position and Halocline × Position. None of the models contained the interaction term Diet × Halocline because the number of larvae from each diet that were introduced into each halocline treatment were the result of our experimental manipulation and not an experimental effect. We did not compute the full model with the three-way interaction term Diet × Halocline × Position because it yields expected values equal to the observed values (Fienberg 1970).

For *Echinometra lucunter*, the most complex model did not fit the data well (i.e. *G*-values were significant), and we examined the independence of the factors Diet and Position within each level of the factor Halocline, and the independence of the factors Halocline and Position within each level of the factor Diet by two-way models. In all cases but one, the two-way models also yielded significant *G*-values, and we examined the independence of all possible pairs of levels of the factors Halocline and Diet. For *Arbacia punctulata*, the most complex model fit the data (i.e. *G*-value was not significant). Therefore, we assessed the significance of each two-way interaction term by determining the difference in *G*-values between models that did and did not contain that term (as recommended by Fienberg 1970). As for *E. lucunter*, when the two-way models yielded significant *G*-values, we examined the independence of all possible pairs of levels of the factors Halocline and Diet.

Prior to this analysis, homogeneous replicates (established by two-way contingency tables with Replicate and Position as the two factors) of each Diet × Halocline treatment combination were pooled (Sokal and Rohlf 1981). In cases of heterogeneity, we removed from the analysis the replicate in which percentage of larvae in any one of the three vertical positions in the cylinder was outside the 95% confidence intervals of the mean percentage of larvae in that position for all four replicates. We then confirmed homogeneity in the absence of this replicate and pooled the remaining three replicates. Generally, *G*-tests were considered significant at $\alpha = 0.05$ (but see "Results").

Salinity measurements

Salinity was measured daily at the sea-surface on the south side of Fort Pierce Inlet, ~800 m from the collection site of the adult urchins spawned in our study, with a temperature-corrected refractometer (Aquatic Eco-systems Inc.) from 10 October 1994 to 22 November 1995. The depth of the water column at the sampling site was ~4 m, and tidal, wave and weather conditions varied over the sampling period.

Results

Larval dietary conditioning

The rate of larval development of both urchin species varied among diet treatments, but only by 15 d after fertilization. For *Echinometra lucunter*, 100% of the larvae from all diet treatments were in the two-arm stage 2 and 5 d after fertilization and in the four-arm stage 8 and 10 d after fertilization, making analysis by contingency tables impossible (Fig. 1). The number of larvae in each developmental stage was dependent on Diet 15 d ($G = 44.11$, $df = 1$, $P < 0.001$), 18 d ($G = 115.1$, $df = 9$, $P < 0.001$) and 23 d ($G = 151.0$, $df = 5$, $P < 0.001$) after fertilization. In all cases, more larvae from high-ration treatments were at more advanced developmental stages than larvae from low-ration treatments. On Day 23, 70 to 80% of larvae from low-ration treatments were in the four-arm stage, whereas 95 to 100% of larvae from high-ration treatments were in the last larval stage. To determine whether larvae from low-ration treatments could develop past the four-arm stage, we doubled the food concentration in these cultures and observed some development to the eight-arm stage by 31 d. However, 35 to 60% of larvae remained in the four-arm stage.

For *Arbacia punctulata*, 100% of the larvae from all diet treatments were in the two-arm stage 2 d after fertilization and 85 to 100% of the larvae from all diets were in the four-arm stage 6 and 15 d after fertilization, making analysis by contingency tables impossible (Fig. 2). The number of larvae in each developmental stage was dependent on Diet 20 d ($G = 18.38$, $df = 7$, $P = 0.01$), 26 d ($G = 25.03$, $df = 3$, $P < 0.001$), 32 d ($G = 20.35$, $df = 3$, $P < 0.001$) and 46 d ($G = 37.57$, $df = 7$, $P < 0.001$) after fertilization. On Day 20, 60% of larvae from the mixed-high treatment were in the

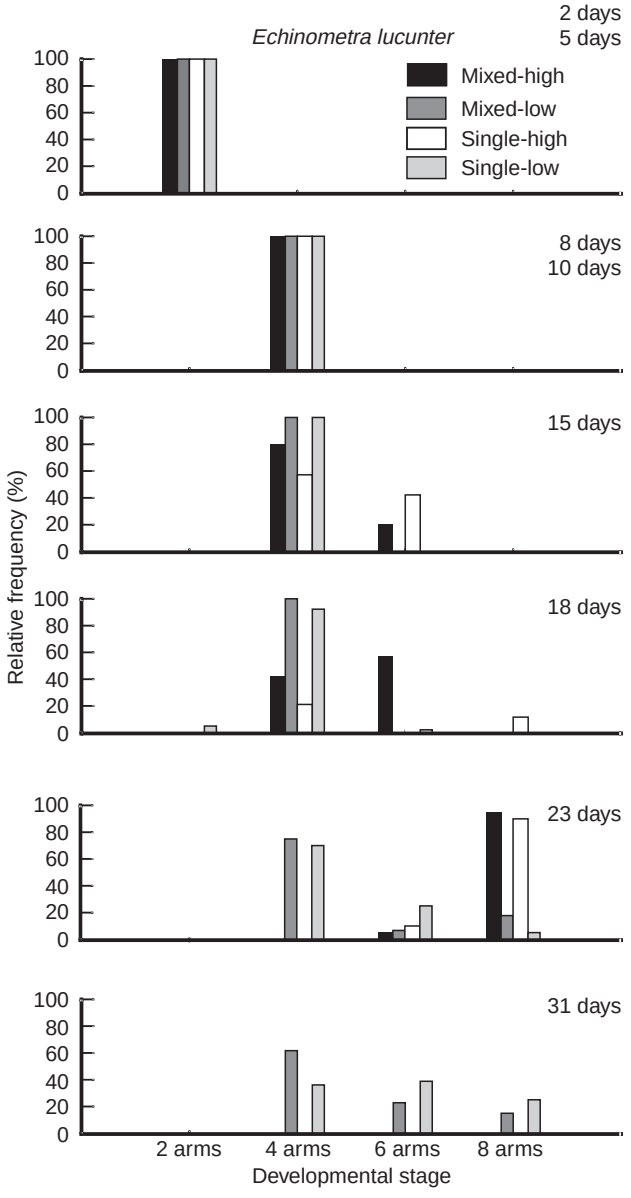

Fig. 1 *Echinometra lucunter*. Percentage of larvae in different developmental stages under four diet treatments at eight ages after fertilization (see "Materials and methods" for treatment abbreviations). Percentage was identical 2 and 5 d, and 8 and 10 d after fertilization. On Day 31, we only show results for larvae from low-ration diets. We made no measurements in high-ration treatments since these larvae had started settling out

four-arm stage, whereas 55 to 60% of larvae from the mixed-low and single-high treatments were in the six-arm stage. On Day 32, more larvae from mixed-species treatments were at more advanced developmental stages than larvae from single-species treatments. On Day 16, 60 to 80% of larvae from high-ration treatments were in the last developmental stage, whereas 70% of larvae from the single-low treatments were still in the eight-arm stage.

Larval length and larval width of both sea urchin species varied among diets but not at all ages. For *Echinometra lucunter*, length and width varied significantly by 8 and 5 d after fertilization, respectively (length: $F_{\text{Diet}(3,1004)} = 46.69$, $F_{\text{Age}(5,1004)} = 1453$, $F_{\text{Diet} \times \text{Age}\ (15,1004)} = 16.06$; width: $F_{\text{Diet}(3,1004)} = 100.7$, $F_{\text{Age}(5,1004)} = 1242$, $F_{\text{Diet} \times \text{Age}(15,1004)} = 28.37$, $P < 0.001$ for all F-tests) (Fig. 3). On Day 5, width was smaller in the mixed-high than all other diets. On Day 8, length was greater in the mixed-high than in the mixed-low and single-low diets; width was greater in the mixed-high than all other diets. On Day 10, both length and width were greater in the single-high than all other diets. On Day 15, length was greater in the mixed-high and single-high than in the mixed-low and single-low diets; width varied among all treatments as follows: in decreasing order from single-high, mixed-high, mixed-low, single-low diet. On Day 23, both length and width varied among all treatments as follows: in decreasing order from single-high, mixed-high, single-low, mixed-low diet (SNK-tests, $P < 0.05$).

For *Arbacia punctulata*, larval length varied significantly among diets 15 and 46 d after fertilization and larval width varied significantly among diets by 15 d after fertilization (length: $F_{\text{Diet}(3,395)} = 21.67$, $F_{\text{Age}(3,395)} = 1512$, $F_{\text{Diet} \times \text{Age}(9,395)} = 5.78$; width: $F_{\text{Diet}(3,395)} = 7.33$, $F_{\text{Age}(3,395)} = 541$, $F_{\text{Diet} \times \text{Age}(9,395)} = 6.41$; $P < 0.001$ for all F-tests) (Fig. 4). On Day 15, length was greater in the mixed-high and single-high than in the mixed-low and single-low diets; width was smaller in the mixed-low than all other diets. On Day 32, width was greater in the single-high than all other diets. On Day 46, both length and width were smaller in the single-low than all other diets (SNK-tests, $P < 0.05$).

Larval response to haloclines

The position of larvae in the cylinders was dependent on the diet and halocline treatments for both sea urchin species and all developmental stages tested. For the two- and four-arm stages of *Echinometra lucunter*, non-independence was indicated by the poor fit to the data of the most complex log-linear model (Tables 2, 3). For *Arbacia punctulata*, non-independence was indicated by the significant G-values obtained when each of the interaction terms was removed from the complex model (Table 4). For both species, we also detected significant Halocline × Position and Diet × Position interactions by two-way contingency tables. For these two-way tables, we used a more conservative G_{critical} because we compared all possible pairs of levels of the factors Diet and Halocline. G-values were significant when $G > \chi^2_{\alpha[k,\nu]}$ with $k = b(b-1)/2$, where $\alpha = 0.05$, k is the number of intended comparisons, ν is the degrees of freedom of the test and b the number of levels of the factor (Sokal and Rohlf 1981).

In the absence of a halocline, 50 to 60% of the larvae of both species immediately swam upwards to the water surface upon introduction to the cylinders, where they remained for the duration of the experiment (Figs. 5, 6; unpublished data for *Arbacia punctulata*). In treatments

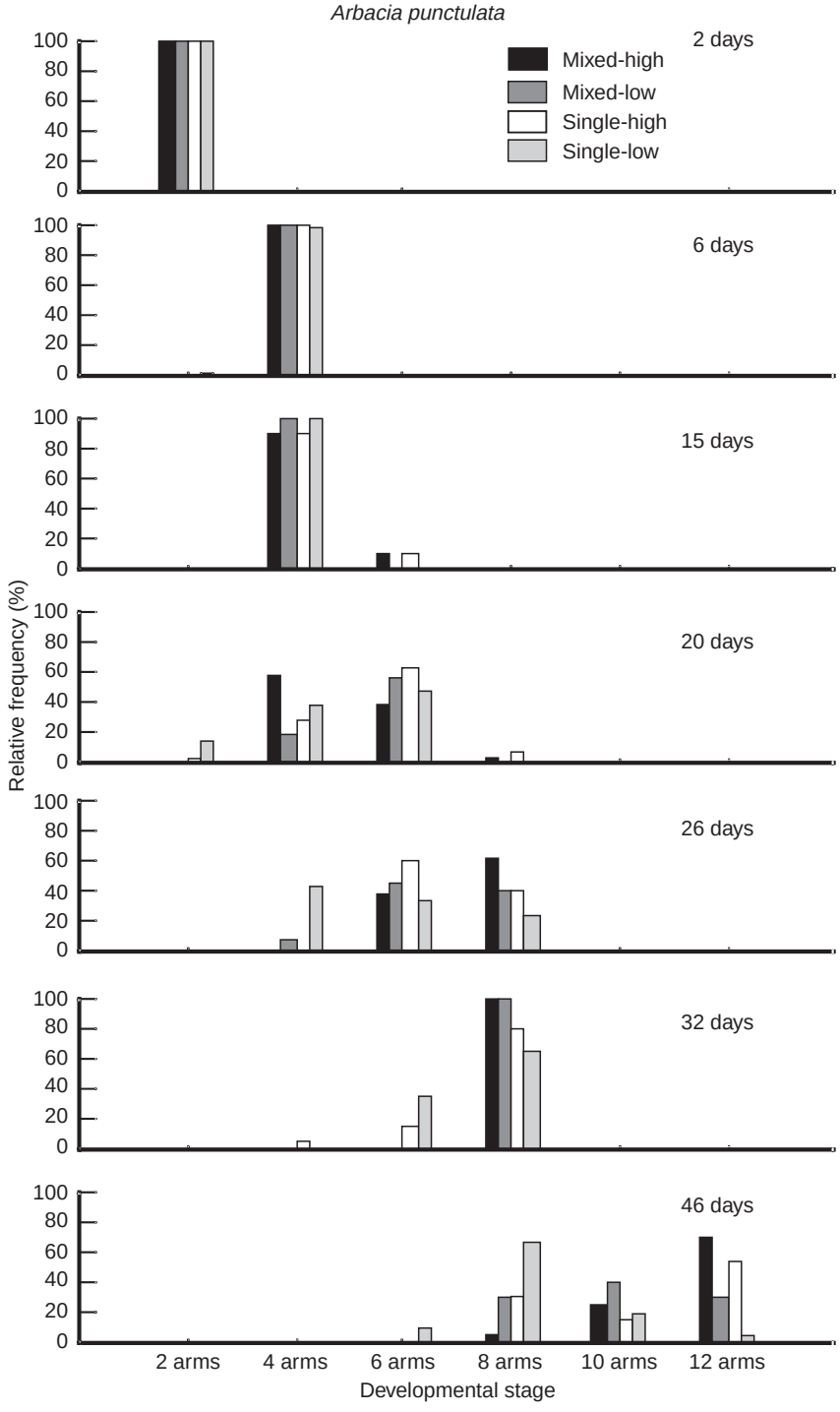

Fig. 2 *Arbacia punctulata*. Percentage of larvae in different developmental stages under four diet treatments at seven ages after fertilization

with a halocline, most larvae that we recorded in the halocline layer swam upwards until they reached the top of the halocline just a few minutes after their introduction to the bottom layer, stopped and remained in that position for the duration of the experiment. Most larvae that crossed the halocline did so after remaining at the halocline layer for a few minutes, and some of these larvae stopped their upward movement before they reached the water surface. For both urchins and all stages tested, the proportion of larvae above the halocline increased with increasing salinity of the top water layer (Figs. 5 to 7). For *Echinometra lucunter*, although Position differed significantly between the 21/33 and 21/30 treatments for two-arm larvae from high-ration diets (Table 2), the difference was too small to be biologically meaningful. Position in each of the 21/33 and 21/30 treatments differed significantly from Position in both the 24/33 and 27/33 treatments, for both stages of this species and most diets (Tables 2, 3). Position also differed significantly between the 24/33 and 27/33 treat-

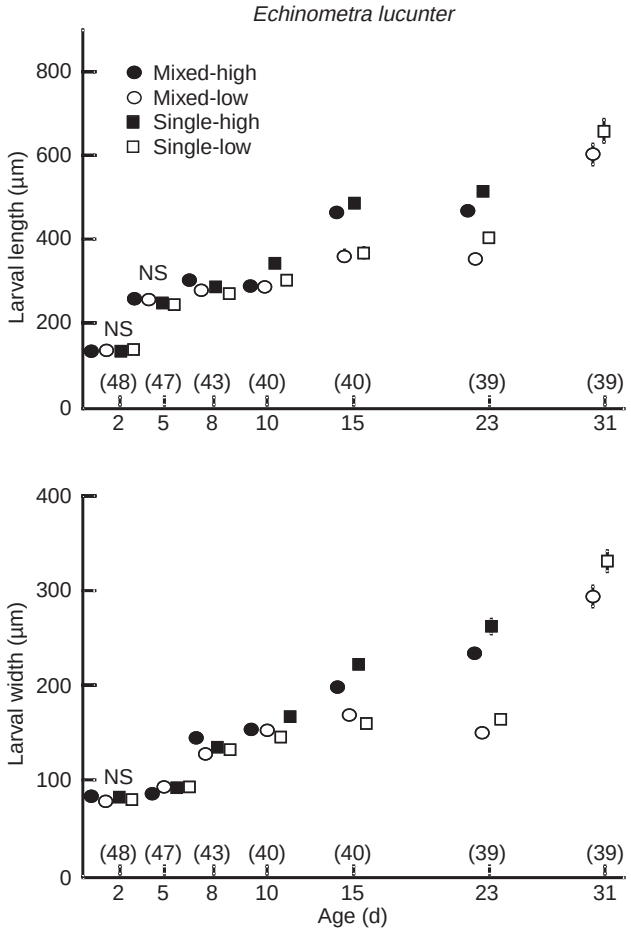

Fig. 3 *Echinometra lucunter*. Larval length and width under four different diet treatments at seven different ages after fertilization. Error bars are standard errors of the mean and are within the symbols when not shown. *n*-values used to calculate the harmonic means of each treatment at each age are shown above the *x*-axis (*NS* non-significant differences among treatments, as indicated by SNK-test within the level of the factor Age)

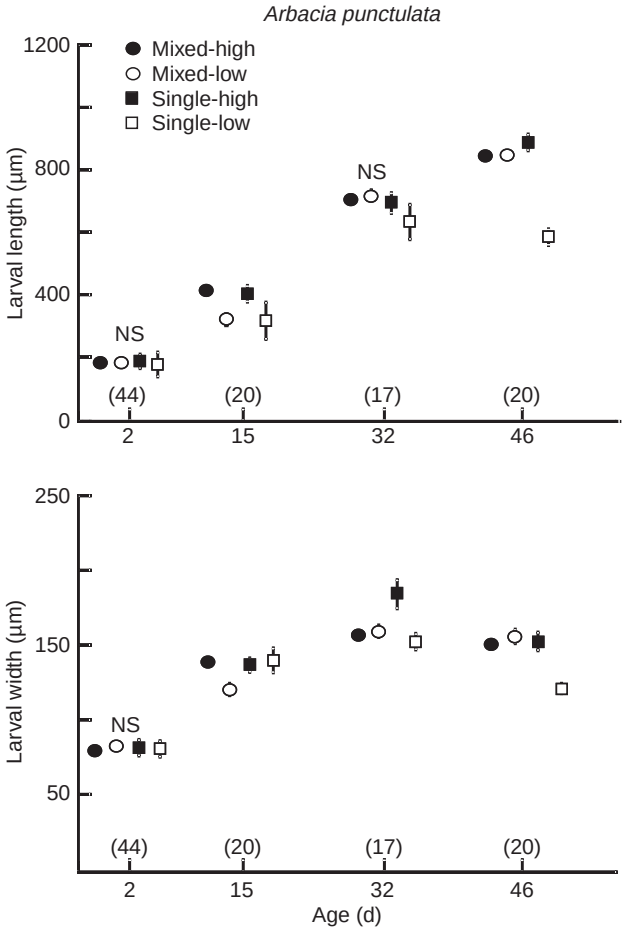

Fig. 4 *Arbacia punctulata*. Larval length and width under four different diet treatments at four different ages after fertilization. Error bars are standard errors of the mean and are within the symbols when not shown. *n*-values used to calculate the harmonic means of each treatment at each age are shown above the *x*-axis (*NS* non-significant differences among treatments, as indicated by SNK-test within the level of the factor Age)

ments, for high-ration diets for both stages and for the mixed-low diet for the four-arm stage. For *A. punctulata,* Position did not differ between the 21/33 and 21/30, and between the 24/33 and 27/33 treatments, for either level of Diet (Table 4). However, Position in each of the 21/33 and 21/30 treatments differed significantly from Position in both the 24/33 and 27/33 treatments.

For both species, we observed an effect of Diet on the position of larvae in the cylinders and this effect varied among levels of the factor Halocline (Figs. 5 to 7). For *Echinometra lucunter*, there was an effect of food quantity (not tested for *Arbacia punctulata*). Position was significantly different between the high- and low-ration diets in the 24/33 treatments for both developmental stages from both mixed- and single-species diets, and in the 27/33 treatments for four-arm larvae from mixed-species diets (Tables 2, 3). In all cases, a greater proportion of larvae from low- than from high-ration diets were present above the halocline. Position of larvae in the cylinders also varied with food quality for both species: for high-ration treatments, more larvae from single- than from mixed-species diets were present above the halocline. We observed this for four-arm larvae of *E. lucunter* in the 24/33 treatments, and of *A. punctulata* in the 21/30 treatments (Table 4). For *E. lucunter*, Position also varied between the mixed-high and single-low diets for two-arm larvae in the 24/33 treatment and for four-arm larvae in the 24/33 and 27/33 treatments: in all cases, more larvae from single-low than mixed-high diets were present above the halocline. Lastly, for both stages of *E. lucunter* in the 24/33 treatment, there were either more larvae above the halocline or fewer larvae at the halocline from mixed-low than from single-high diets. For *E. lucunter*, there also were significant differences: (1) between rations in the 21/33 treatments for two-arm larvae from the mixed-species diets, (2) between the mixed- and single-species diets in the 21/33 treatments for two-arm larvae from high-ration diets, and (3) between the mixed-high and single-low diets for four-arm larvae in the 21/30 treatments. However, in all cases the

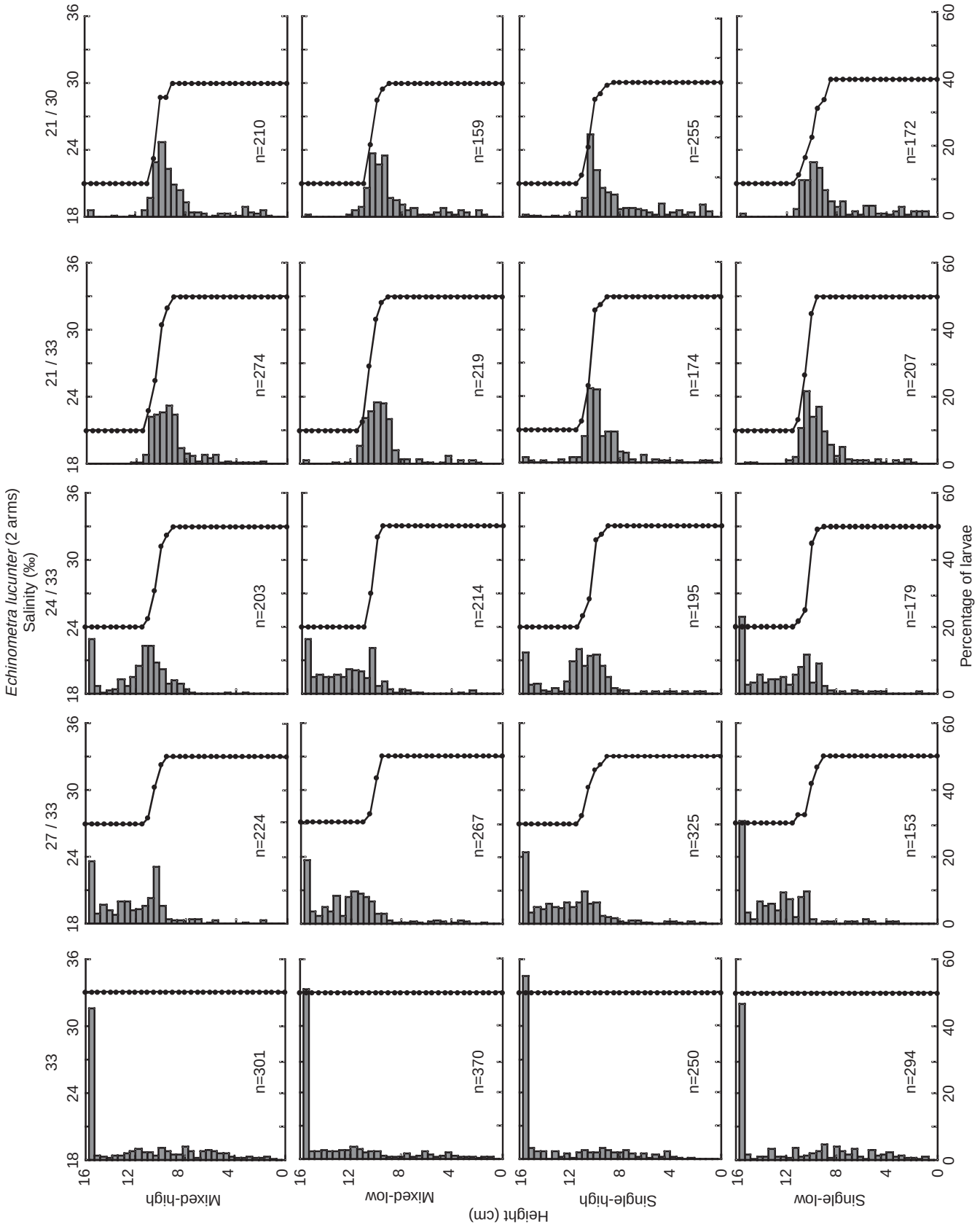

Fig. 5 *Echinometra lucunter*. Vertical distribution in the experimental cylinders of two-arm larvae from four diet treatments placed in four halocline treatments and in cylinders with no halocline but filled with sea water 33‰ in salinity (see "Materials and methods" for treatment abbreviations). Percentages were calculated on total number of larvae, shown as *n*-values, pooled from three or four replicates. Superimposed line-plots show mean salinity profiles in the pooled replicate cylinders

Table 2 *Echinometra lucunter*. Analysis by log-linear models of independence between the position in the experimental cylinders of two-arm larvae (*P*), and the Diet (*D*) and Halocline (*H*) treatments. Because the most complex model provided a significant value for *G*, simpler models were examined within each level of the factors D and H. Mean percentages of larvae in each of three pooled positions [below (*B*), at (*H*) and above (*A*) the halocline] are shown for each treatment [*NS* non-significant *P*-values, adjusted for the number of comparisons among all possible pairs of levels of the factors H and D (critical $G_{0.05[6,\ 2]} = 9.53$); * significant *P*-values, adjusted for the number of comparisons among all possible pairs of levels of the factors H and D; see "Materials and methods" for treatment abbreviations]

Model	Treatment means		*G*	*df*	*P*
	B:H:A	B:H:A			
D + H + P + [D × P] + [H × P]			136.2	27	< 0.001
H + P					
Mixed-high:			270.5	6	< 0.001
21/33 vs 21/30	17:83:0	15:82:3	10.22	2	0.006*
21/33 vs 24/33	17:83:0	3:70:27	118.8	2	< 0.001*
21/33 vs 27/33	17:83:0	6:47:45	195.2	2	< 0.001*
21/30 vs 24/33	15:82:3	3:70:27	66.25	2	< 0.001*
21/30 vs 27/33	15:82:3	6:47:45	123.1	2	< 0.001*
24/33 vs 27/33	3:70:27	6:47:45	21.34	2	< 0.001*
Mixed-low:			320.9	6	< 0.001
21/33 vs 21/30	7:90:1	18:82:1	7.04	2	0.030^{NS}
21/33 vs 24/33	7:90:1	4:48:48	154.2	2	< 0.001*
21/33 vs 27/33	7:90:1	7:43:51	180.1	2	< 0.001*
21/30 vs 24/33	18:82:1	4:48:48	139.7	2	< 0.001*
21/30 vs 27/33	18:82:1	7:43:51	152.3	2	< 0.001*
24/33 vs 27/33	4:48:48	7:43:51	2.97	2	0.227^{NS}
Single-high:			278.3	6	< 0.001
21/33 vs 21/30	14:83:3	26:71:2	10.21	2	0.006*
21/33 vs 24/33	14:83:3	6:72:22	35.28	2	< 0.001*
21/33 vs 27/33	14:83:3	6:46:49	130.1	2	< 0.001*
21/30 vs 24/33	26:71:2	6:72:22	73.36	2	< 0.001*
21/30 vs 27/33	26:71:2	6:46:49	199.3	2	< 0.001*
24/33 vs 27/33	6:72:22	6:46:49	39.44	2	< 0.001*
Single-low:			312.1	6	< 0.001
21/33 vs 21/30	16:83:1	26:73:1	5.47	2	0.065^{NS}
21/33 vs 24/33	16:83:1	4:47:49	149.5	2	< 0.001*
21/33 vs 27/33	16:83:1	5:39:56	169.5	2	< 0.001*
21/30 vs 24/33	26:73:1	4:47:49	140.0	2	< 0.001*
21/30 vs 27/33	26:73:1	5:39:56	155.6	2	< 0.001*
24/33 vs 27/33	4:47:49	5:39:56	2.05	2	0.358^{NS}
D + P					
21/33:			19.56	6	0.003
mixed-high vs mixed-low	17:83:0	7:90:1	11.84	2	0.003*
mixed-high vs single-high	17:83:0	14:83:3	11.97	2	0.003*
mixed-high vs single-low	17:83:0	16:83:1	3.42	2	0.180^{NS}
mixed-low vs single-high	7:90:1	14:83:3	4.71	2	0.095^{NS}
mixed-low vs single-low	7:90:1	16:83:1	5.37	2	0.068^{NS}
single-high vs single-low	14:83:3	16:83:1	3.14	2	0.208^{NS}
21/30:			15.25	6	0.018
mixed-high vs mixed-low	15:82:3	18:82:1	3.02	2	0.221^{NS}
mixed-high vs single-high	15:82:3	26:71:2	8.57	2	0.014^{NS}
mixed-high vs single-low	15:82:3	26:73:1	7.34	2	0.025^{NS}
mixed-low vs single-high	18:82:1	26:71:2	6.70	2	0.035^{NS}
mixed-low vs single-low	18:82:1	26:73:1	3.48	2	0.176^{NS}
single-high vs single-low	26:71:2	26:73:1	0.90	2	0.639^{NS}
24/33:			53.56	6	< 0.001
mixed-high vs mixed-low	3:70:27	4:48:48	22.08	2	< 0.001*
mixed-high vs single-high	3:70:27	6:72:22	2.60	2	0.273^{NS}
mixed-high vs single-low	3:70:27	4:47:49	22.09	2	< 0.001*
mixed-low vs single-high	4:48:48	6:72:22	30.94	2	< 0.001*
mixed-low vs single-low	4:48:48	4:47:49	0.16	2	0.922^{NS}
single-high vs single-low	6:72:22	4:47:49	29.58	2	< 0.001*
27/33:			5.31	6	0.505

differences among means were too small to be biologically meaningful.

Field salinity measurements

Salinity at the collection site of the adults generally ranged between 30 and 35‰ for most of the sampling period (Fig. 8). However, the range in salinity increased from July to November 1995, when values as low as 15‰ were recorded. Salinity was greater during flood than ebb tides, reflecting intrusion of more saline water from the open ocean at flood tide and drainage of riverine-influenced, less saline water from the Indian River Lagoon at ebb tide to the adult urchin habitat.

Discussion

Effect of the salinity gradient

Larval response to the haloclines was pronounced and the strength of the response increased with decreasing salinity in the top water layer. In the absence of a halocline, larvae swam straight to the water surface. We do not believe this to be a phototactic response because the cylinders had caps that did not permit light penetration from above, but rather it is probably the result of negative geotaxis (Pennington and Emlet 1986; Young 1995). For both species and all developmental stages and diet treatments, few or no larvae crossed the halocline into the top layer when salinity in that layer was 21‰. In most cases, there was no difference in larval distribution between the 21/33 and 21/30 treatments, but there was a difference between the 21/30 and 24/33 treatments. This result suggests that in the 21/33 treatment larvae were responding to the salinity of the top layer rather than to the salinity difference between the top and bottom layers. Overall, more larvae crossed the halocline when the salinity of the top layer was 24 and 27‰ than when it was 21‰. For high-ration treatments for both stages of *Echinometra lucunter*, more larvae crossed the halocline in the 27/33 than in the 24/33 treatment. Larval aggregation at haloclines and/or avoidance of the lower salinity layer has been documented for other taxa including ascidian tadpoles (Vázquez and Young 1996), barnacle nauplii (Harder 1968), bivalve larvae (Mann et al. 1991), blue crab zoea (Sulkin and Van Heukelem 1982), hermit crab zoea (Roberts 1971), littorinid larvae (Harder 1968) and lobster zoea (Scarratt and Raine 1967).

Changes in salinity may alter larval swimming performance by affecting ciliary activity. Podolsky and Emlet (1993) showed that swimming speed of larvae of the sand dollar *Dendraster excentricus*, as well as rate of water movement through the cilia, decreased with increasing viscosity of the medium. They examined the effects of a 27% change in viscosity (from 1.02 to 1.30 cP) resulting from a change in temperature. However, salinity does not have as great an effect on viscosity as temperature, and a difference between 20 and 30‰ results in a difference in viscosity of 2.2% (from 1.047 to 1.07 cP at 20 °C: Walton Smith 1981). Therefore, we do not believe that the larval response to the haloclines in our study was the result of the difference in viscosity between the two water layers.

There is some evidence that salinity can have a pronounced effect on rates of development and survival of urchin larvae but this effect is species-specific (for review see Stickle and Diehl 1987). For example, larvae of *Strongylocentrotus pallidus*, *S. purpuratus* and *Lytechinus variegatus* do not survive in salinities <25‰, but larval survival in *S. droebachiensis* is not affected by salinities as low as 20‰ (Roller and Stickle 1985, 1993). Larval development is retarded or inhibited in salinities <27.5‰ for *S. pallidus*, *S. purpuratus* and *L. variegatus*, and in salinities <22‰ for *S. droebachiensis* and *S. nudus* (Roller and Stickle 1985, 1993, 1994; Yaroslavtseva and Sergeeva 1993). Little is known about the effect of salinity on larval development and survival in the genera *Echinometra* and *Arbacia*. Petersen and Almeida (1976) suggested that normal larval development can occur at salinities as low as 22‰ but that larvae do not survive in salinity of 18‰. We found that development of *E. lucunter* can be arrested, and both long- and short-term (24 h) survival can be reduced in salinities as high as 27‰ (Metaxas and Young in preparation). The increasing strength of larval response to haloclines with decreasing salinity in the top water layer in this study may reflect the tolerance (and therefore possible osmotic stress) of the two species to reduced salinity.

Larval response to the haloclines was similar in the two developmental stages in *Echinometra lucunter*. In preliminary experiments, larval stages with more than four arms of both sea urchin species showed reduced swimming activity and tended to remain near the bottom of the experimental vessels, probably because of increased larval density, and therefore sinking rate. Thus, the larval response to haloclines that we recorded is pertinent only to early developmental stages that swim upwards from the bottom water layer. Larval response to haloclines also may affect the ability of later developmental stages found in the top water layer, to re-enter the bottom layer that overlies potential settlement sites. This may be the case if the change in salinity at the halocline induces larvae to stop swimming and thus inhibits their descent to the bottom; however, older larvae probably are less capable of controlled swimming behaviour because of their great density. We have observed eight-arm larvae of *E. lucunter* and ten- and twelve-arm larvae of *Arbacia punctulata* sink straight to the bottom upon their introduction into the surface layer of an experimental cylinder.

In Florida, the peak of the reproductive cycle of *Echinometra lucunter* is from late May to late October and *Arbacia punctulata* spawns thoughout the year (personal observation). During most of the active reproductive period of both species, salinity in the adult

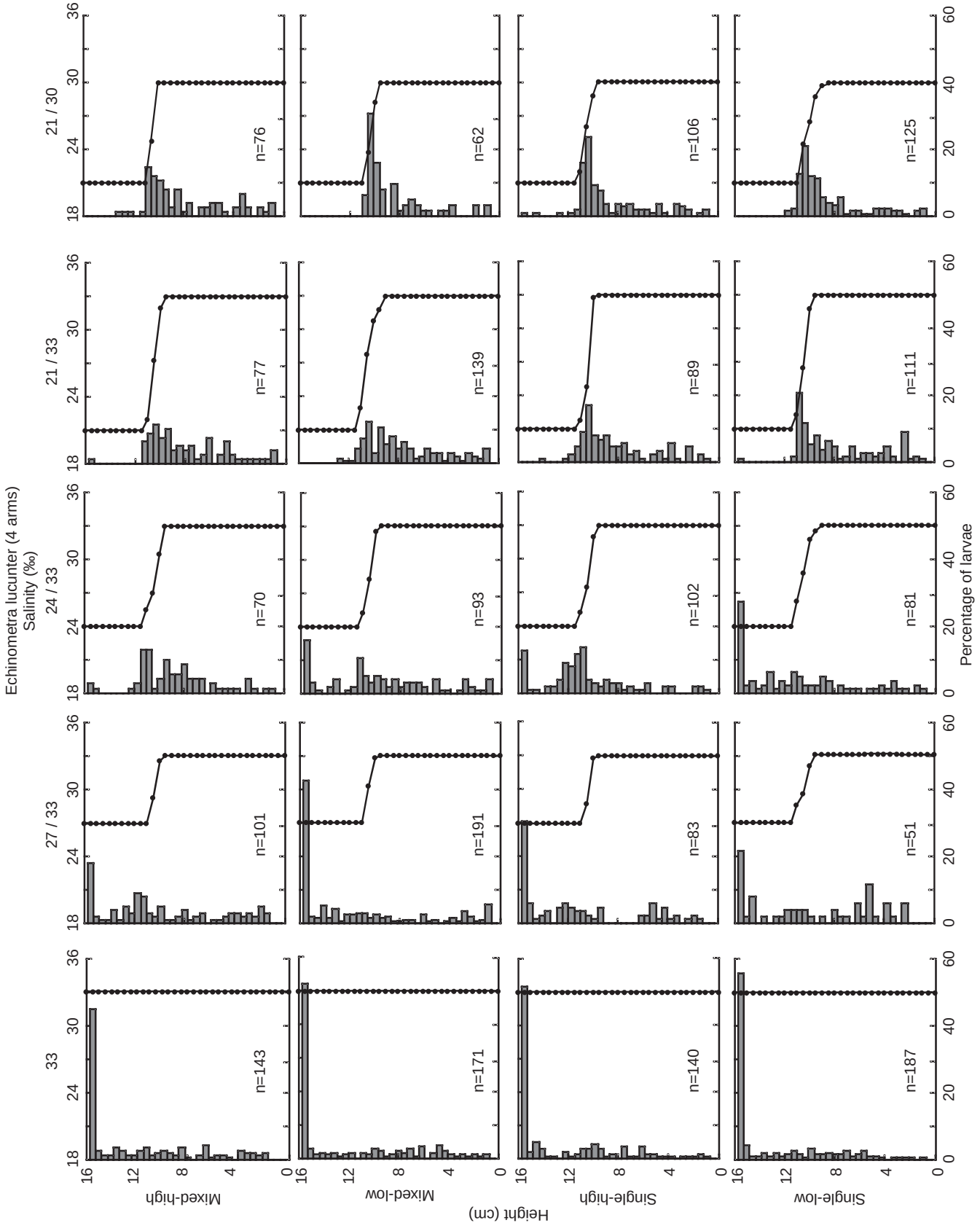

Fig. 6 *Echinometra lucunter*. Vertical distribution in the experimental cylinders of four-arm larvae from four diet treatments placed in four halocline treatments and in cylinders with no halocline but filled with sea water 33‰ in salinity (see "Materials and methods" for treatment abbreviations). Percentages were calculated on total number of larvae, shown as *n*-values, pooled from three or four replicates. Superimposed line-plots show mean salinity profiles in the pooled replicate cylinders

Table 3 *Echinometra lucunter*. Analysis by log-linear models of independence between the position in the experimental cylinders of four-arm larvae (*P*), and the Diet (*D*) and Halocline (*H*) treatments. Because the most complex model provided a significant value for *G*, simpler models were examined within each level of the factors D and H. Mean percentage of larvae in each of three pooled positions [below (*B*), at (*H*) and above (*A*) the halocline] is shown for each treatment [*NS* non-significant *P*-values, adjusted for the number of comparisons among all possible pairs of levels of the factors H and D (critical $G_{0.05[6,\ 2]} = 9.53$); * significant *P*-values, adjusted for the number of comparisons among all possible pairs of levels of the factors H and D; see "Materials and methods" for treatment abbreviations]

Model	Treatment means		*G*	*df*	*P*
	B:H:A	B:H:A			
D + H + P + [D × P] + [H × P]			128.8	27	< 0.001
H + P					
Mixed-high:			58.82	6	< 0.001
21/33 vs 21/30	45:53:1	47:51:1	0.06	2	0.972^{NS}
21/33 vs 24/33	45:53:1	40:56:4	1.54	2	0.462^{NS}
21/33 vs 27/33	45:53:1	34:35:32	34.03	2	< 0.001*
21/30 vs 24/33	47:51:1	40:56:4	1.80	2	0.406^{NS}
21/30 vs 27/33	47:51:1	34:35:32	33.52	2	< 0.001*
24/33 vs 27/33	40:56:4	34:35:32	23.19	2	< 0.001*
Mixed-low:			207.3	6	< 0.001
21/33 vs 21/30	42:57:1	27:73:0	5.13	2	0.077^{NS}
21/33 vs 24/33	42:57:1	39:34:27	44.55	2	< 0.001*
21/33 vs 27/33	42:57:1	23:20:58	151.4	2	< 0.001*
21/30 vs 24/33	27:73:0	39:34:27	37.59	2	< 0.001*
21/30 vs 27/33	27:73:0	23:20:58	95.76	2	< 0.001*
24/33 vs 27/33	39:34:27	23:20:58	24.39	2	< 0.001*
Single-high:			101.1	6	< 0.001
21/33 vs 21/30	38:61:1	31:66:3	1.65	2	0.439^{NS}
21/33 vs 24/33	38:61:1	18:63:20	26.04	2	< 0.001*
21/33 vs 27/33	38:61:1	25:27:48	64.23	2	< 0.001*
21/30 vs 24/33	31:66:3	18:63:20	18.74	2	< 0.001*
21/30 vs 27/33	31:66:3	25:27:48	64.06	2	< 0.001*
24/33 vs 27/33	18:63:20	25:27:48	26.49	2	< 0.001*
Single-low:			202.9	6	< 0.001
21/33 vs 21/30	41:59:1	24:75:1	7.51	2	0.023^{NS}
21/33 vs 24/33	41:59:1	22:32:46	67.95	2	< 0.001*
21/33 vs 27/33	41:59:1	20:16:64	111.8	2	< 0.001*
21/30 vs 24/33	24:75:1	22:32:46	77.91	2	< 0.001*
21/30 vs 27/33	24:75:1	20:16:64	129.2	2	< 0.001*
24/33 vs 27/33	22:32:46	20:16:64	7.28	2	0.026^{NS}
D + P					
21/33:			1.21	6	0.976
21/30:			15.88	6	0.014
mixed-high vs mixed-low	47:51:1	27:73:0	7.36	2	0.025^{NS}
mixed-high vs single-high	47:51:1	31:66:3	5.15	2	0.076^{NS}
mixed-high vs single-low	47:51:1	24:75:1	11.92	2	0.003*
mixed-low vs single-high	27:73:0	31:66:3	3.19	2	0.203^{NS}
mixed-low vs single-low	27:73:0	24:75:1	1.03	2	0.596^{NS}
single-high vs single-low	31:66:3	24:75:1	3.15	2	0.207^{NS}
24/33:			56.61	6	< 0.001
mixed-high vs mixed-low	40:56:4	39:34:27	18.19	2	< 0.001*
mixed-high vs single-high	40:56:4	18:63:20	16.41	2	< 0.001*
mixed-high vs single-low	40:56:4	22:32:46	38.15	2	< 0.001*
mixed-low vs single-high	39:34:27	18:63:20	17.13	2	< 0.001*
mixed-low vs single-low	39:34:27	22:32:46	8.25	2	0.016^{NS}
single-high vs single-low	18:63:20	22:32:46	19.29	2	< 0.001*
27/33:			25.21	6	< 0.001
mixed-high vs mixed-low	34:35:32	23:20:56	18.29	2	< 0.001*
mixed-high vs single-high	34:35:32	25:26:48	5.22	2	0.074^{NS}
mixed-high vs single-low	34:35:32	20:16:64	19.73	2	< 0.001*
mixed-low vs single-high	23:20:56	25:26:48	2.26	2	0.323^{NS}
mixed-low vs single-low	23:20:56	20:16:64	1.06	2	0.588^{NS}
single-high vs single-low	25:26:48	20:16:64	4.59	2	0.101^{NS}

habitat can vary from 15 to 40‰, reflecting strong tidal circulation. Therefore, while in the plankton, larvae often will encounter the range of salinities used in our experiments. To our knowledge, there are no data available on the stratification of Fort Pierce Inlet or the Indian River Lagoon. However, fresh- and saltwater

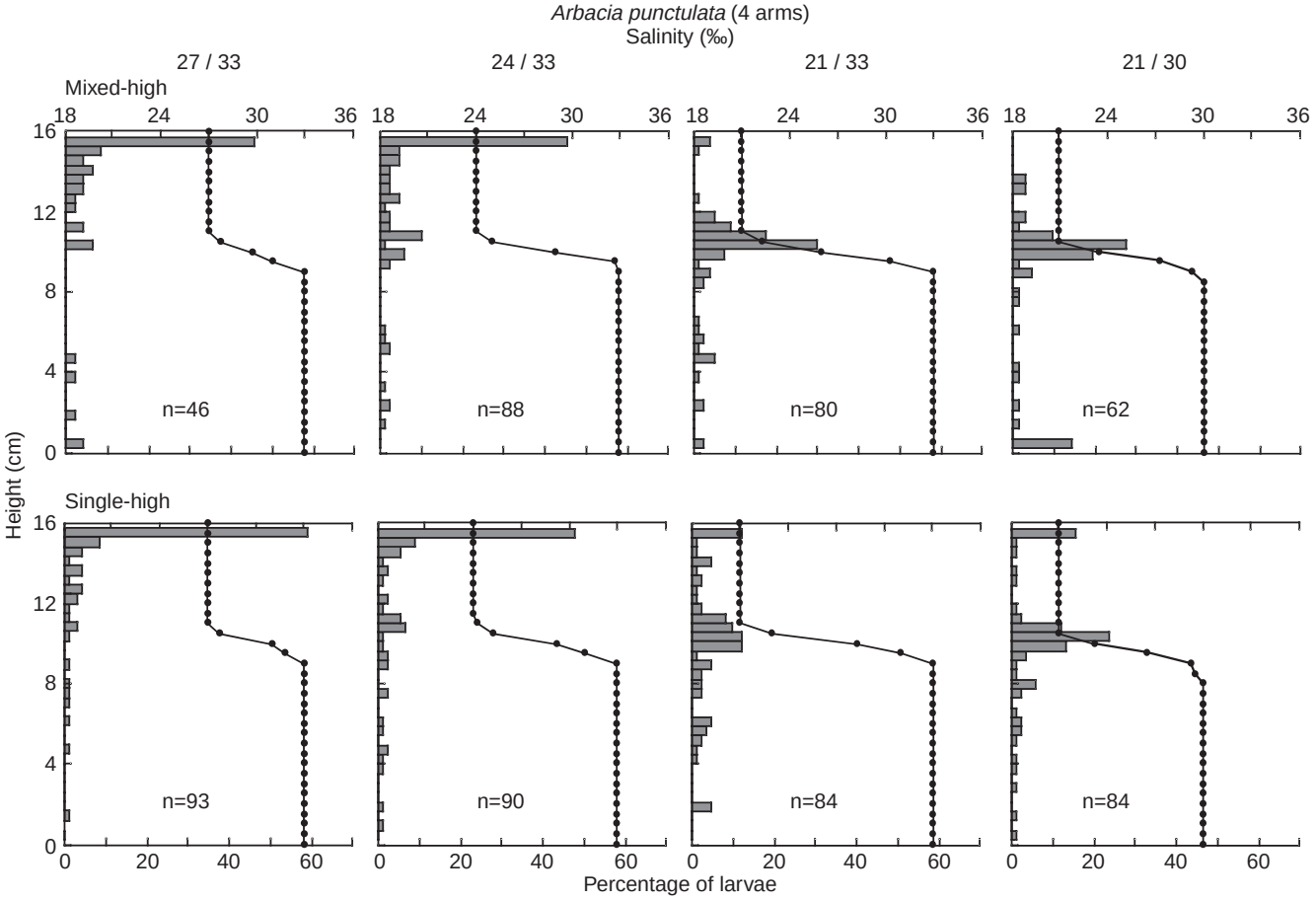

Fig. 7 *Arbacia punctulata*. Vertical distribution in the experimental cylinders of four-arm larvae from two diet treatments placed in four halocline treatments (see "Materials and methods" for treatment abbreviations). Percentages were calculated on total number of larvae, shown as *n*-values, pooled from three or four replicates. Superimposed line-plots show mean salinity profiles in the pooled replicate cylinders

lenses most likely are formed during tidal exchange, and they can result in the development of haloclines. In general, these two sea urchin species occur most commonly in shallow coastal habitats on rocky and sandy bottoms, on coral reefs, and in grass beds (Hendler et al. 1995), where temporary haloclines also may develop during periods of increased rainfall and runoff. Although the weak swimming ability of larvae precludes the possibility of controlled horizontal dispersal (e.g. Emlet 1986), it has been shown that larval swimming can influence vertical distribution (e.g. Sulkin and Van Heukelem 1982; Raby et al. 1994). Our experiments support this suggestion: although weak swimmers, echinoderm larvae can actively avoid crossing haloclines, and therefore show an ability to maintain their position within the high-salinity bottom water. This ability will reduce the probability of young larvae being introduced to outflowing low-salinity water that (1) may be physiologically detrimental and (2) may carry them to the open ocean, away from the adult habitat. It should be pointed out, however, that the ability of larvae to remain within a particular water mass will be highly influenced by the flow characteristics of that mass, such as flow speed and turbulence. The effects of the interaction between flow characteristics and larval behaviour in the water column remain largely unknown.

Effect of dietary conditioning

For both *Echinometra lucunter* and *Arbacia punctulata,* different diets resulted in larvae of different nutritional condition as evidenced by the rates of development and sizes at age. For both species, larvae developed faster when reared under high than under low rations. In particular, the failure of $> 50\%$ of larvae to develop past the four-arm stage in low rations implies that a certain food quantity may be required for successful progression through some stages of development (e.g. for *E. lucunter*, the four- but not the two-arm stage). Similar positive effects of increasing food quantity on the rate of larval development have been shown for a number of invertebrate taxa (e.g. George 1994; Basch 1996; Basch and Pearse 1996). For *A. punctulata*, we also found an effect of food quality; development was faster when larvae were reared in mixed- than in single-species microalgal diets. Other studies have shown that mixed-species diets can result in faster growth and earlier settlement than single-species diets for sea urchins and sea stars (e.g.

Table 4 *Arbacia punctulata*. Analysis by log-linear models of independence between the position in the experimental cylinders of four-arm larvae (*P*), and the Diet (*D*) and Halocline (*H*) treatments. Because the models that contained the interactions D × P and H × P provided significant values for *G*, simpler models were examined within each level of the factors D and H. Mean percentage of larvae in each of three pooled positions [below (*B*), at (*H*) and above (*A*) the halocline] are shown for each treatment [*NS* non-significant *P*-values, adjusted for the number of comparisons among all possible pairs of levels of the factor H (critical $G_{0.05[6,\ 2]} = 9.53$); * significant *P*-values, adjusted for the number of comparisons among all possible pairs of levels of the factor H; see "Materials and methods" for treatment abbreviations]

Model	Treatment means		*G*	*df*	*P*
	B:H:A	B:H:A			
D + H + P + [D × P] + [H × P]			15.73	9	0.073
D + H + P + [D × P]			287.3	15	< 0.001
D + H + P + [H × P]			22.98	11	0.018
H + P					
Mixed-high:			139.4	6	< 0.001
21/33 vs 21/30	18:76:6	23:69:8	0.84	2	0.656^{NS}
21/33 vs 24/33	18:76:6	9:24:67	75.28	2	< 0.001*
21/33 vs 27/33	18:76:6	11:11:78	77.66	2	< 0.001*
21/30 vs 24/33	23:69:8	9:24:67	58.48	2	< 0.001*
21/30 vs 27/33	23:69:8	11:11:78	62.96	2	< 0.001*
24/33 vs 27/33	9:24:67	11:11:78	3.52	2	0.172^{NS}
Single-high:			137.2	6	< 0.001
21/33 vs 21/30	15:69:15	13:65:21	1.06	2	0.590^{NS}
21/33 vs 24/33	15:69:15	11:22:67	52.31	2	< 0.001*
21/33 vs 27/33	15:69:15	4:11:85	94.63	2	< 0.001*
21/30 vs 24/33	13:65:21	11:22:67	40.68	2	< 0.001*
21/30 vs 27/33	13:65:21	4:11:85	78.64	2	< 0.001*
24/33 vs 27/33	11:22:67	4:11:85	8.61	2	0.013^{NS}
D + P					
21/33:			3.70	2	0.157
21/30:			6.31	2	0.043
mixed-high vs single-high	23:69:8	13:65:21			
24/33:			0.23	2	0.890
27/33:			2.08	2	0.354

Fenaux et al. 1988; Basch 1996). Larval size varied with dietary conditioning as early as 2 d post-fertilization, and the differences became progressively more pronounced with age. Generally, larvae reared in high rations were longer and wider, and therefore in better nutritional condition, than larvae reared in low rations.

Larvae were less likely to cross the halocline if reared under a better diet, i.e. high ration or mixed-species diet. For *Echinometra lucunter*, differences in size that reflected differences in nutritional condition first occurred 2 or 5 d after fertilization and became statistically significant 5 or 8 d after fertilization (for larval width and length, respectively). We observed an effect of diet on larval vertical distribution around the haloclines in 3- to 6-d-old larvae. For *Arbacia punctulata*, there were significant differences in size 15 d after fertilization, and we detected a dietary effect on vertical distribution in 16- to 19-d old larvae.

Poor dietary conditions have been shown to alter phototactic behaviour in crab zoea (Cronin and Forward 1980), diel vertical migration in scallops (A. Silva personal communication), and substrate selection behaviour in sea star larvae (Basch and Pearse 1996). Dietary conditioning can affect larval shape of echinoids through arm elongation (e.g. Strathmann 1987; Hart and Strathmann 1994), larval density through changes in chemical composition (e.g. Pechenik 1987; Thompson and Harrison 1992) and locomotory activity through changes in ciliary movement (e.g. Strathmann 1987). These changes in turn may alter larval buoyancy and sinking rates, and therefore can affect greatly the passive dispersal of larvae through haloclines. In our study, larvae from all treatments exhibited controlled swimming activity, and very few larvae swam straight through the halocline, suggesting that the larval response to haloclines is the result of active behaviour and not passive dispersal. The observed differences in larval response among diets suggest that nutritional condition probably operates directly on larval behaviour rather than indirectly on larval buoyancy.

Ecological significance

Larval dispersal away from the parental habitat allows for colonization of new sites and genetic mixing, but it also can result in high rates of larval mortality. Thus, it may be beneficial for larvae not to disperse but rather to remain within the water mass overlying suitable settlement sites in the adult habitat. Larvae in later stages of development tend to sink, probably because of increased density, and therefore will remain near the bottom. In contrast, young larvae tend to swim towards the sea surface and are likely to encounter a water mass that can carry them away from the parental habitat. A density

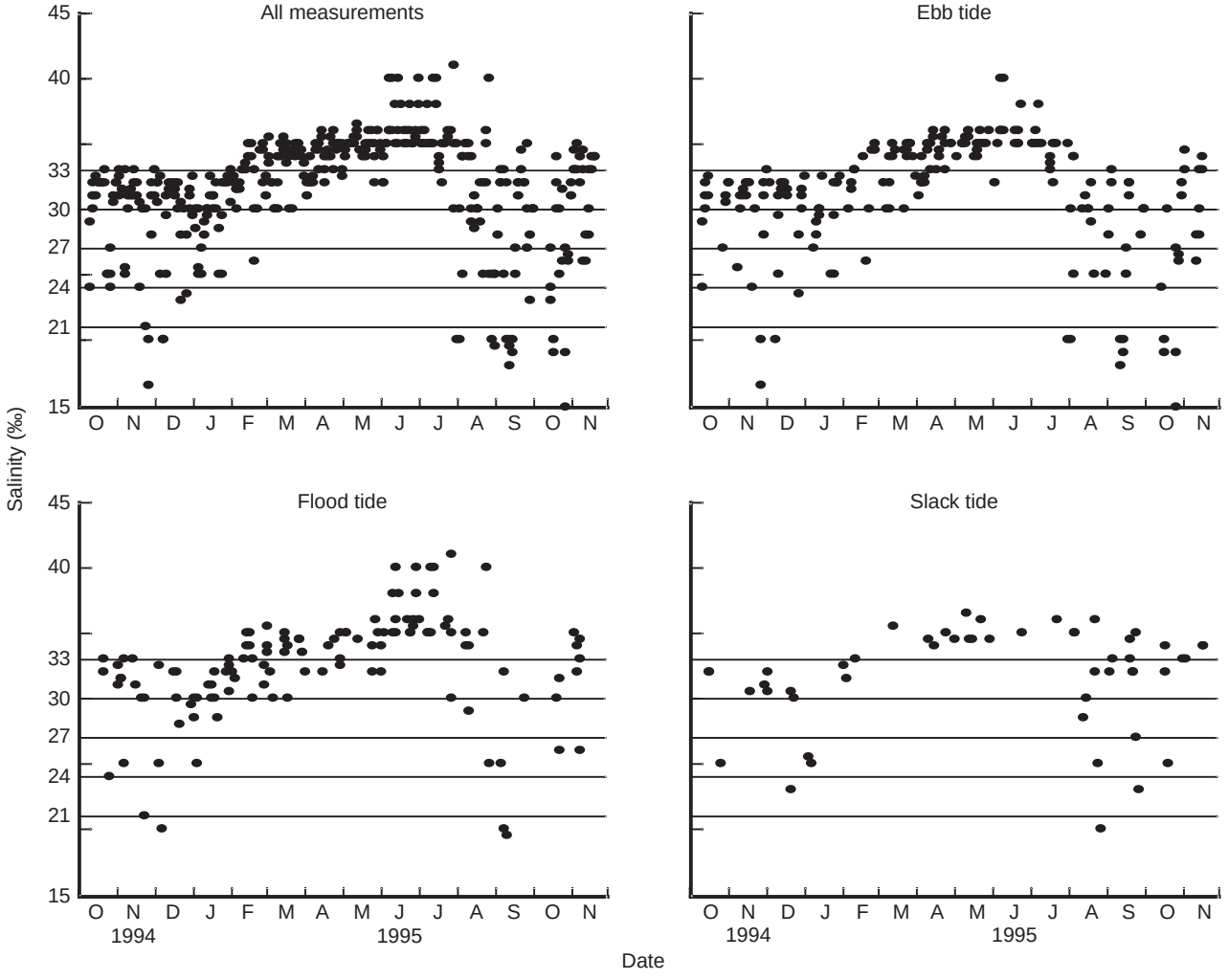

Fig. 8 Surface salinity measurements taken at the south side of Fort Pierce Inlet, Fort Pierce, Florida from October 1994 to November 1995. Values are shown for all tidal conditions combined, and separately for flood, ebb and slack tidal states during sampling. Horizontal lines indicate the values of salinity used in our experiments

discontinuity, such as a halocline, usually characterizes the interface between two water masses. Larval response to such a discontinuity will affect vertical dispersal. We showed that young, well-fed larvae can actively maintain their position with respect to a halocline, and therefore remain within a high-salinity water mass. However, if larvae cross a halocline, they will encounter water of lower salinity which, in addition to carrying them away from suitable habitats, may induce high osmotic stress. The physiological cost because of osmotic stress will increase with decreasing salinity to a threshold lethal value. Above that threshold (which in our study was between 24 and 21‰), other factors such as poor nutritional status also will become important in determining larval survival and behaviour. Poorly fed larvae could actively cross a halocline to increase their probability of survival either by reaching the near-surface more productive area of the water-column or by being introduced into a new water mass that can carry them away from an altogether nutritionally poor environment.

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