# The ups and downs of life in a halocline: The response of *Pisaster ochraceus* larvae to food patches and prior exposure to low salinity

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Salinity fluctuations are expected to become increasingly frequent in the Salish Sea due to glacial melting caused by global warming. *Pisaster ochraceus,* a keystone predator of the intertidal zone will be especially affected by these fluctuations since they lack the ability to ion- or osmo-regulate. We examined the impact of prior exposure to low salinity and the presence of a food patch on the behavior of *P. ochraceus* larvae in a halocline. In the gastrula stage, *P. ochraceus* larvae were split into a low salinity treatment (reared in 20-22‰ filtered sea water) and a control treatment (reared in 30-32‰ filtered sea water). In the first experiment, bipinnariae were introduced into the top or bottom of 20/30 haloclines. In the second experiment brachiolariae were introduced into control columns with and without the algae *Isochrysis galbana,* and into 20/30 haloclines with and without *I. galbana* patches at the halocline. The halocline posed a major barrier to larval vertical migration, especially for the younger bipinnaria stage. Low salinity larvae changed their distribution in the water column at a much slower rate than control larvae, indicating that they may have impaired swimming abilities. Brachiolariae from the controls aggregated in the halocline when a food patch was present but those from low salinity swam right through the food patch to the top of the column. Lowered salinity in the Salish Sea could result in larvae arriving in unsuitable habitats since their different vertical distribution in the water column may result in currents carrying them to unsuitable habitats. Smaller adult *P. ochraceus* populations in the rocky intertidal could result in drastic changes to the ecosystem including a reduction in species diversity.

## Introduction

Global warming will cause drastic changes in the world's oceans, including the expansion of the oceans, rising ocean temperatures, changing ocean circulation, and decreasing oceanic pH (Harley et al. 2006). Changes to the terrestrial environment will also have significant consequences for marine ecosystems. An important example is glacial melting, which will result in increased freshwater runoff into the ocean. Freshwater enters the Salish Sea from 14 different rivers, the most important of which is the Fraser River in Canada (Khangaonkar et al. 2011). This influx of freshwater can result in the formation of a halocline, where a layer of less dense, lower salinity water rests on top of the greater mass of denser ocean water. Haloclines commonly form in the Salish Sea of the Pacific Northwest with a 5-10m thick layer of brackish water (~20‰) resting above the saltier lower layer (~30‰) (Khangaonkar et al. 2011).

Haloclines pose a challenge to sea stars such as *Pisaster ochraceus,* a keystone predator of the intertidal zone. Sea stars are exposed to a wide range of salinities (Stickle and Diehl 1987), yet they lack the ability to ion- or osmo-regulate (Held and Harley 2009). Thus, adult *P. ochraceus* actively avoid regions of low salinity (Garza and Robles 2010). In addition to affecting adult sea stars, haloclines also have major implications for planktonic sea star larvae. The effect of these haloclines on the vertical position of larvae in the water column will determine where they are swept horizontally by currents, thereby influencing the density and distribution of adult populations (see Miller and Emlet 1997). In the case of *P. ochraceus,* this would have great consequences for the structure of the intertidal ecosystem as a whole.

Previous studies have shown that plankton aggregate in the water column in response to haloclines (e.g. copepods, Lougee et al. 2002). A study by Arellano et al.

(2012) found that sand dollar larvae behaviorally respond to the low salinity environment of a sharp halocline by aggregating in or below the halocline and a study by Vazquez and Young (1996) found that ascidian larvae actively avoid regions of low salinity in a halocline. Very few studies have looked into the behavior of sea star larvae in haloclines. Since sea stars are voracious predators and, in some cases (including *P. ochraceus*) keystone predators, it is vitally important to understand the behavior of their larvae in haloclines.

A study by Lee and George (2011) found that *P. ochraceus* larvae in a simulated halocline (a layer of 20‰ over 30‰ seawater) first cluster around the halocline and then gradually move to the top of the water column, presumably in search of food. Recent studies have discovered morphological differences between *P. ochraceus* larvae reared at 20‰ and 30‰ seawater. *P. ochraceus* larvae exposed to 20‰ for 7 or 14 days develop into wider and shorter brachiolariae than larvae reared at 30‰ (Pia et al. 2012). Very few studies have looked into the differential behavior of these larval morphs in haloclines. An exception is the study by Lee and George(2011), which found that *P. ochraceus* larvae exposed to low salinity throughout development were less able to actively avoid unfavorable salinities. A question not addressed in Lee and George's study is which salinity these low salinity reared larvae prefer. If they were raised in 20‰ seawater and have adapted morphologically to 20‰ seawater, it is possible that they would prefer the salinity to which they have acclimated rather than always preferring natural 30‰ seawater.

*P. ochraceus* larvae must swim actively in order to maintain their position in the water column so they must feed on algae to meet their energy demands. Since maximum

light exposure occurs at the top of the water column, it would make sense for larvae to gradually move upwards when looking for food. If the larvae encounter food before reaching the surface, it follows that many larvae would stop at the food patch rather than continue swimming to the surface of the column. A study by Sameoto and Metaxas (2008) found that more sea star (*Asterias rubens*) and mussel larvae aggregated at a halocline when a food patch was present in the water column and Metaxas and Young (1998) found that fewer sea urchin larvae swim to the top of a halocline when a food patch is present. No studies of this kind have been conducted on *P. ochraceus.*

Most studies on larval behavior in haloclines stop after less than an hour of observation. In order to truly understand the behavior of larvae in haloclines, it is vitally important to conduct more long-term studies to fully comprehend where in the water column they will end up.

In this study, we will observe the behavior of low salinity reared larvae and control larvae in 10/20 and 20/30 haloclines with and without food patches for a period of 24 hours. While 20/30 haloclines occur naturally in the Salish Sea (Khangaonkar et al. 2011), the 10/20 halocline will enable us to determine whether larvae prefer the salinity they have acclimated to, regardless of whether it is above or below the halocline.

In this study we addressed the following questions: 1) Is the halocline a barrier to larval movement? 2) Do larvae raised in low salinity water behave differently in a halocline over time than those raised in normal salinity ocean water? and 3) would the presence of a food patch affect larval behavior in a halocline?

In response to these questions we developed 3 main hypotheses. 1) Since previous studies have shown that the halocline is an effective barrier to planktonic vertical

migration (Harder 1968, Lougee et al. 2002), we hypothesize that larvae introduced above or below a halocline will have trouble passing through to the other side. 2) Since larvae raised in lower salinity develop slower and are morphologically distinct from control larvae (Pia et al. 2012), their swimming abilities may be impaired and their behavior may be different. Therefore, we predict that the vertical distribution of low salinity reared larvae will differ from that of control larvae. 3) Since the search for food is presumably the explanation for why larvae swim upwards in water columns, larvae in columns with food patches or dispersed food will stay in the food region and not bother swimming further upwards than necessary.

## Methods

### Collection and spawning of organisms

Eight Adult *Pisaster ochraceus* were collected in front of Friday Harbor Laboratory on San Juan Island, Washington (48° 32′ 46″ N, 123° 0′ 46″ W) on June 8th, 2012 during a -1.5 ft low tide. They were maintained in a tank of flowing seawater until spawning. Two days after collection, 7 sea stars were injected with 2ml of $10^{-4}$M 1-methyl adenine to induce spawning. The first adult spawned 1.5 hours after injection and the last adult finished spawning 3 hours after that. Gametes were collected and examined under the microscope. In total, 2 females and 1 male spawned. Eggs were fertilized naturally within the adults' tank with 99% fertilization success. The average egg diameter for the first female was 154 +/-7.45 μm and for the second female it was 159 +/- 6.30 μm. Zygotes were divided equally among 4 stock jars and 2000ml of 0.45μm filtered seawater was added to each jar.

Two days later, swimming embryos were collected and observed under the microscope. All swimming embryos were at the gastrula stage. Three samples were taken to calculate the number of swimming embryos. The first sample had eight 5ml subsamples ranging from 8-16 embryos, the second, six 5ml subsamples ranging from 6-14 embryos, and the third, five 5ml subsamples ranging from 29-36 embryos. From these 3 samples, the number of healthy embryos was calculated to be approximately 24,285.

Experimental Design

The healthy embryos were divided equally into 2 treatments (low salinity and control) with 6 replicates per treatment. Each replicate was housed in a 1-gallon jar. Embryos in the low salinity treatment (hereafter referred to as LS) were reared in 20-22‰ water (made by mixing 0.45µm filtered seawater with reverse osmosis water) and embryos in the control treatment were reared in 30-32‰ 0.45µm filtered seawater. All 12 jars were placed in a 78.7 X 129.5 X 16.5 centimeter tank filled with flowing seawater (pumped directly from the ocean) to maintain a mean temperature of 12.5°C with a range of 11-14°C.

At 2 days old, all embryos were fed an algal combination of *Rhodomonas*, *Isochrysis galbana,* and *Dunaliella tretiolecta*. The embryos and food were kept in suspension by a system of swinging paddles (Strathmann 1987).

Algal Cultures

Three algal species (*Rhodomonas sp.*, *Isochrysis galbana,* and *Dunaliella tertiolecta*) were grown in the lab as food for the larvae. Algal cultures were kept under florescent light and used in their exponential phase. Before feeding, algal cells were measured using an ocular micrometer and counted using a hemocytometer under the

microscope. Based on these data, we then calculated the amount of each algal culture to add to each jar. Both treatments received equal food. Larvae were fed 4000-17,500 cells/ml *I. galbana*, 625-4435 cells/ml *Rhodomonas sp.*, and 1100-3000 cells/ml *D. tertiolecta*. These concentrations are similar to those used by Schiopu et al. (2006) and George et al. (2008).

Halocline Setup

Haloclines were prepared in cylindrical columns 45cm tall with an inner diameter of 10.3cm. Needles connected to eight 3-way valves were attached at depths 40, 35, 30, 25, 20, 17.5, 15, and 10 cm to take water samples.

Control water columns were established by pouring 40cm of 30‰ or 20‰ filtered seawater into the column. Haloclines were established by first pouring 12.5cm of low salinity water into the bottom of the column, then gravity feeding 22.5cm of high salinity water into the bottom (up to the 5cm mark) via 1/8 X 1/4 inch tubing at a slow rate to prevent mixing. The columns filled up to the 5cm mark in about 1 hour. Gravity feeding was accomplished using a PVC tube with 2 clamps on it: one to function as an on/off switch and one to regulate flow speed (figure 1). For halocline columns containing food, *I. galbana* cultured in 24.5‰ FSW was gravity fed into the bottom of the water column after the addition of low salinity water but before the addition of high salinity water. For control columns containing food, *I. galbana* cultured in either 30‰ FSW or 20‰ FSW (same salinity as the column) was stirred into the water column at the same time as food was gravity fed to haloclines in the experiment. The columns were kept in a sea table with flowing seawater to maintain a constant temperature of ~12-13°C. However, columns were only submerged in the sea table up to a 20cm depth, so a thermocline

formed (table 1). An experiment was run comparing the distribution of larvae in haloclines with this thermocline and in haloclines in a cold room with a constant temperature of 10°C. There were differences among replicates but not between the presence or absence of a thermocline.

Experiment 1: Introduction of larvae at the top or bottom of haloclines

Control larvae (reared in 30-32‰ FSW) were introduced into 3 different halocline treatments with 2 replicates per treatment: a control water column (30‰) with larvae introduced into the bottom a 20/30 halocline with larvae introduced into the bottom, and a 20/30 halocline with larvae introduced into the top. Low salinity reared larvae (reared at 20-22‰) were introduced into 4 different halocline treatments with 2 replicates per treatment: a control water column (30‰) with larvae introduced into the bottom, a 20/30 halocline with larvae introduced into the bottom, a 20/30 halocline with larvae introduced into the top, and a 10/20 halocline with larvae introduced into the bottom. Columns with a 20/30 halocline and larvae introduced at the top will hereafter be referred to as 20/30 top columns while 20/30 columns with larvae introduced at the bottom will hereafter be referred to as 20/30 bottom columns (figure 2).

At least an hour after haloclines were established, ~100 24-29 day old {stage XI late bipinnaria (George 1999)} *P. ochraceus* larvae (figure 3) in 150 ml of FSW were gravity fed into the bottom of the column using the same tube previously used to drip in high salinity water. For control columns, a new tube was inserted for this purpose. Larvae were counted in each vertical section in 5cm intervals except around the halocline where they were counted in two 2.5cm increments at 1, 3, 7, 19, and 24 hours after larvae were introduced. Thirty seconds were allotted for each count of a 5cm section, so the 15-

17.5cm and 17.5-20cm sections were each counted in 15 seconds. To count larvae, the columns were placed in a small chamber coated in black plastic, lit from behind with a fluorescent light to improve visibility. Salinity was measured with a portable refractometer at 1, 7, and 24 hours after the introduction of larvae by taking water samples from each valve in the column.

Experiment 2 Effect of food patch on the vertical distribution of larvae

Control larvae were introduced into 4 different halocline treatments: a control column (30‰) with no food, a control column (30‰) with *I. galbana* dispersed throughout, a 20/30 halocline with no food, and a 20/30 halocline with a food patch (initially $2.3*10^6$ cells/ml *I. galbana*) at the halocline. Low salinity reared larvae were introduced into the same 4 halocline treatments except control columns were 20‰ FSW. All larvae were introduced into the bottom of the column (figure 3).

At least an hour after haloclines were established, ~100 34-36 day old *P. ochraceus* brachiolariae (figure 5) in 150 ml of FSW were gravity fed into the bottom of the column using the same tube previously used to drip in high salinity water. For control columns, a new tube was inserted into the water column at this point to add larvae. Larvae were counted in each vertical section as in experiment 1 at 1, 7, 19, and 24 hours after larvae were introduced. Salinity was measured with a portable refractometer at 1 and 24 hours after larvae were introduced. Samples of 5 larvae were taken from the halocline in all columns with haloclines at 7 hours and 24 hours after introduction. These larvae were then observed under the microscope to determine whether they had empty or full stomachs.

Statistical Analysis

Based on the location of the halocline, each column was divided into 4 sections for statistical analysis: top, halocline, below and bottom. To determine whether vertical distribution of larvae differed between columns, we ran Pearson chi squared tests with JMP 9.0. In experiment 1, there was no significant difference between replicates in 60% of measurements. In experiment 2, there was no significant difference between replicates in 62.5% of measurements.

## Results

Experiment 1: Introduction of larvae at the top or bottom of haloclines

The distribution of larvae in halocline columns differed significantly from the distribution in control columns 96% of the time (figure 6,7).

### Comparison of halocline treatments

The distribution of low salinity (LS) and control larvae in 20/30 haloclines differed significantly depending on whether they were introduced at the top or bottom of the column ($p<0.0001$). Larvae introduced at the bottom swam up to the halocline and stayed there while those introduced at the top sank down to the halocline then swam back up to the top of the water column (figure 6, 7).

The vertical distribution of LS larvae in 10/20 haloclines differed significantly from the distribution in 20/30 top haloclines 100% of the time ($p<0.0001$) and from 20/30 bottom haloclines 60% of the time ($p<0.003$). For larvae introduced into the bottom of the water column, those in 20/30s moved up faster than those in 10/20s and fewer of the larvae in 10/20s swam up to the halocline than those in 20/30s. Larvae in 10/20 haloclines moved up to the halocline slower than those in 20/30 bottom treatments and

fewer larvae in 10/20 treatments swam up to the halocline than those in 20/30 bottoms (figure 6).

**Comparison of LS and Control Larvae**

In control columns, larval distribution was significantly different between LS and control larva treatments ($p<0.0001$). Control larvae moved upwards in the column faster than LS larvae and 78.5% made it to the top after 24 hours, compared to 23.3% for LS larvae (figure 6, 7).

In 20/30 bottom treatments, the distribution of LS larvae was significantly different from that of control larvae until ($p<0.0059$) 24 hours after introduction ($p=0.089$). Control larvae moved into the upper sections of the water column faster than LS larvae, but the LS larvae caught up by 24 hours when 72% of control larvae and 84% of LS larvae were at the halocline (figure 6,7).

LS larvae in 20/30 top water columns were distributed significantly differently than control larvae at 1, 3, and 7 hours after introduction ($p<0.0001$) but there was no significant difference at 19 and 24 hours ($p=0.4161$ and $p=0.0854$ respectively). More control larvae swam down to the halocline than LS larvae and they stayed there longer before returning to the top of the water column. A few (12.5%) LS larvae swam to the bottom of the water column but they migrated upwards by 19 hours (figure 6, 7).

<u>Experiment 2 Effect of food patch on the vertical distribution of larvae</u>

For haloclines with and without food, the distribution of larvae differed significantly from control columns 87.5% of the time (figure 8, 9).

**Comparison of halocline treatments**

For both LS and control larvae, the vertical distribution within haloclines was significantly different in columns with and without food patches ($p<0.0209$). Larvae in food columns generally stayed in the lower regions of the water column for a while before swimming to the top of the column. By 7 hours, more LS larvae were distributed to the top of the water column in columns with food than in columns without food. By 19 hours, the distribution of control larvae held the same pattern (figure 8, 9).

LS and control larvae in haloclines without food aggregated in the halocline before breaking through to the top section of the water column (figure 8, 9).

**Comparison of control and LS larvae**

The vertical distribution of LS larvae was significantly different from that of control larvae in control columns without food ($p<0.0145$). LS larvae generally lagged behind control larvae in swimming to the top of the column (figure 8,9).

The distribution of LS larvae was significantly different from that of control larvae in control columns with food ($p<0.0038$). The majority of LS larvae were initially at the bottom of the column while control larvae were scattered throughout. After 7 hours, the majority of LS larvae had migrated to the top. A majority of control larvae also swam to the top, but not as large a proportion as the LS larvae (figure 8,9).

LS larvae were distributed significantly differently ($p<0.0233$) than control larvae in haloclines columns without food in all measurements except 7 hours ($p=0.3615$). Initially, many more LS larvae had made it to the top of the water column, but by 7 hours control larvae had caught up and in the 19 and 24 hour counts, there were many more control larvae in the top of the water column (figure 8,9).

The distribution of LS larvae was significantly different from that of control larvae in halocline columns with a food patch ($p<0.0001$). Control larvae immediately swam up to the halocline and stayed there for almost the entire experiment, although 31% had moved to the top by 24 hours. However, LS larvae seemed to swim right through the halocline food patch and congregate at the top. At the 1 hour measurement 37% were in the halocline, and at the 7 hour measurement 20% were at the halocline, but only 10% remained by 19 hours (figure 8, 9).

**Observations of larval feeding in haloclines**

LS and control larvae in haloclines with no food had empty stomachs at 7 and 24 hours after introduction.

Control larvae in haloclines with food had stomachs packed full of food 7 hours after introduction. Most LS larvae in haloclines with food had stomachs filled with food, but they appeared to have eaten less than control larvae.

Both LS and control larvae in haloclines with food had empty stomachs 24 hours after introduction.

## Discussion

**The halocline is a barrier**

Our results supported the hypothesis that the halocline is a barrier to the vertical migration of *P. ochraceus* larvae. Very few of the larvae introduced either above or below the halocline passed through to the other side. This was true of both LS and control larvae, controlling for any bias the larvae may have had for the salinity they were reared in. My results are similar to those of Harder (1968). Harder found that a wide diversity of planktonic organisms aggregate at haloclines, and that the primary factor driving this

behavior is the density gradient associated with haloclines: in columns with a salinity gradient but no density gradient, organisms did not aggregate at the halocline. It is very likely that the density gradient in our haloclines, due to the density difference between 20‰ and 30‰ water, was a major factor inhibiting larval movement, in addition to the salinity stress associated with a region of rapid salinity change

Larvae in control columns with no halocline appeared to gradually move to the top of the water column. This is consistent with the results of many other studies which have found that echinoderm larvae aggregate at the top of the water column in the absence of other cues (Lee and George 2012, Metaxas and Young 1998, Burdett-Coutts and Metaxas 2004, Sameoto and Metaxas 2008, Sameoto et al. 2008). Since larvae in control columns aggregated at the top of the column while those in 20/30 bottom columns stopped at the halocline, this further confirms our hypothesis that the halocline is a barrier to larval vertical migration.

LS larvae introduced into 10/20 columns lagged behind larvae in all other halocline treatments in swimming to the top and only a few individuals even made it to the top section. It seems clear that these larvae actively avoid water of very low salinity. Many of them even seemed to avoid the halocline region, preferring to remain in the lower regions of the water column. This column was meant to test whether the larvae prefer the salinity they have acclimated to but the halocline proved to be such a strong barrier and the larvae disliked 10‰ water so strongly that this question could not be addressed. A better way to answer this question would be to set up 2 different 20/30 halocline treatments with no change in density by adding sucrose or another non-electrolyte to the 20‰ water (after Harder 1968). Larvae would be added to the top of

one treatment and to the bottom of the other. The salinity in which most larvae aggregate would indicate the one they prefer the most.

**LS vs control larvae**

Throughout all halocline treatments, control larvae changed position in the water column much faster than LS larvae, supporting our hypothesis that their vertical distributions would differ. In control columns and 20/30 bottom columns, control larvae moved to the upper regions of the water column faster than LS larvae. These results agree with those of Lee and George (2011), who found that larvae exposed to low salinity during development are slower than control larvae in making it to the surface of halocline and control columns. In 20/30 top columns, a greater number of control larvae migrated down to the halocline and stayed there for longer. The results of the 20/30 top column may have been confounded by other variables since the larvae seem naturally inclined to swim upwards. The LS larvae didn't have much reason to swim downwards since they had been raised in the 20‰ water they were currently immersed in and since they prefer moving upwards to downwards. Control larvae may have swum downwards in order to find higher salinity water.

Since LS larvae in 20/30 bottom columns were introduced into higher salinity water than they were used to, and since they apparently have a natural desire to swim upwards, one would expect them to have a greater impulse to swim upwards than control larvae, which were only motivated by the natural desire to swim upwards. Yet it was the control larvae that first moved into the halocline. This suggests that the swimming abilities of LS larvae are impaired. Previous studies (Pia et al. 2012, Lee and George 2011) have shown that larvae raised in low salinity are morphologically distinct from

those raised in normal salinity ocean water. Larvae exposed to lower salinity water are significantly wider and shorter than controls (Pia et al. 2012). It is likely that this altered morphology results in the impaired swimming abilities of LS larvae. Larvae raised in lower salinity may grow wider and shorter as a physiological adaption enabling them to survive lower salinity seawater but the downside seems to be an impaired swimming ability.

**The effect of food on larval distribution**

It should be noted that the initial concentration of cells in halocline food patches ($2.3*10^5$ cells/ml) was greater than that used in most other studies, with the exception of a study by Menden-Deuer and Grünbaum (2006) in which they introduced a layer of $10^4$ cells/ml *I. galbana*.

Control larvae in haloclines and control columns and LS larvae in control columns remained in the food region rather than expending excess energy swimming upwards when they were surrounded by food, supporting my hypothesis that larvae will hang around food in the water columns rather than swimming upwards. Control larvae in haloclines with food patches swam up to the food patch and stayed there for almost 24 hours, although by 24 hours some of them had swum to the top. Larvae in control columns with dispersed food stayed in the bottom regions of the column for a while before swimming upwards, although once they decided to swim upwards, larvae in food columns had greater numbers in the upper sections than larvae in control columns without food. My results agree with previous studies which have found that zooplankton aggregate in food patches [sea urchin larvae, Burdett-Coutts and Metaxas 2004; sea star

(*Asterias rubins*) larvae, Sameoto and Metaxas 2008; protists, Menden-Deuer and Grünbaum 2006).

However, LS larvae in haloclines with food patches did not perform as expected. They swam right to the top of the water column, and greater numbers of larvae in haloclines with food patches made it to the top than did larvae in haloclines without food patches. A possible explanation is that the LS larvae prefer the 20‰ water they've acclimated to and their need to return to 20‰ water is initially stronger than their desire to feed. In haloclines without food, more LS than control larvae were at the top after 1 hour, but by 7 hours the control larvae had caught up and by 19 hours more control larvae were at the top since some LS larvae had migrated back down to the halocline. Since LS larvae in haloclines with food patches did feed, it is likely that they fed as they swam upwards, relieving the immediate need for food and thus allowing all larvae to return to 20‰ water. But LS larvae in haloclines without food did not have the opportunity to feed as they swam upwards, so after the majority had reached the top of the water column, their need for food overpowered their desire to remain in 20‰ water and they swam back down to the halocline in search of food.

**Response of *P. ochraceus larvae to haloclines***

*P. ochraceus* bipinnariae and brachiolariae both aggregated at the halocline. Planktonic aggregation at halcoclines is a widespread behavior described by many other researchers (copepods, Lougee et al. 2002; sand dollars, Arellano et al. 2012; zooplankton, Harder 1968; sea urchins, Metaxas and Young 1998).

However, brachiolariae seemed more able to break free of the halocline and swim to the top of the water column than bipinnariae since greater proportions of brachiolariae

made it to the top of the water column in halocline treatments. This is likely due to the improved swimming ability of larvae as they grow older, larger, and stronger. These results are consistent with those Arellano et al. (2012) who found that sand dollar embryos had more trouble passing through density gradients than larvae.

Since the halocline is a barrier to larval vertical migration, larvae above the halocline may be stuck there until the two water layers mix and the halocline dissipates. Larvae stuck above the halocline may be temporarily separated from food if food patches are present below the halocline. To investigate this implication further, future studies with *P. ochraceus* larvae in water columns with food patches should be conducted. In particular, a study should be conducted with a food patch below the halocline and larvae introduced above the halocline, to see if they can cross through the density barrier in response to a food cue. Also, the reciprocal situation should be tested, with food above the halocline and larvae introduced below the halocline to test in what direction larvae can most easily pass through the halocline in response to food. It would be particularly interesting to conduct this study with many different stages of *P. ochraceus* larvae in order to discover at which developmental stage they are first capable of swimming through a halocline.

My results show that LS larvae are distributed differently in the water column than control larvae. Since the larvae will be at a different vertical positions in the wild, they will be swept in different directions by the currents (see Miller and Emlet 1997) and will metamorphose in different, potentially unsuitable, habitats.

In addition, larvae raised in lower salinity seem to be weaker swimmers than their counterparts reared in normal salinity ocean water. Weaker larvae may be less able to

migrate to food patches and will likely develop even slower than they normally would (Pia et al. 2012). These slower developing larvae will be floating around in the currents for a longer time than usual and will therefore have a small chance of ending up in a suitable habitat.

With global warming and the expected drastic changes to the world's oceans (Harley et al. 2006), many ecosystems and organisms will be affected. As global warming melts glaciers and the runoff decreases the salinity of estuarine systems like the Salish Sea, organisms will be forced to live in lower salinity water. This study indicates that changes to *P. ochraceus* larvae as a result of lowered salinity may result in smaller larval recruitment to the rocky intertidal of the Pacific Northwest. Since *P. ochraceus* is a keystone predator of this ecosystem, a reduced population will result in drastic changes to the whole ecosystem. A study by Paine (1966) showed that the artificial removal of *P. ochraceus* from the intertidal zone resulted in a marked decrease in species diversity as organisms such as mussels and gooseneck barnacles took up most of the space, leaving little room for other creatures. If global warming continues melting glaciers unabated, we can expect to see a decrease in species diversity throughout the intertidal zones of the Pacific Northwest as *P. ochraceus* larvae are taken elsewhere by currents and fewer juveniles are recruited to suitable habitats.

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## Tables and Figures

**Table 1.** Temperature range of halocline columns partially submerged in the sea table.

| Depth (cm) | Temp (°C) |
|---|---|
| 10 | 16 |
| 15 | 16 |
| 17.5 | 15 |
| 20 | 15 |
| 45 | 13 |

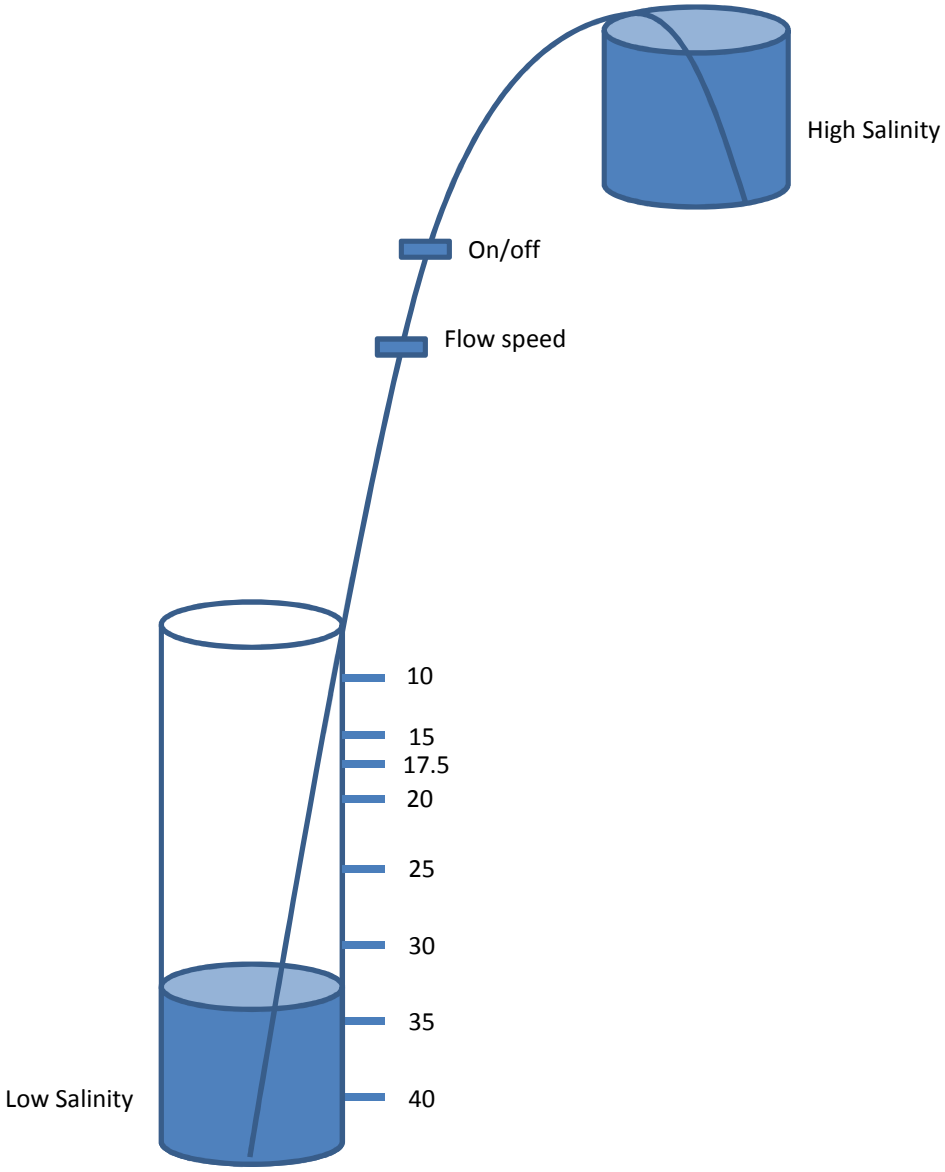



**Figure 1.** Diagram of the halocline setup. Numbers on the side of the halocline column indicate depths in centimeters. High salinity water was gravity fed below the layer of low salinity water.

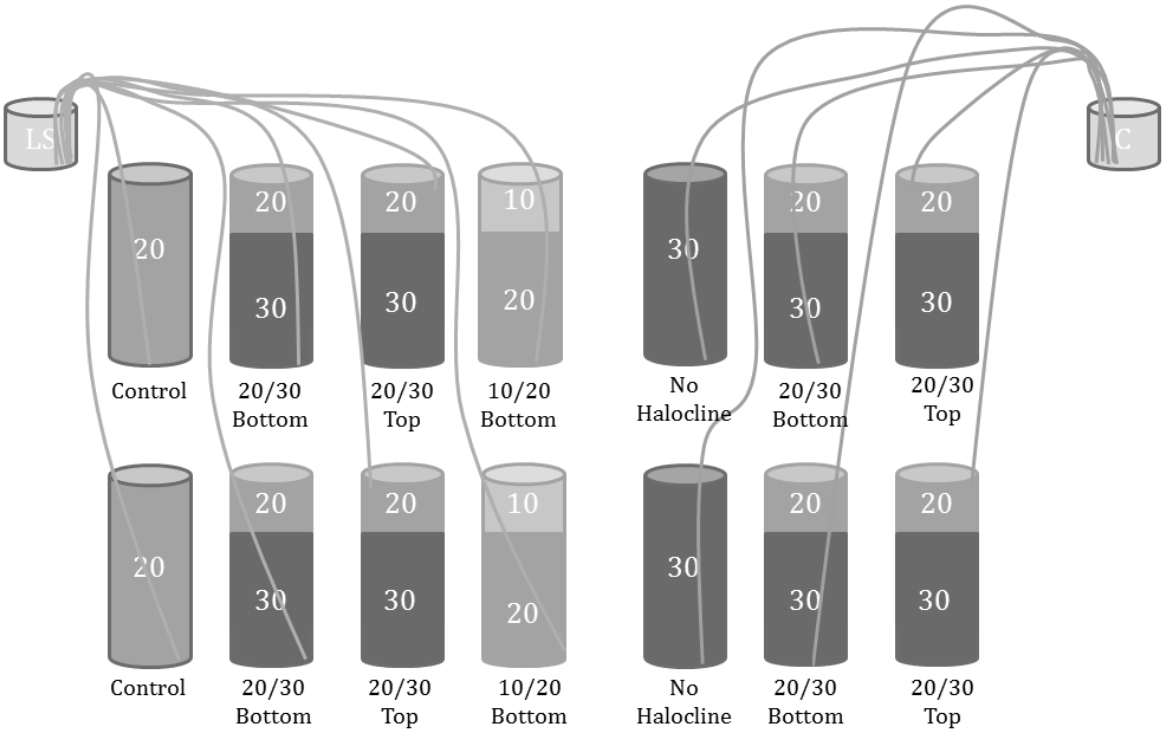



**Figure 2.** Experimental design for experiment 1. Numbers represent salinities in parts per thousand.

**a** **b**

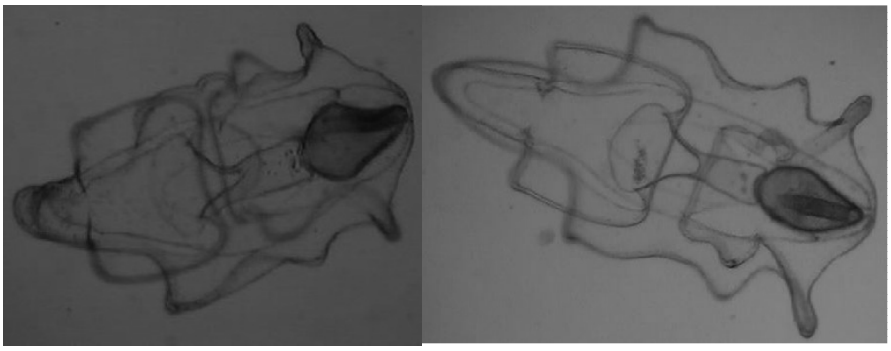


**Figure 3.** Twenty-three day old a) LS and b) control *P. ochraceus* bipinnaria.

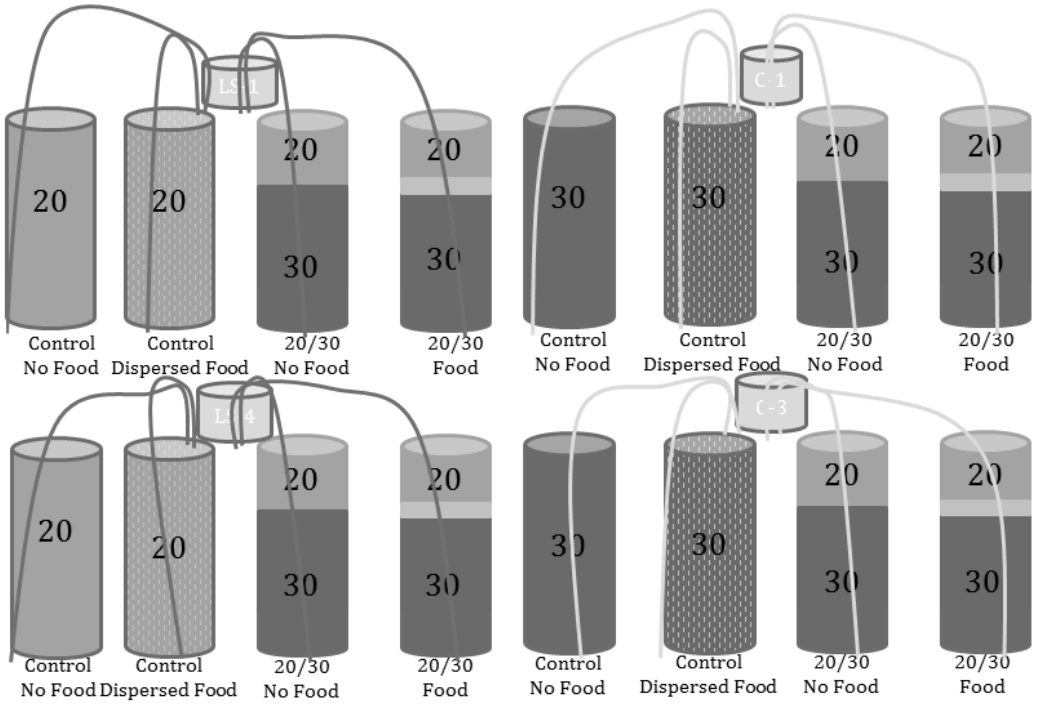
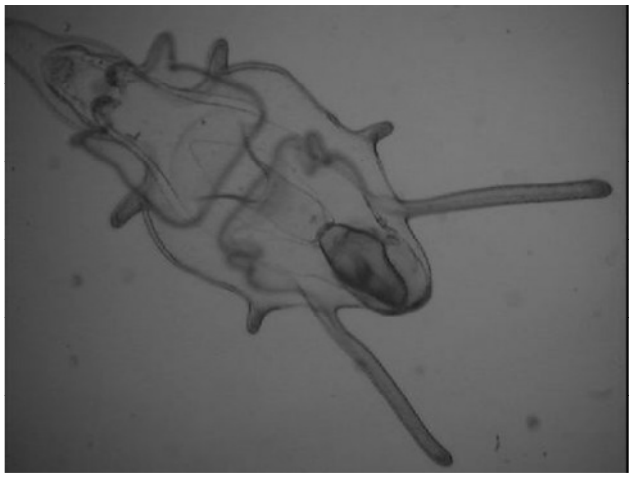



**Figure 4.** Experimental design for experiment 2. Numbers indicate salinities in parts per thousand. Bands at the halocline or dots in the column indicate the presence of *I. galbana.*

**a**



**b**

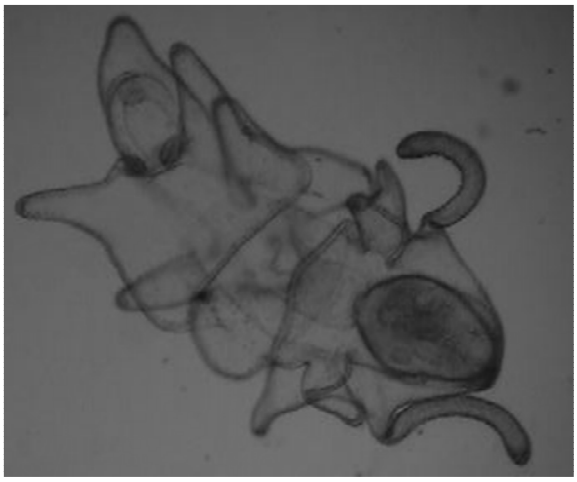
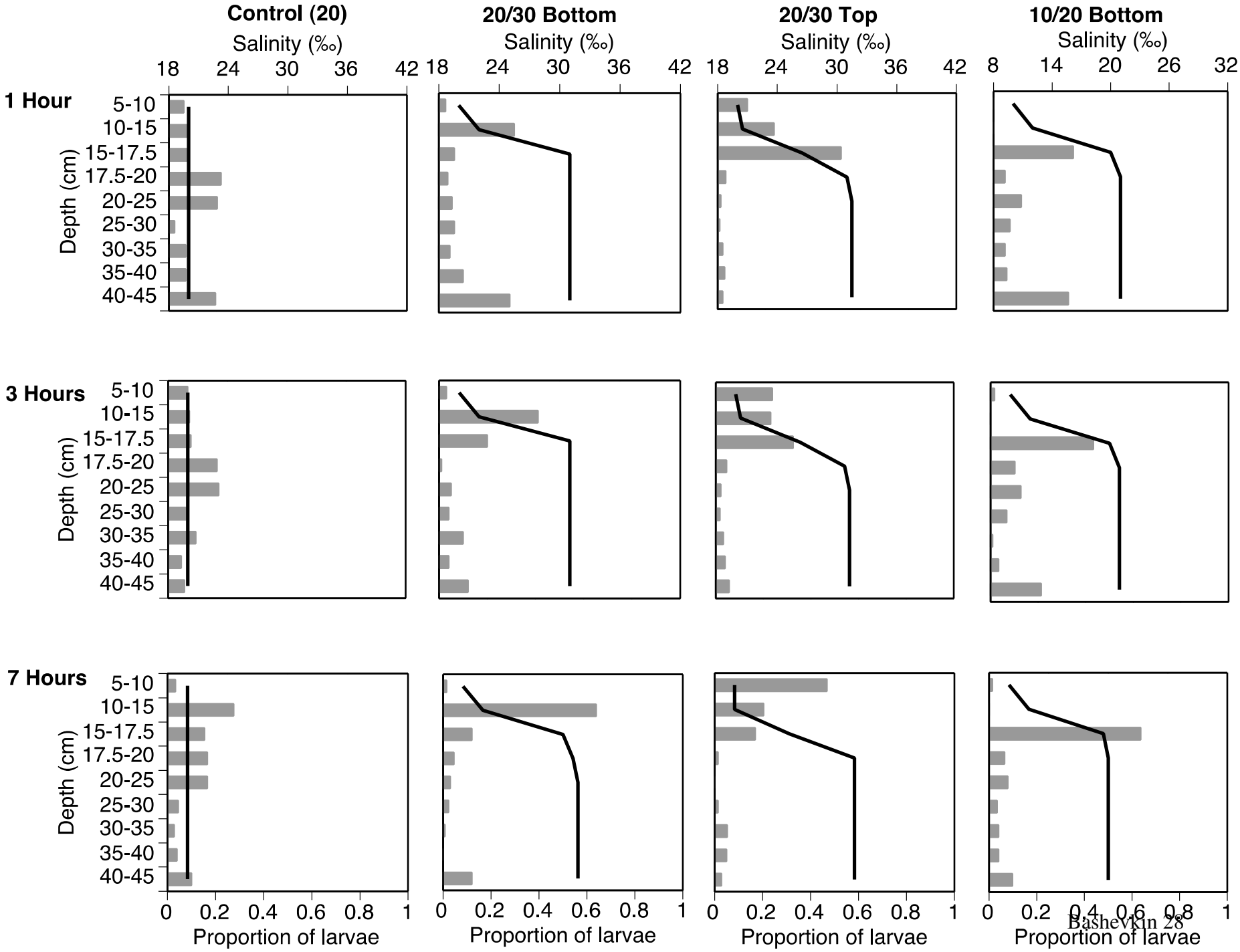


**Figure 5.** a) 34 day old control and b) 35 day old LS *P. ochraceus* brachiolariae.


Control (20)
20/30 Bottom
20/30 Top
10/20 Bottom
Salinity (‰)
1 Hour
3 Hours
7 Hours
Depth (cm)
5-10
10-15
15-17.5
17.5-20
20-25
25-30
30-35
35-40
40-45
Proportion of larvae

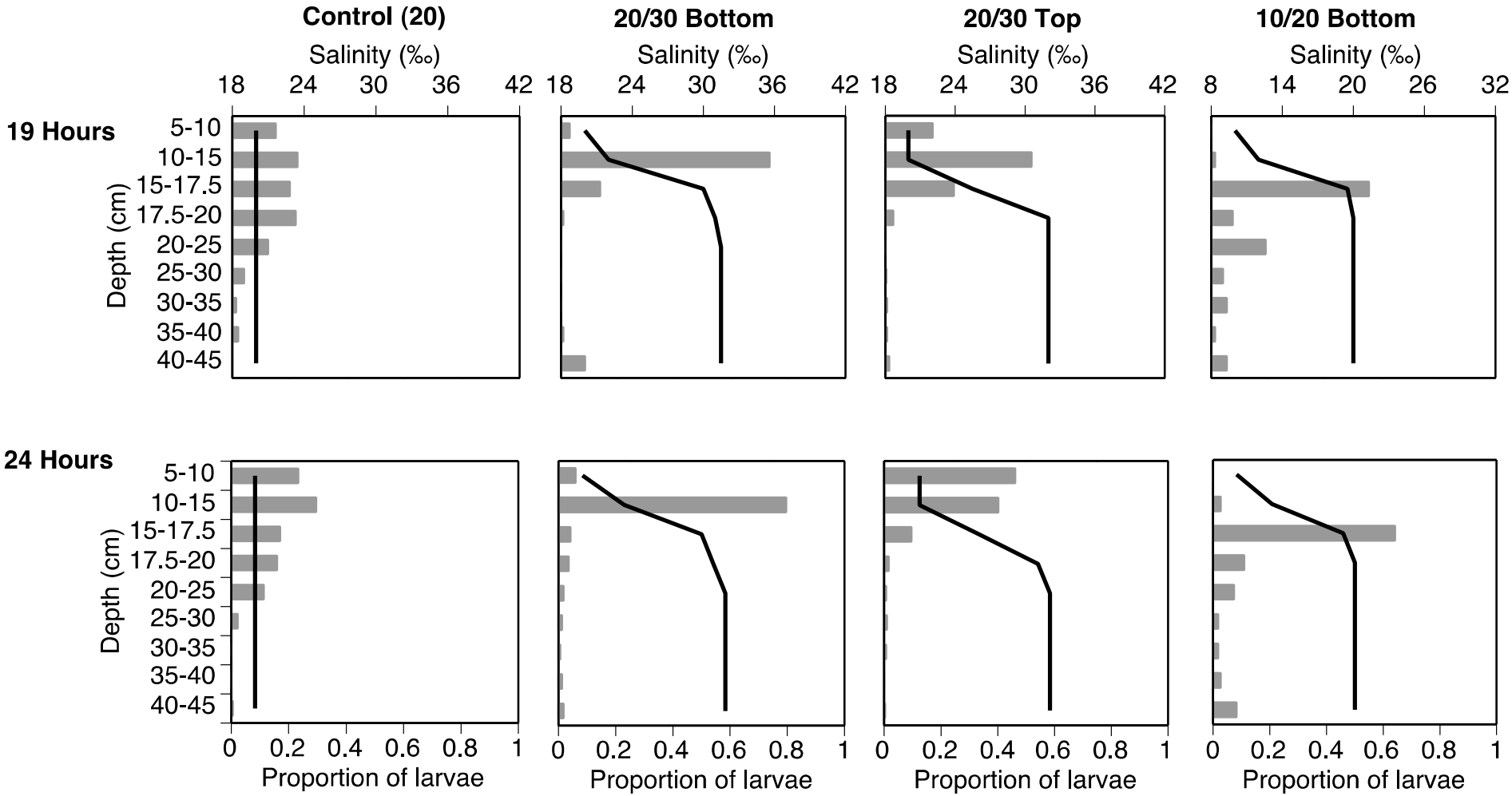
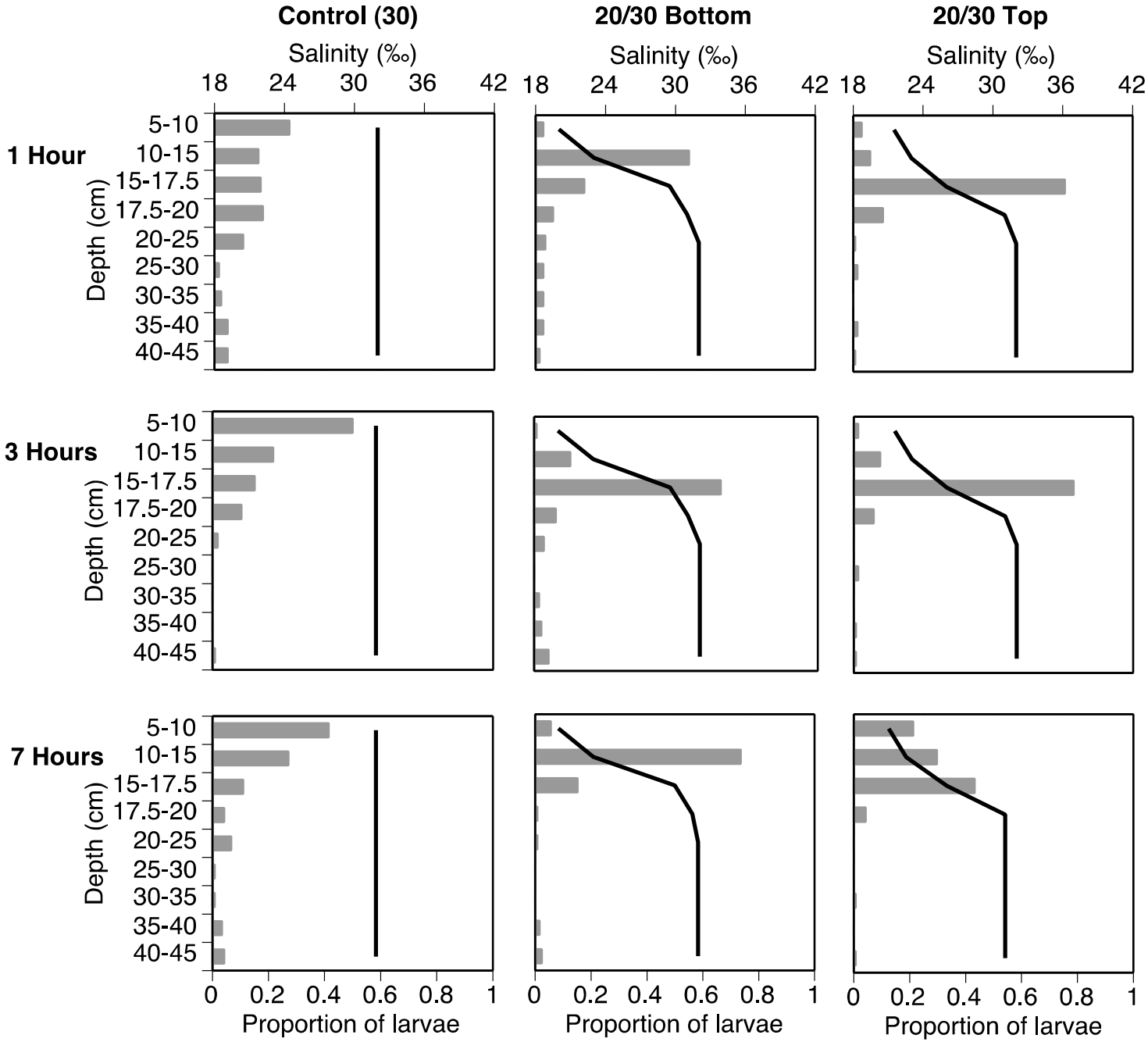



**Figure 6**. Distribution of low salinity *P. ochraceus* larvae after introduction to the top or bottom of a halocline. Bipinnaria were 24-27 days old. Larvae were raised in 20-22‰ FSW. Each graph consists of the combined results of 2 replicate columns. Between 50 and 170 larvae were introduced into each column at least an hour after creation of the halocline. The line represents salinity measurements while the horizontal bars represent the proportion of larvae in each vertical section.


Control (30)
20/30 Bottom
20/30 Top
Salinity (‰)
18
24
30
36
42
1 Hour
3 Hours
7 Hours
Depth (cm)
5-10
10-15
15-17.5
17.5-20
20-25
25-30
30-35
35-40
40-45
0
0.2
0.4
0.6
0.8
1
Proportion of larvae

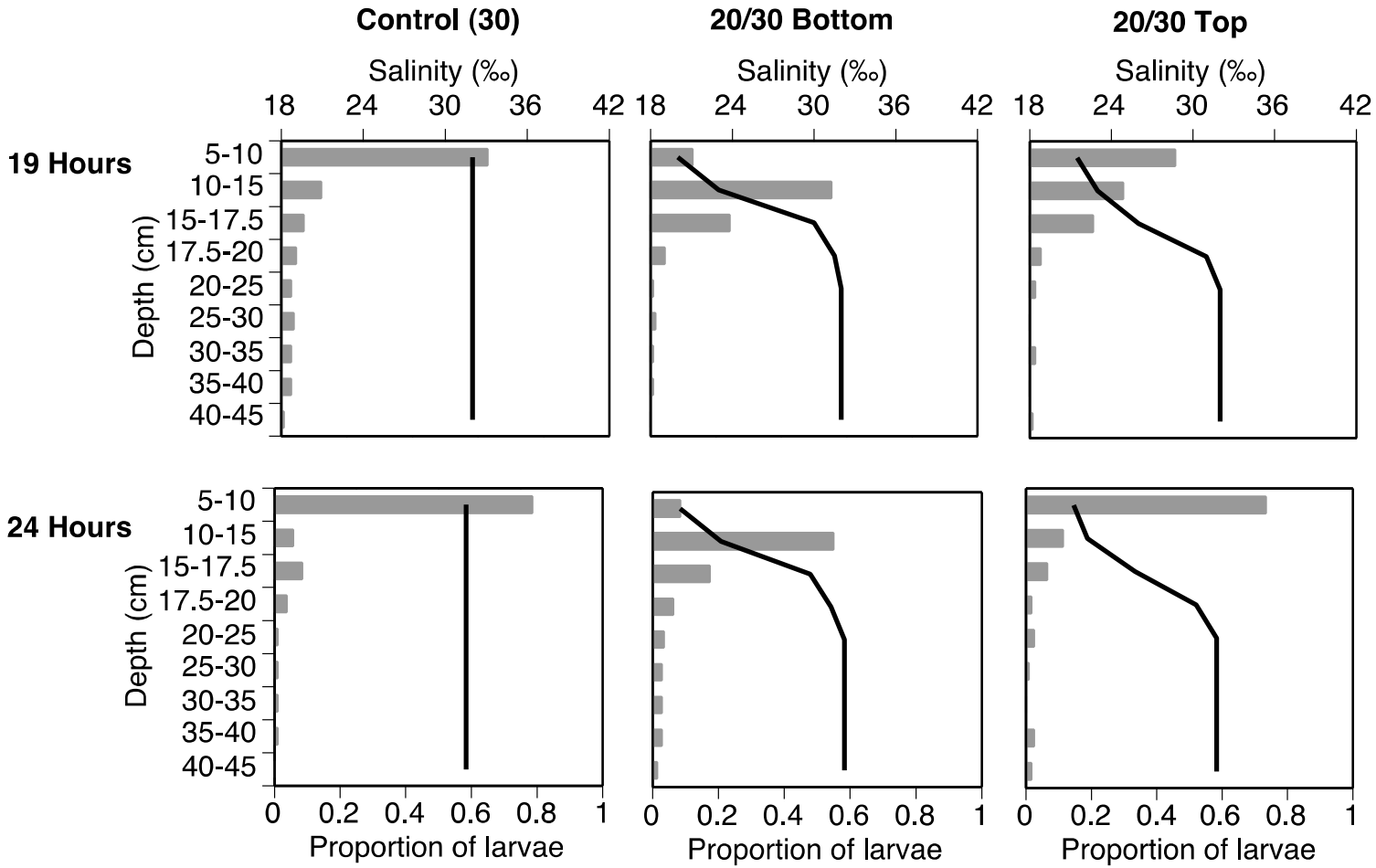
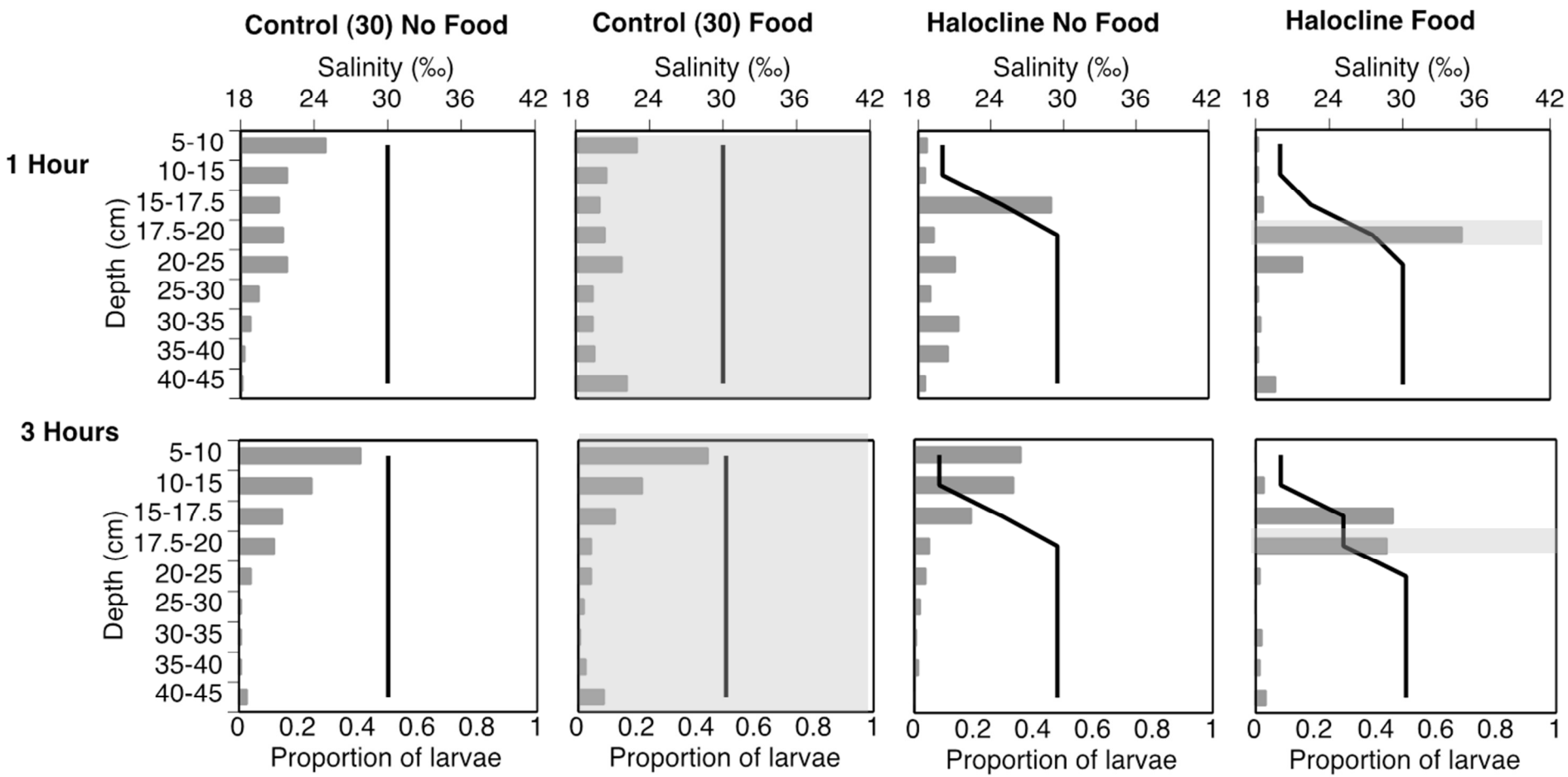



**Figure 7**. Distribution of control *P. ochraceus* larvae after introduction to the top or bottom of a halocline. Bipinnaria were 27-29 days old. Larvae were raised in 30-32‰ FSW. Each graph consists of the combined results of 2 replicate columns. Between 60 and 120 larvae were introduced into each column at least an hour after creation of the halocline. The line represents salinity measurements while the horizontal bars represent the proportion of larvae in each vertical section.


Control (30) No Food
Control (30) Food
Halocline No Food
Halocline Food
Salinity (‰)
18
24
30
36
42
1 Hour
3 Hours
Depth (cm)
5-10
10-15
15-17.5
17.5-20
20-25
25-30
30-35
35-40
40-45
0
0.2
0.4
0.6
0.8
1
Proportion of larvae

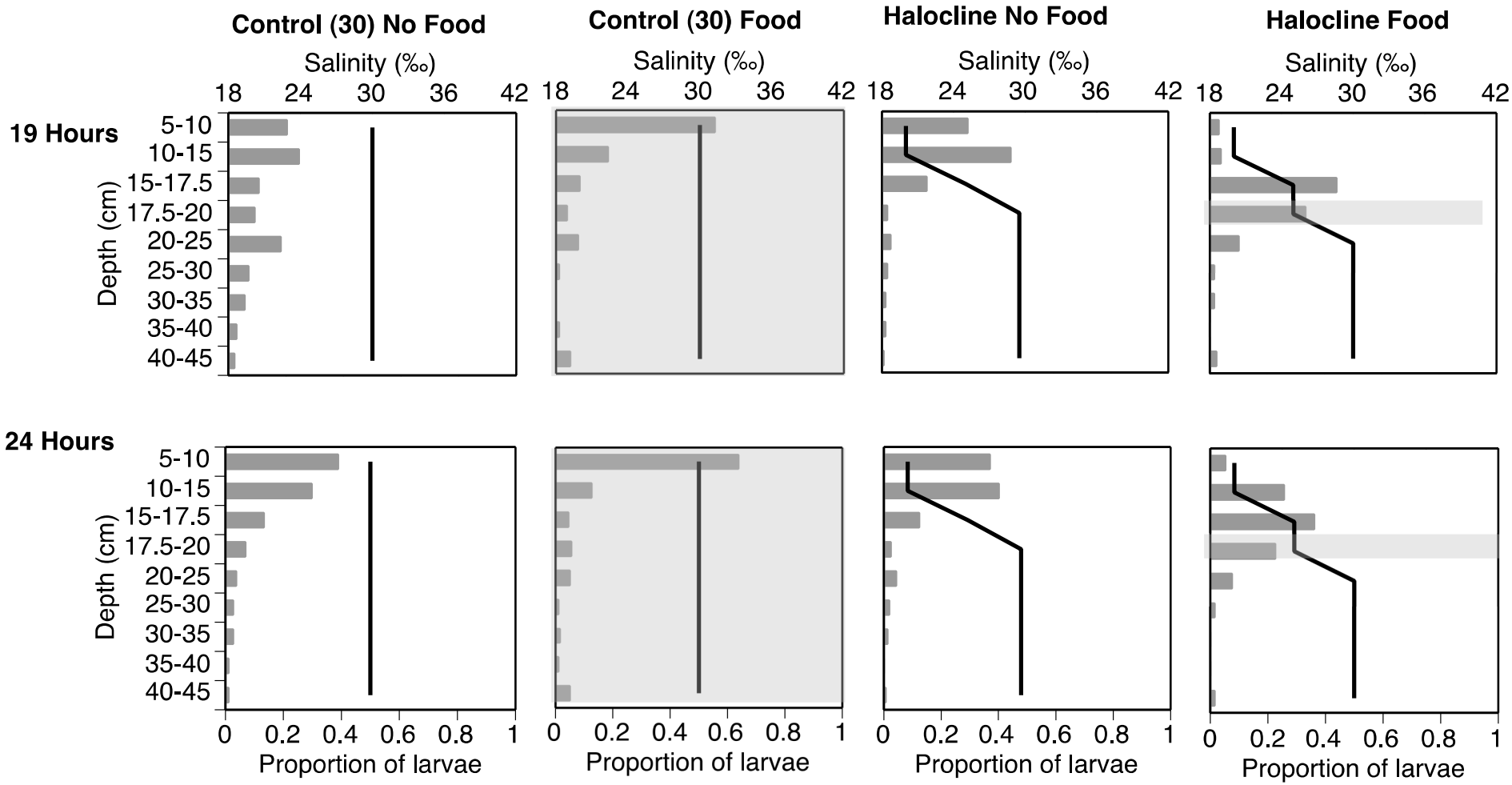
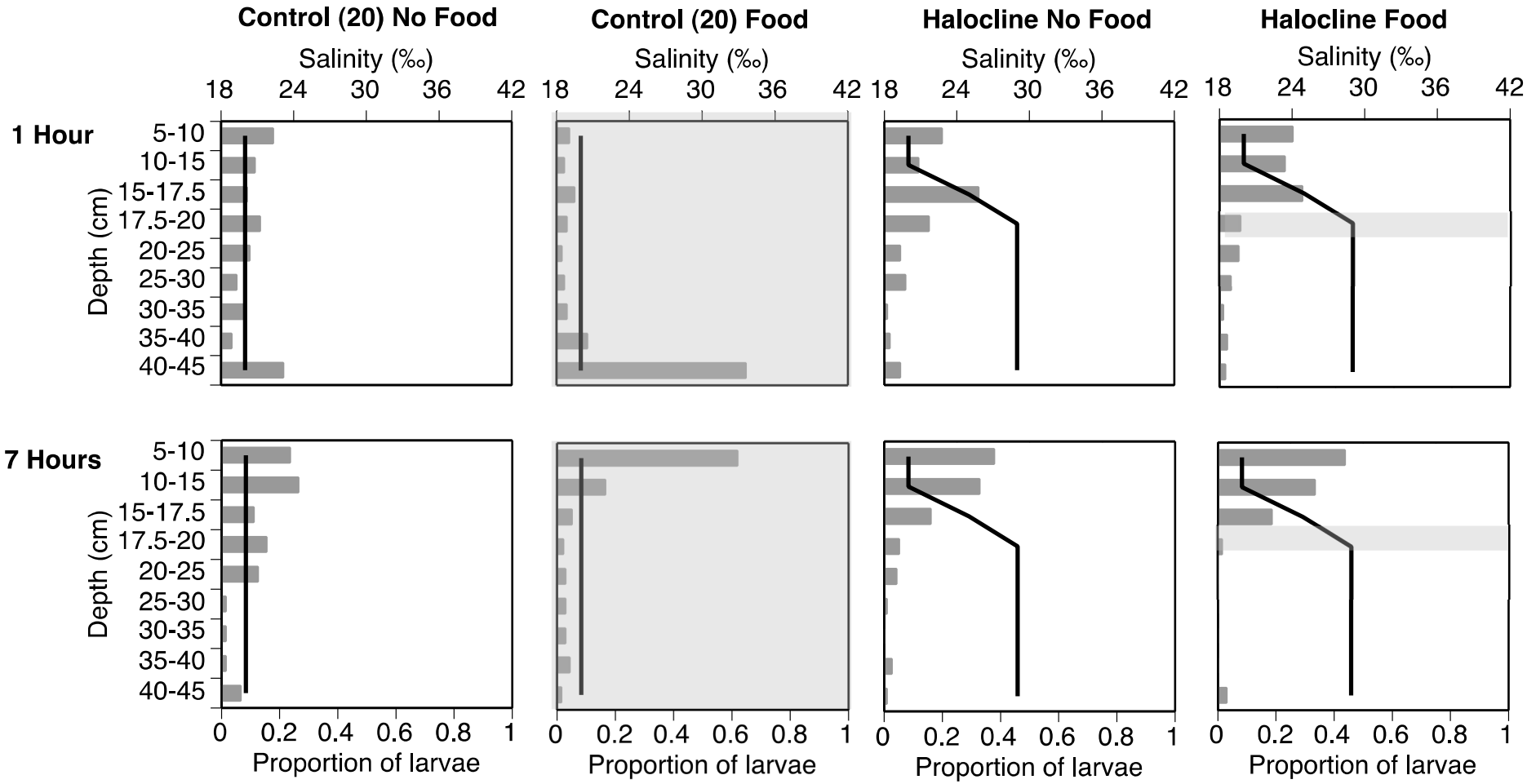



**Figure 8**. Distribution of control *P. ochraceus* larvae in water columns with and without food. Brachiolaria were 34-36 days old. Larvae were raised in 30-32‰ FSW. Each graph consists of the combined results of 2 replicate columns. Between 60 and 130 larvae were introduced into each column at least an hour after creation of the halocline. The shaded region indicates the initial location of food within the column. The line represents salinity measurements while the horizontal bars represent the proportion of larvae in each vertical section.


Control (20) No Food
Control (20) Food
Halocline No Food
Halocline Food
Salinity (‰)
18
24
30
36
42
1 Hour
7 Hours
Depth (cm)
5-10
10-15
15-17.5
17.5-20
20-25
25-30
30-35
35-40
40-45
0
0.2
0.4
0.6
0.8
1
Proportion of larvae

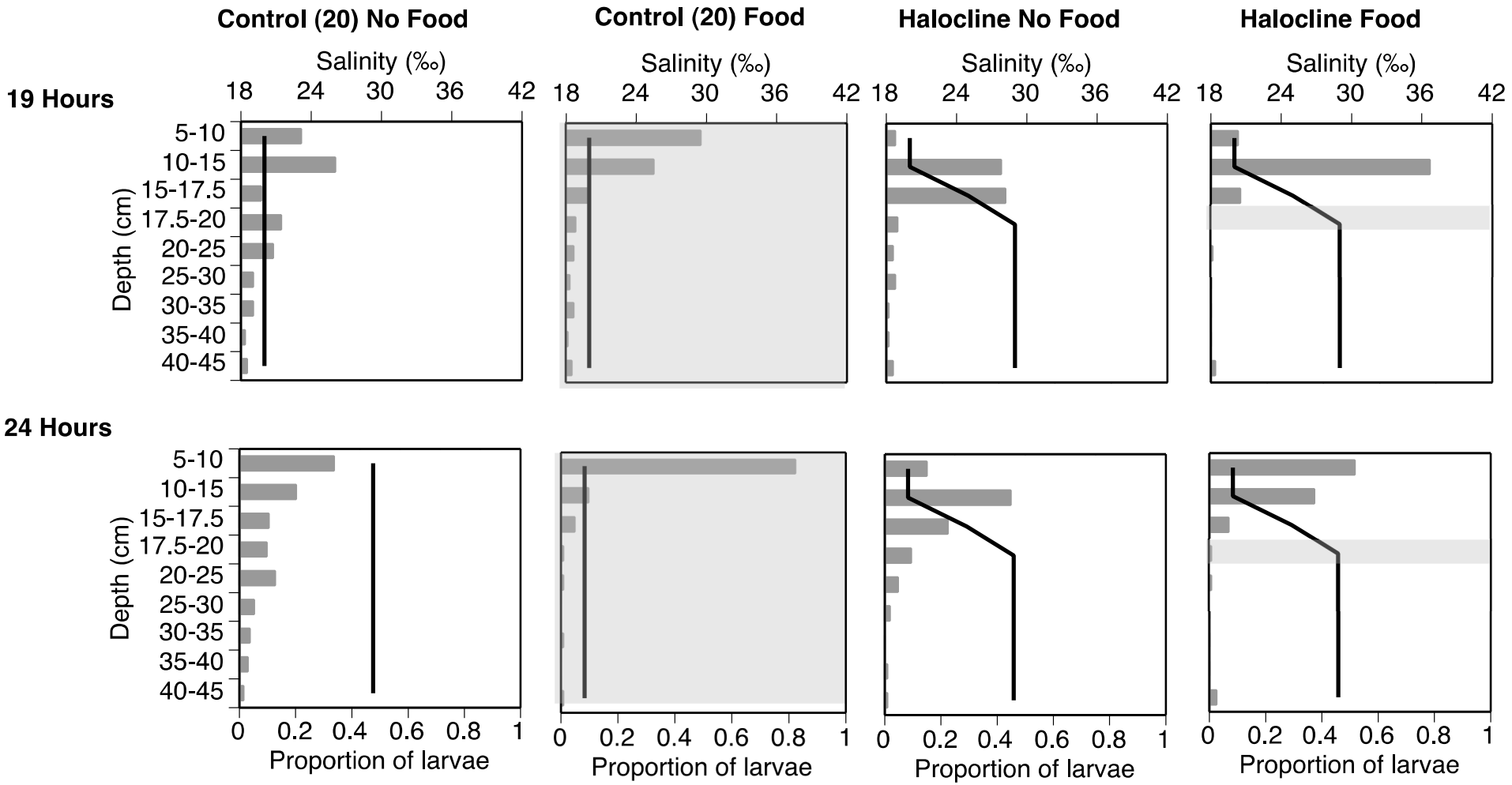



**Figure 9**. Distribution of low salinity reared *P. ochraceus* larvae in water columns with and without food. Brachiolaria were 36 days old. Larvae were raised in 20-22‰ FSW. Each graph consists of the combined results of 2 replicate columns. Between 40 and 110 larvae were introduced into each column at least an hour after creation of the halocline. The shaded region indicates the initial location of food within the column. The line represents salinity measurements while the horizontal bars represent the proportion of larvae in each vertical section.