

# Substrate preference and delayed settlement in northern rock sole larvae *Lepidopsetta polyxystra*

Benjamin J. Laurel<sup>1,\*</sup>, Anthony J. Basilio<sup>2</sup>, Courtney Danley<sup>3</sup>, Clifford H. Ryer<sup>1</sup>,  
Mara Spencer<sup>1</sup>

<sup>1</sup>Fisheries Behavioral Ecology Program, Resource Assessment and Conservation Engineering Division, Alaska Fisheries Science Center, National Marine Fisheries Service, NOAA, Hatfield Marine Science Center, Newport, OR 97365, USA

<sup>2</sup>Department of Biology, California State University, Monterey Bay, 100 Campus Center, Seaside, CA 93955-8001, USA

<sup>3</sup>Cooperative Institute for Marine Resources Studies (CIMRS), Hatfield Marine Science Center, Newport, OR 97365, USA

**ABSTRACT:** We addressed the hypothesis that larval flatfish have behavioral control over the timing and habitat in which they settle. Annual and seasonal settlement patterns of northern rock sole *Lepidopsetta polyxystra* were characterized across varying depths and sediments in 2 nursery areas around Kodiak, Alaska, USA. These data were compared to experimental data from the laboratory, where northern rock sole larvae were reared and exposed to varying sediment sizes to determine: (1) the earliest ontogenetic stage of habitat selection, and (2) whether settlement was delayed when preferred sediment sizes were unavailable. Field data indicated that newly settled rock sole were not selecting habitat based on sediment characteristics. Rather, rock sole settled in shallow-water regions (~5 m depth) of the nursery, where sediments were indistinguishable from surrounding sediment types and notably coarser than those in the deepest areas of the nursery. At 1 to 2 mo post-settlement, the distribution of juvenile rock sole shifted to deeper regions of the nursery, as predicted by habitat selection experiments from the laboratory. However, laboratory experiments indicated that habitat selection occurs earlier at the time of settlement, with preference for fine sediment sizes. Rock sole larvae in the laboratory also delayed settlement when exposed to coarse sediments, resulting in a significant increase in both the size- and condition-at-settlement. Therefore, despite evidence of behavioral control at settlement, biological or larger-scale physical processes may ultimately regulate quality and access to preferred benthic habitat in juvenile northern rock sole.

**KEY WORDS:** Flatfish · Metamorphosis · Nursery area · Habitat selection · Settlement behavior · Larval transport · Dispersal

Resale or republication not permitted without written consent of the publisher

## INTRODUCTION

Flatfishes (Pleuronectiformes) are a diverse group of species that are easily identifiable by their uniquely flat body types and strong links to benthic habitats. Like most marine fish species, flatfish begin life with a dispersive, planktonic larval phase that plays a significant role in transport to juvenile nursery areas. However, at the end of the larval period, flatfish undergo a distinct metamorphosis and transition to

the benthos (i.e. settlement) to begin a near 2-dimensional demersal way of life. From a dispersal perspective, this transition is an important period, as it approximates the point in time where active behavior (e.g. horizontal swimming, habitat selection, etc.) prevails over large-scale, oceanographic processes. From a survival perspective, larvae need to initiate settlement when transported to suitable nursery habitats, as they have limited movement capability to relocate to more favorable habitats following meta-

morphosis (McCormick 1999). Therefore, the physiological and behavioral factors controlling pelagic larval duration and settlement cues in flatfish are ecologically important and play a broader role in habitat use patterns, population dynamics, and species persistence (Bradbury et al. 2008).

Northern rock sole *Lepidopsetta polyxystra* (hereafter referred to as NRS) is an abundant flatfish species distributed throughout the western and eastern North Pacific, with high commercial importance in the Bering Sea and Gulf of Alaska (Orr & Matarese 2000). NRS have been a focal species for modeling larval transport under changing environmental conditions in the eastern Bering Sea (Wilderbuer et al. 2002, Lanksbury et al. 2007, Cooper et al. 2014) as well as for understanding growth and distribution in post-settled stages in coastal areas of the Gulf of Alaska (Stoner & Ottmar 2003, Ryer et al. 2004, Hurst et al. 2010). Field and laboratory studies have indicated that sediment type (i.e. particulate grain size) is a key habitat variable for newly settled NRS (Stoner & Ottmar 2003, Stoner & Titgen 2003, Laurel et al. 2007), as post-settled NRS must be able to bury in loose substrate or cryptically match the sediments to avoid predation (Ryer et al. 2004). However, ichthyoplankton studies suggest that NRS may be restricted or delayed from accessing suitable benthic habitats by the presence of a 'cold pool' in some years (Cooper et al. 2014). The fate of these larvae is uncertain, as the settlement dynamics of NRS have neither been captured in the field nor examined explicitly in the laboratory. Questions still remain as to whether sediment characteristics are important during settlement and whether NRS can delay settlement when favorable habitat is unavailable. Such plasticity would be an important component of larval transport and survival for this species (Wilderbuer et al. 2002, Lanksbury et al. 2007) as well as for understanding annually variability in the use of nursery habitat in Alaskan waters.

The goal of this study was to determine the degree of behavioral plasticity in the timing and location of settlement in NRS. In the field, sampling was conducted over a 4 mo period to characterize the distribution of sediments and newly settled NRS in 2 coastal nurseries around Kodiak, Alaska, USA. In the laboratory, 2 experiments were conducted to test whether pre- and post-settled larvae show preference for certain sediments and whether NRS larvae can delay settlement in the absence of preferred habitat. These results were compared to determine whether small-scale settlement dynamics measured in the laboratory support larger-scale settlement patterns observed in the field.

## MATERIALS AND METHODS

### Field sampling

A series of scrapes at varying depths were completed during May to August of 2010 in 2 known nursery areas, Holiday Beach and Pillar Creek Cove, in Chiniak Bay, Kodiak, Alaska (Fig. 1). These 2 nursery areas have been the location for a series of studies describing habitat use in post-settled NRS juveniles using a 2 m beam trawl and camera sled (Stoner et al. 2007, Ryer et al. 2010, 2013, Laurel et al. 2012). In this study, the camera sled was modified into a benthic scrape and outfitted with 3 mm mesh plankton net. The towed apparatus (1.2 m long, 0.7 m wide) was designed to disturb the uppermost layer of benthic habitat and effectively collect the smallest size range of newly settled crab and flatfishes to provide a relative estimate of species density by depth region. The sled was towed along a depth contour approximately 30 m during each scrape. Depth and position were measured and maintained using an electronic depth finder and GPS chartplotter. The total number of age-0 yr flatfish collected was recorded for each scrape. Age-0 fish were easily discriminated from age-1 or older flatfish by a clear separation in length frequencies. Data from trawls towed in the same region in previous years revealed that juveniles were >95% NRS, with lesser catches of Pacific halibut and English sole (Hurst et al. 2007, Stoner et al. 2007, Ryer et al. 2010). The catch per unit effort (CPUE) was calculated using number of animals caught per the standardized area of 20.42 m<sup>2</sup> for each scrape. In 2010, a total of 3 to 6 replicate scrapes were performed at each depth contour (~2, 4, 6, 10, 12, 16, 18, 24 m) at each site (n = 2) during every month of sampling (n = 4; May, June, July, and August). These depths represent >95% of the age-0 NRS distribution in these nurseries based on earlier July and August surveys (Stoner et al. 2007, Ryer et al. 2013). However, 2 additional deeper transects were conducted at the 28 and 31 m depth contour of Pillar Creek Cove to confirm these observations where deep-water habitat was available. In 2011, a total of 3 to 4 replicate scrapes were performed at coarser-scaled depth contours (~3, 7, 11, 16, 23, 26 m) at each site in every month.

Based on a previous study conducted at Holiday Beach and Pillar Creek Cove, depth should be negatively correlated with mean grain size and % sand composition in the sediment (Stoner et al. 2007). To confirm these patterns, 3 replicate sediment samples were taken with a Ponar grab (400 cm<sup>2</sup>) across

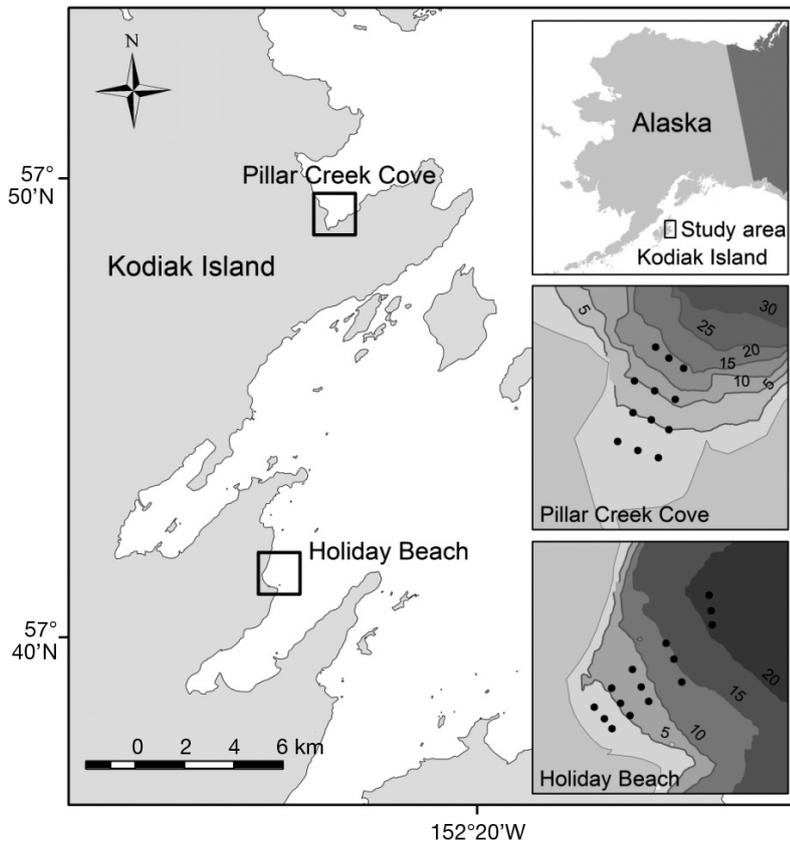


Fig. 1. Two coastal nursery areas around Kodiak Island, Alaska, USA, where settlement and post-settlement distributions of juvenile northern rock sole *Lepidopsetta polyxystra* were characterized using a towed benthic scrape at varying depth strata. Points represent locations where sediment samples were collected with a Ponar grab

discrete depths at each site (3–4, 6–7, 10–12, 15–16 [Holiday Beach only], and 21–23 m). Two subsamples were taken from the upper 2 cm of sediment in each sample and frozen for later analysis. Analysis was conducted on 50 g of thawed sediment by drying to a constant weight at 50°C. Samples were then burned for 8 h at 550°C to determine the % dry weight comprised of organic content. Another subsample of sediment was washed through a 0.062 mm mesh sieve to separate the silt-clay and sand fractions. The filtrate was dried at 50°C to determine the silt-clay fraction. The sand fraction was dried and then fractionated on a mechanical shaker with sieves ranging from –4 to 4 on the phi scale (16 to 0.062 mm) at 0.5 phi increments. Mean grain size was calculated using product moment statistics (McBride 1971), and silt-clay, sand, and granule fractions were determined based on relative proportions.

Field data were analyzed using ANOVA under the general linear model (GLM). CPUE data were log-transformed prior to analysis, and residuals were

checked to ensure data met the assumptions of normality of the statistical model.

### Larviculture

The larval rock sole were reared in the laboratory using eggs strip-spawned from NRS adults held at the Hatfield Marine Science Center (HMSC) in Newport, Oregon, USA. Adults were originally collected from Chiniak Bay and live-shipped to the HMSC in September 2009. In 2010, males and females showed signs of ripening starting in February. The gametes of ripe males ( $n = 3$ ) and a female ( $n = 1$ ) were combined into a clean, dry container for a 1 min period before the addition of ambient seawater. Seawater was repeatedly added and decanted from egg batches to clean them of excess milt and tissue. Fertilized eggs were thinly dispersed as a single layer in a series of 4 l containers with 220  $\mu\text{m}$  mesh sides and solid bottoms. Incubating containers were suspended in  $1 \times 1 \times 0.5$  m square tanks and supplied with temperature-controlled seawater (6°C) at a rate of 2 to 3  $\text{l min}^{-1}$ . Each container was gently lifted and lowered within

the seawater bath twice daily to achieve seawater exchange. The onset of hatch occurred following 16 d of incubation, with high daily hatching occurring for an additional 6 d.

Yolk-sac larvae were counted and removed from the containers every day and distributed evenly across a series of 100 l round fiberglass upwelling tanks ( $n = 12$ ) at a density of 600 larvae  $\text{tank}^{-1}$ . Temperatures were maintained between 7 and 10°C on a 12 h light:12 h dark photoperiod to approximate day length conditions experienced by NRS larvae in the Gulf of Alaska in March and April. Water was supplied at a rate of 250  $\text{ml min}^{-1}$  through central-bottom intake to minimize disturbance to the larvae. Gentle aeration was provided by an airstone placed on the bottom center of the tank. Tanks were supplied with a combination of algae (*Nannochloropsis* spp.) and rotifers (*Brachionus plicatilis*) enriched with Algamac 3050. Rotifers were supplied at a density of 5 prey  $\text{ml}^{-1}$  twice daily. Otohime microparticulate dry food was also added to tanks twice daily at 0.3 g  $\text{tank}^{-1}$ .

Table 1. Substrate types placed at the bottom of each test arena in equally sized wedges. Composition is percent by weight. DE: diatomaceous earth

Substrate number	Substrate type	Mean grain size (mm)	Approximate phi value	Composition
1	Sandy mud	~0.04	4.5	30% fine sand (0.2 mm grain size) + 70% DE
2	Muddy sand	~0.1	3.25	70% fine sand (0.2 mm) + 30% DE
3	Fine sand	0.2	2.25	Silica sand
4	Medium sand	0.5	1.0	Silica sand
5	Coarse sand	1	0	Silica sand
6	Gravel	2–4	–1.0	River gravel
7	Pebble	~10	–3.0	River gravel

The microparticulate diet is larger in diameter than mean rotifer length (220–350  $\mu\text{m}$ ) and was anticipated to be important for faster-growing fish requiring larger prey sizes.

### Laboratory Expt 1

This experiment tested the onset and ontogenetic changes in habitat selection of pre- and post-settling NRS larvae. Trials were run on individual larvae in a series of 5 round black 19 l tanks kept at 8°C in a cold room. Seven different substrate types (Table 1) were randomly organized on the bottom of each tank into equally sized wedges. For comparative purposes, the substrate types and arrangement were identical to a previous experiment by Stoner & Ottmar (2003) that examined habitat selection in older juvenile stages of NRS. The use of a broad range of sediment types also insured that we captured the range of sediments experienced by settling NRS within and outside our focal nursery areas. Arenas were drained and filled with fresh seawater after every 2 trials. Each substrate was replaced every 2 wk to avoid it becoming anaerobic.

Prior to the start of any experimental trial, an individual larva was collected from a rearing tank using a small net and a 1 l container with ambient seawater. Larvae were collected from 2 to 3 different rearing tanks on each trial day to ensure that all 12 rearing tanks were evenly used throughout the experimental period. Larvae were considered 'pre-settled' if they were swimming in the water column of the rearing tanks (8–17 mm total length, TL) and 'post-settled' if they were lying flat on the bottom (14–30 mm TL). Each 1 l container holding an individual larva was suspended inside a foam ring float on the surface of an arena for a 15 min period of acclimation. Following acclimation, larvae were introduced to the arena by gently tipping the con-

tainer below the water surface. Larvae were then allowed full access to the substrate and water column of the arena for a 24 h period with no disturbance. Light levels were set at 4.73  $\mu\text{E m}^{-2} \text{s}^{-1}$  for daytime conditions on a 12 h light:12 h dark cycle. After 24 h, an observer with the aid of a small flashlight noted the position of larvae in the water column (settled or not settled), and if settled, which of the 7 substrates were occupied. Larvae were subsequently collected using a small siphon and anesthetized in a solution of tricaine methanesulfonate (MS-222) for measurement with a digital camera attached to a stereo microscope and ImagePro® software (Media Cybernetics). In total, 183 trials were run on individual larvae of varying sizes and pre-conditioned settlement state.

Analysis of the data duplicates the methods of Stoner & Ottmar (2003), who examined sediment choice among different sizes of flatfish. Larvae that did not settle during the experimental period were removed from the analysis, leaving a total of 172 trials for analysis. The frequency distribution of substrate choices was tested with a likelihood-ratio *G*-test with the null hypothesis being that choices are equally distributed among the 7 substrate types presented. Larvae were binned into 3 size categories (12–14, 15–17, and 18–30 mm TL) for each analysis. Independent trials were used as replicates in accordance with the statistical assumptions of the model. Because age and size of fish were tightly coupled, the small size category of fish represented younger larvae (earlier trials) compared to the large size category (later trials). However, previous experiments indicated that settlement competency in NRS larvae is a size- rather than age-dependent process (Laurel et al. 2014). Significant differences in substrate selection between pre- and post-settled individuals were also examined using a 2 × 7 contingency table for the 15–17 mm size bin where pre- and post-settled larvae were represented.

## Laboratory Expt 2

The second laboratory experiment measured the rate of settlement as a function of fish size and habitat type. Fifteen 19 l arenas (see Expt 1) were used during each trial run, all of which were filled with either fine sand (0.2 mm grain size) or gravel (2–4 mm grain size). Based on post-settlement habitat selection experiments, fine sand represents the preferred habitat for juvenile NRS (Stoner & Ottmar 2003). The substrate depth was approximately 1.3 cm. Arenas were filled with seawater and stored in the same cold room and light conditions described in Expt 1. These arenas were also drained and filled with fresh seawater after every 2 trials, and the sediments were changed every 2 wk.

Individual larvae were collected and introduced to arenas in the same manner as described in Expt 1. However, all larvae used for this experiment were collected from the water column and considered 'pre-settled' prior to any trial. After larvae were in the arena for 24 h, an observer recorded whether the larvae had settled (0 = settled, 1 = not settled). A second observation was conducted 30 min later to develop a proportional settlement score from both observations (0.0, 0.5, and 1.0). Following observations, all larvae were collected and measured using image analysis. Larval dry mass was then measured for each larva by placing individuals onto pre-weighed aluminum foil sheets and placing them in a drying oven (60°C) for 72 h. All larvae were rinsed with ammonium formate to remove excess salts prior to drying. Dried larvae were cooled for 5 min at room temperature (~20°C) and then weighed to the nearest microgram using a Mettler Toledo MT5 microbalance.

Data were not normally distributed and were instead analyzed using a 2-way general linearized model (GLZM) with a logit data link (GLZM; SPSS 10.0) to determine whether the size-dependency of settlement shifted between preferred (sand) and non-preferred (gravel) habitats. A GLZM was used in place of a GLM because data could not be transformed to meet the assumptions of normality. A significant effect of 'habitat' in the model would indicate behavioral control over settlement. Data from each habitat trial were also plotted using a 3-parameter logistic function using Sigmaplot 10.0 to both visualize the patterns and describe the relative likelihood of settlement as a function of size and habitat. However, given that the model was generated from small-scale arenas in the laboratory, we made no attempt to apply these rates or generate a predictive model for the field. Finally, the effects of larval condition on

settlement within each habitat were examined. Condition was calculated by using the residuals of the length–dry mass relationship for all larvae used in the laboratory trials. Mean values of the length–dry mass residuals for larvae from Expt 2 were plotted and examined by settlement score (0.0, 0.5, 1.0) and habitat type (gravel or sand) to determine whether the energetic state of larvae contributed to settlement likelihood in the experiment.

## RESULTS

### Settlement patterns (field data)

No age-0 NRS were caught in May sampling during 2010 and 2011, so data analyses were restricted to June, July, and August months. Average CPUE values were calculated for each depth interval by site location and month (Fig. 2). Years were analyzed separately because depth intervals were sampled more coarsely in 2011 and NRS abundance was much higher than in 2010. In both years, the GLM detected a significant 3-way interaction between site, depth bin, and month (2010 sampling,  $F_{16,205} = 2.01$ ,  $p = 0.037$ ; 2011 sampling,  $F_{10,104} = 1.31$ ,  $p = 0.048$ ). Larvae were observed settling in the shallowest depth regions at both sites in June (3–5 m), followed by a post-settlement shift in distribution to deeper areas of the nursery later in the summer. However, the shift to deeper water was variable between sites in July, likely causing the 3-way interaction in the models. By August, peak abundance of juvenile NRS was between the 15 and 20 m depth contour of Pillar Creek Cove and across a broader depth range of 10 to 24 m at Holiday Beach. Although 'year' was not analyzed in the statistical model, the deeper water distribution of fish in August was more distinct in 2011 compared to 2010.

Sizes of NRS in June ranged between 13.7 and 25.6 mm TL (mean = 18.4 mm) in 2010 and between 14.6 and 22.1 mm TL (mean = 16.4 mm) in 2011. Average sizes of NRS were approximately 10 to 15 mm TL larger in each successive month following the June settlement period, but a freezer failure containing July and August samples precluded a detailed length–frequency analysis. However, juvenile NRS size and growth data are well-described for the July and August period in Pillar Creek Cove and Holiday Beach in earlier years (2004–2007; Hurst et al. 2010).

Sediment type within the nurseries was essentially uniform except at the deepest depth regions (21–23 m; Fig. 3). There, sediment grain size (phi

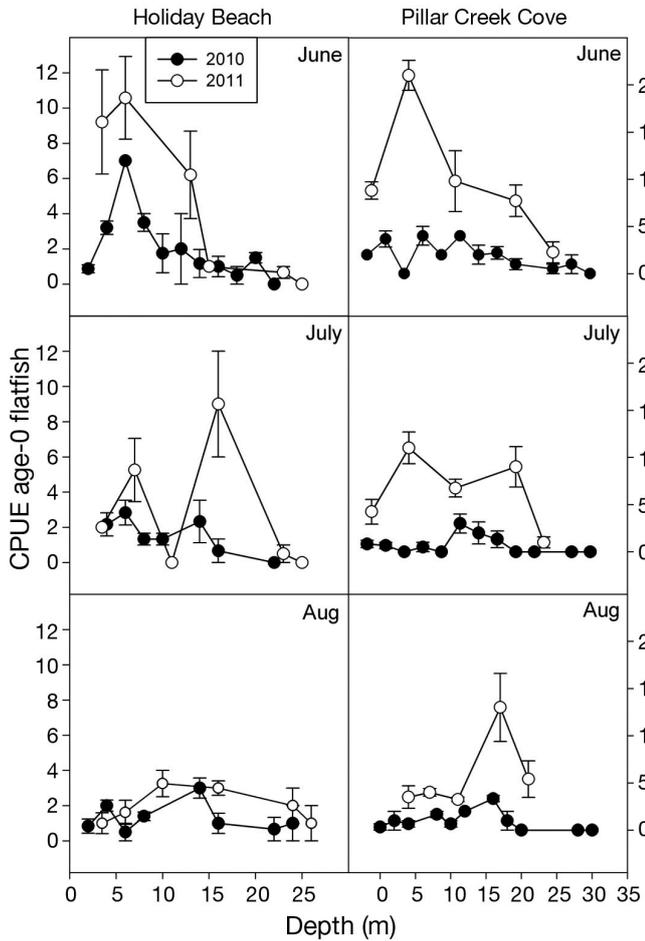


Fig. 2. Catch per unit effort (CPUE) of northern rock sole *Lepidopsetta polyxystra* at varying depths at 2 Alaskan sites in 2010 and 2011. Values are based on mean catches ( $n = 2-6$ )  $\pm 1$  SE of rock sole collected by a towed benthic scrape over ~30 m on the bottom and standardized to number of fish per 20.42 m<sup>2</sup>. Note difference in scales between Holiday Beach and Pillar Creek Cove

scale) increased significantly at Pillar Creek Cove (ANOVA,  $F_{3,8} = 6.482$ ;  $p = 0.016$ ) and Holiday Beach (ANOVA,  $F_{3,8} = 28.988$ ;  $p < 0.001$ ). There was also a significant decrease in the % sand (ANOVAs; Pillar Creek Cove,  $F_{3,8} = 46.431$ ,  $p < 0.001$ ; Holiday Beach,  $F_{3,8} = 183.642$ ,  $p < 0.001$ ) and an increase in the % organic matter (ANOVAs; Pillar Creek Cove,  $F_{3,8} = 10.788$ ,  $p = 0.003$ ; Holiday Beach,  $F_{3,8} = 110.812$ ,  $p < 0.001$ ) at the deepest depths.

**Habitat selection (Expt 1)**

NRS larvae showed a preference for finer sediment types for all size classes and settlement stages considered (Fig. 4). *G*-tests within each size and settlement state indicated that these patterns were sig-

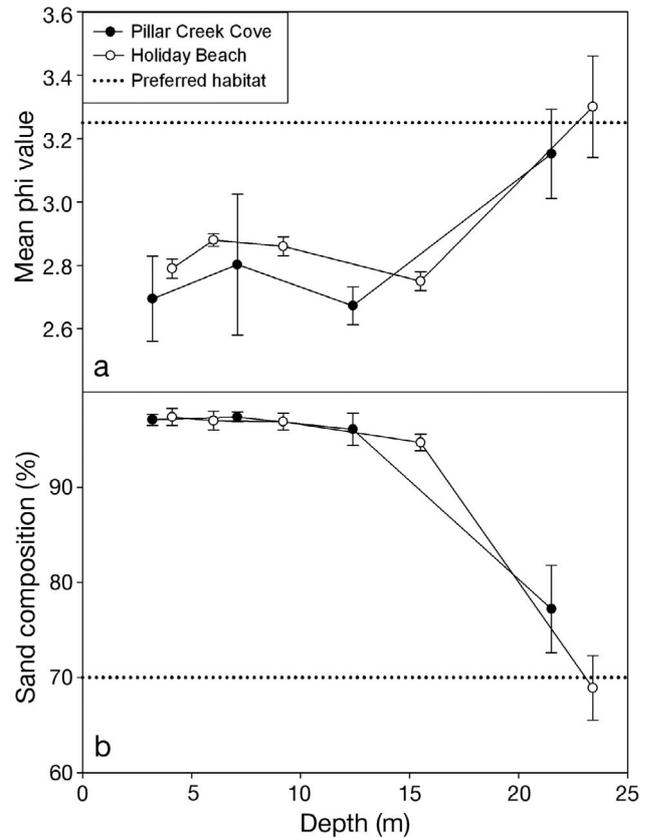


Fig. 3. Sediment characteristics of Holiday Beach and Pillar Creek Cove in terms of (a) mean granule size (phi scale) and (b) % sand composition. Values are based on means  $\pm 1$  SE ( $n = 3$ ). Dotted lines reflect habitat preference of northern rock sole *Lepidopsetta polyxystra* from experimental trials (see Fig. 4). Note that high phi values indicate smaller sediment granule size (see Table 1)

nificant ( $p < 0.05$ ), supporting the hypothesis that NRS larvae choose habitat at the time of settlement. A reference line added to Fig. 3 shows where these preferred habitats were found within the nursery based on mean phi values (grain size) and % sand composition. The contingency table comparing non-settled and settled larvae indicated no difference in sediment size preference ( $\chi^2 = 10.441$ ,  $df = 5$ ,  $n = 90$ ,  $p = 0.064$ ). There was also no difference in sediment size preference between the largest class and smallest size class ( $\chi^2 = 8.059$ ,  $df = 5$ ,  $n = 55$ ,  $p = 0.153$ ) despite an apparent ontogenetic shift from muddy sand to fine sand (Fig. 4).

**Delayed settlement (Expt 2)**

NRS settled in the habitat arenas across a range of sizes (11–18 mm TL), but the overall likelihood of settlement increased with size (Fig. 5). Settlement was

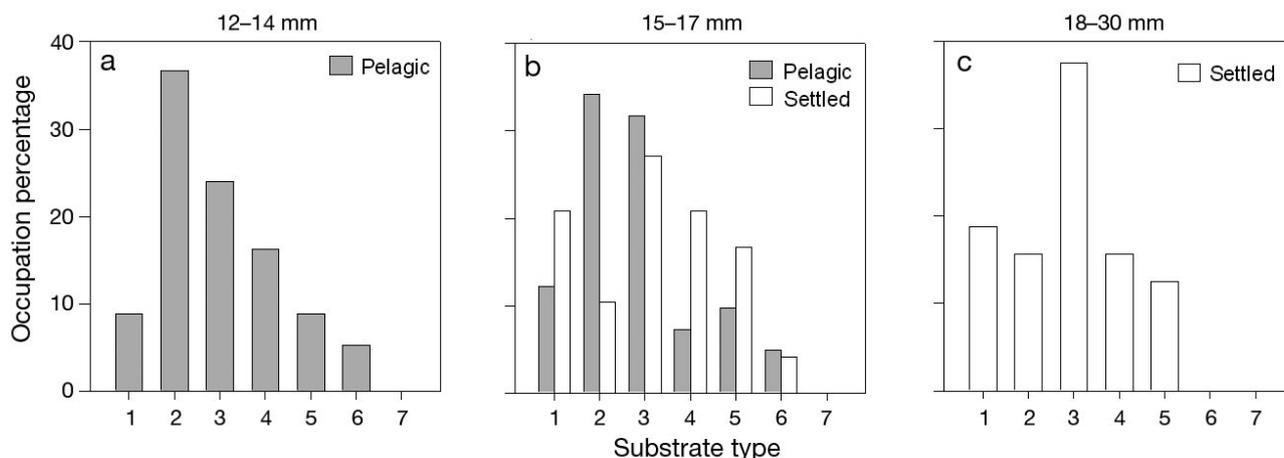


Fig. 4. Habitat selection by (a) small (pelagic), (b) medium (pelagic and settled), and (c) large settled northern rock sole *Lepidopsetta polyxystra* larvae (mm total length) over a 24 h period in experimental arenas. Sediment type listed from finest to largest (i.e. 1 = sandy mud, 2 = muddy sand, 3 = fine sand, etc; see Table 1). Data are presented as percentages of trials where fish chose a particular habitat (n = 172 total trials)

further influenced by habitat type, such that NRS larvae were more likely to settle at a smaller size in preferred habitat (sand) compared to less preferable habitat (gravel) (GLZM:  $\chi^2 = 11.472$ , df = 1, n = 484, p < 0.001; Fig. 5). As an example, the logistic model indicated that a 14 mm larvae encountering sand habitat was ~4 times more likely to settle than an equivalent-sized larvae encountering gravel habitat. These habitat effects were less apparent as larvae grew, and at ~18 mm TL, settlement in the arenas

occurred regardless of habitat type. However, several large pelagic larvae (20–21 mm TL) were sampled from the general larval rearing tanks, suggesting that NRS larvae can delay settlement even further when suitable habitat is not encountered.

Larvae >20 mm TL appeared to be in poorer condition, as indicated by the change in trajectory of the length–weight relationship of NRS larvae (Fig. 6). Therefore, the regression used to calculate length–weight residuals (larval condition) was restricted to larvae used in the settlement experiment (up to 20 mm TL) where the data were linear following log transformation. From those data, it was evident that the larvae that settled within the 24 h experimental period were in better condition than those larvae that remained pelagic (Fig. 7). This pattern was most evident in the gravel habitat, suggesting only larvae in the best condition chose to settle in unfavorable habitat (Fig. 7).

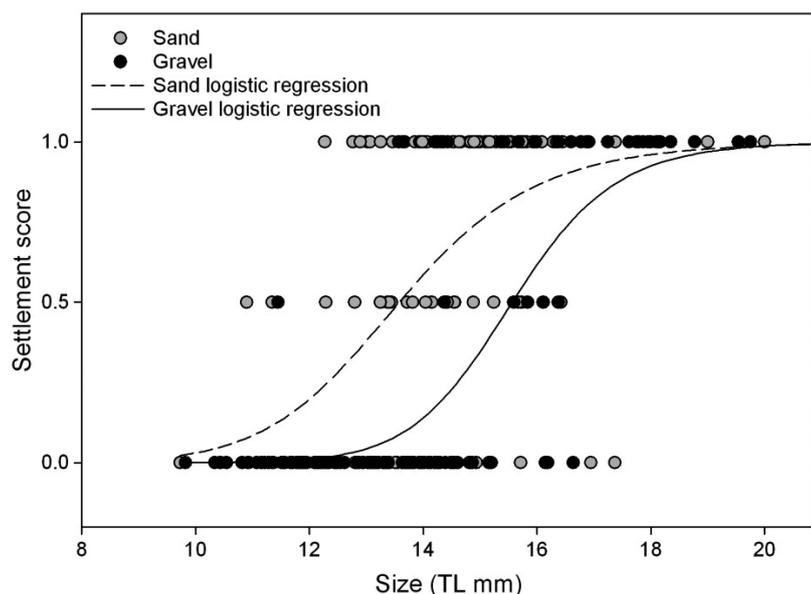


Fig. 5. Size-dependent settlement models for northern rock sole *Lepidopsetta polyxystra* exposed to fine sand or gravel habitat over a 24 h period in experimental arenas. Settlement scores (y-axis) indicate the proportion of observations (n = 2) where settlement occurred in a trial. Model fits were conducted using a 3-parameter, logistic regression. TL: total length

## DISCUSSION

The results from this study support a series of studies examining processes regulating transport and distribution of NRS larvae to and within nursery areas in Alaskan waters (e.g. Stoner et al. 2007, Ryer et al. 2010, Cooper et al. 2014). Laboratory results indicate that NRS larvae prefer

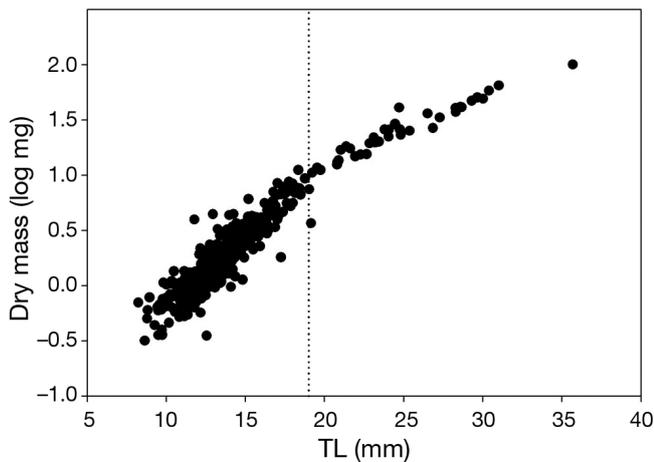


Fig. 6. Length–dry mass relationship of northern rock sole *Lepidopsetta polyxystra* larvae used in experimental trials. Dotted line represents the maximum size at which larvae delayed settlement. Note that the decrease in the slope of the length–mass relationship (>20 mm) suggests energetic stress following settlement. TL: total length

finer sediments at settlement and will delay settlement when such habitats are unavailable or energetic reserves are insufficient. While similar results have been shown for reef fish (McCormick 1999), to our knowledge this is the first study to show such plasticity in a flatfish species. However, in the field, larval NRS settled in a narrow depth distribution at the shallowest part of the nursery where sediments were coarser, albeit within the range of sizes selected by larvae in the laboratory. Although the central distribution of NRS juveniles shifted to the deeper areas with finer sediments, the initial field distributions of NRS juveniles suggest that other processes in the field may regulate habitat selection at the time of settlement. Below we discuss the implications of settlement plasticity in the context of broader ecological and environmental processes occurring within the nursery areas.

### Environmental processes

Both Pillar Creek Cove and Holiday Beach are well-studied nursery areas for post-settled juvenile NRS, but this is the first study to examine processes and fine-scale distribution of NRS at the time of settlement. Although settlement timing likely varies year to year based on temperature conditions (Hurst et al. 2010), the average size of newly settled flatfish captured in June of each year during this study corresponded with sizes of larvae settling in the experimental arenas. Newly settled NRS were also absent

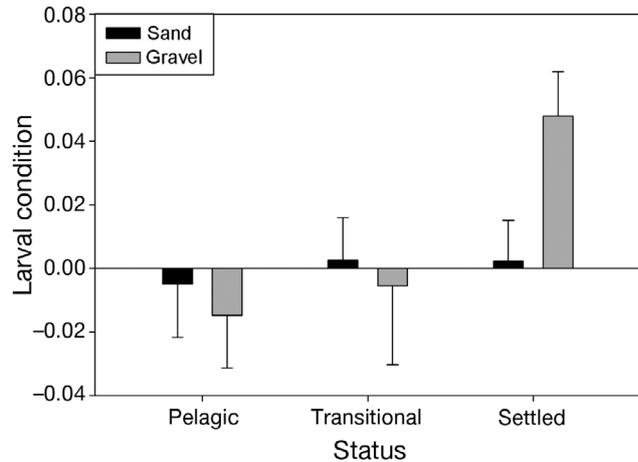


Fig. 7. Comparison of the condition of northern rock sole *Lepidopsetta polyxystra* larvae following the settlement experiment in which individuals were exposed to either sand or gravel habitat in experimental arenas (see Fig. 5). 'Transitional' refers to larvae that were observed in both settled and pelagic states during the 24 h trial period. Condition data are based on residuals of the linear regression of the length–dry mass relationship (Fig. 6) for larvae used in the settlement experiment (up to 20 mm total length)

during earlier May sampling periods. We are therefore confident that field sampling adequately captured the initial settlement distribution.

The habitat cues for settlement in Holiday Beach and Pillar Creek Cove were dependent on depth more so than on sediment type. Sediment characteristics were relatively uniform across a broad depth range (~0.1–0.2 mm granule size based on the phi scale; see Table 1), yet peak settlement occurred in a narrow, shallow depth region (~5 m) at both nurseries. The shallowest regions of the nursery were comprised of slightly coarser sediments (~0.2 mm), but not largely outside the size range rock sole preferred in the laboratory experiment. In the laboratory, rock sole increasingly avoided sediments of considerably larger grain sizes (>0.5 mm), likely because they have limited burial ability (Stoner & Ottmar 2003), leaving them exposed to higher predation risk (Ryer et al. 2010). Therefore, the restricted use of 1 depth region across a broader region of relatively uniform sediment characteristics suggests that other biological and physical processes are factoring into habitat decision-making during settlement. These include increased temperature and lower predator abundance as well as reduced competition for food with larger conspecifics and other flatfish species. The general pattern of shallow-water occupation by juvenile flatfish is well-documented for plaice *Pleuronectes platessa* (e.g. van der Veer & Bergman 1986) and dab *Limanda limanda* (e.g. Gib-

son et al. 2002) as well as several other flatfish species (e.g. Toole 1980, Allen & Baltz 1997, Armstrong 1997). Any impact on burial capabilities of NRS from slightly coarser substrates in the shallow portion of the nursery may be offset by 1 or more of these possible growth and survival advantages. However, the mechanism and cues for NRS settlement in the field are still in question.

Oceanographic conditions in early June may contribute to higher settlement in shallow regions. Although sampling was conducted over a short 3 d period in June, temperature between the surface and bottom of the water column varied by up to 2.5°C in deeper regions (>20 m) of the nurseries, whereas no stratification was detected at the 5 m depth contour where larvae were most abundant. Small decreases in temperature can have profound effects on growth rates of NRS larvae and can negatively impact their condition (Laurel et al. 2014). As such, larval NRS may remain in surface waters in deeper regions of the 2 nurseries. In the eastern Bering Sea, pelagic NRS larvae can encounter a 'cold pool' of <2°C bottom temperature in years of expanded winter ice coverage (Stabeno et al. 2012), which is hypothesized to be an important factor regulating ingress of larval NRS into preferred nursery habitats (Cooper et al. 2014). Follow-up studies on the behavior of NRS around thermoclines and varying sediment types would be useful in understanding the tradeoffs between pelagic and benthic habitat during settlement in this species.

Following the May to June settlement period, there was an apparent migration toward deeper areas of the nursery where finer, preferred sediment types were located. The increase in flatfish density in the deeper regions from June to July suggests that this pattern is due to movement more so than to mortality. However, the shift in distribution occurred gradually over months rather than immediately. Factors such as predator presence (Gibson et al. 2002, Ryer & Hurst 2008), food availability (B. Knoth unpubl.), and worm tube distribution (Laurel et al. 2012, Ryer et al. 2013) may inhibit rapid movement of smaller NRS, keeping them confined to shallow areas with coarser substrates. Gap crossing experiments within the laboratory indicate that newly settled NRS <20 mm TL will generally not move to more favorable habitats even if they are within 2 m of their starting location (B. Laurel & T. Zimmerman unpubl. data). These data reinforce the notion that settlement decisions by NRS larvae have significant influence on juvenile distributions and habitat associations in the nursery, which in turn can impact habitat and resource selec-

tion models currently in place for this species (Norcross et al. 1995, 1999, Stoner et al. 2007).

### Settlement plasticity

The habitat effects on size-at-settlement for NRS larvae observed in the laboratory, both across and within habitat types, have significant implications on dispersal potential. Cold-water species in general have slower growth and development rates and therefore longer pelagic larval durations (Bradbury et al. 2008). Based on the temperature-dependent growth model for NRS larvae, individuals at 7°C would require 5 to 6 wk more growth in the plankton if settlement was delayed from 12 to 18 mm TL (Laurel et al. 2014). As such, behavioral plasticity in settlement can be further exacerbated by environmental fluctuations that impact growth rates and size-at-age (Fernandez-Diaz et al. 2001). Therefore, single parameter estimates of pelagic larval duration and settlement may grossly over- or underestimate oceanographic dispersal in transport models for this species, especially at lower temperatures and across variable benthic habitats.

To our knowledge, our study is a rare example of marine fish larvae being able to extend their pelagic phase until suitable habitats are encountered. We should emphasize that we have no data on whether NRS larvae can also delay metamorphosis. Metamorphosis and settlement are not directly coupled in flatfish species, as one process can occur before or after the other is initiated (Geffen et al. 2007). In contrast, the 2 processes are tightly linked in many reef fish and marine invertebrate species that have a planktonic and benthic stage, such that behavioral control over settlement is synonymous with delayed metamorphosis (e.g. Victor 1986, McCormick 1999, Gebauer et al. 2003). Follow-up studies are therefore needed to examine whether behavioral decisions to settle influence developmental rates in NRS larvae.

The potential to delay settlement beyond 18 mm TL may be possible under other environmental scenarios. The range of settlement sizes observed in this study (~11–18 mm in the experiment; up to 21 mm TL in rearing tanks) is in agreement with an earlier growth study in our laboratory (Laurel et al. 2014), but field studies report occasional catches of larger NRS larvae in plankton nets (>21 mm SL; Orr & Matarese 2000). Therefore, other factors such as genetics or environment (Chambers & Leggett 1992) may contribute to further size variation in NRS settlement. Thermal stratification, ocean currents, or wind

events may restrict access to benthic habitats, forcing continued development in the planktonic environment. The maximum size or development stage to which NRS larvae can delay settlement is still unknown, but failure to reach suitable habitat within the optimal size, set either by physiology or the resources and predators in the nursery habitat, will likely increase mortality risk post-settlement (Morse et al. 1979, Pechenik 1990).

The role of energetics in settlement has not been well studied in flatfish, but changes in the condition of the organism can determine settlement competency in invertebrates (Kramer 1991). Brewster (1987) suggested that stored lipids need to increase prior to metamorphosis and settlement, but this has not been examined explicitly. The condition data from our study suggest that lipids are indeed increasing, but biochemical analyses will need to confirm these trends as length–weight residuals may reflect protein rather than lipid differences. The reason for increased energy demands likely stems from the developmental changes associated with metamorphosis and the corresponding ontogenetic niche shift. In the field, flatfish must often make a foraging switch from pelagic to benthic prey at settlement, resulting in reduced feeding performance by way of increased handling (Yufera et al. 1999) or digestibility of prey (Conway et al. 1993). At the same time, individuals need increased hormone production to undergo a rapid metamorphosis (Geffen et al. 2007). The decreased slope in the length–mass relationship of NRS larvae after 18 mm TL suggests that NRS larvae underwent a period of stress immediately following settlement. Although 2 food types were provided to larvae, this pattern unlikely reflected a prey switch, as larvae were almost exclusively eating dry particulate (Otohime) food after 13 mm TL. More likely, feeding rates decreased because of reduced search and capture ability of food following metamorphosis (e.g. eye migration; Osse & Van den Boogaart 1997) or reduced foraging behavior (e.g. Hamerlynck et al. 1989, Tanaka et al. 1996). Synoptic sampling of pelagic and benthic NRS larvae could determine whether changes in foraging and energetic costs occur naturally. However, post-settlement stress may explain why larvae settling in less favorable habitats were in better condition than those that remained in the water column.

## CONCLUSION

The combination of field sampling and lab experiments indicates that NRS select habitat at the time of

settlement and can delay settlement if suitable habitat is unavailable. Therefore, transport models for this species should consider settlement as a dynamic parameter with behavioral control rather than as a fixed, size-dependent endpoint. Because larvae did not select all regions of the nursery with suitable sediment characteristics, questions remain how larval behavior around oceanographic features and other biological components (prey availability or predators; sensu Gibson et al. 2002) further limits or increases the availability of juvenile nursery habitat. Such data, along with the results from our study, will help determine whether habitat availability at the time of settlement is a bottleneck to nursery recruitment and whether nursery function and productivity are limited by initial settlement dynamics.

*Acknowledgements.* This project was supported in part with funding from NOAA-AFSC's Habitat and Ecological Processes Research (HEPR) Program. We thank Ian Bradbury and Louise Copeman for providing feedback on earlier drafts of this manuscript. Mara Spencer and Paul Iseri collected and shipped broodstock from Kodiak, AK. Boat charters were kindly provided by Tim Tripp aboard the FV 'Miss O.' Whitney Clerf provided assistance in the maintenance and strip-spawning of broodstock in the laboratory, and Scott Haines headed rotifer production, feeding, and larviculture. A.J.B. was supported by a National Science Foundation Research Experience for Undergraduates internship under award OCE-0353083 to the Hatfield Marine Science Center of Oregon State University.

## LITERATURE CITED

- Allen RL, Baltz DM (1997) Distribution and microhabitat use by flatfishes in a Louisiana estuary. *Environ Biol Fishes* 50:85–103
- Armstrong MP (1997) Seasonal and ontogenetic changes in distribution and abundance of smooth flounder, *Pleuronectes putnami*, and winter flounder, *Pleuronectes americanus*, along estuarine and salinity gradients. *Fish Bull* 95:414–430
- Bradbury IR, Laurel B, Snelgrove PV, Bentzen P, Campana SE (2008) Global patterns in dispersal estimates: the influence of geography, taxonomic category and life history. *Proc R Soc Lond B Biol Sci* 275:1803–1809
- Brewster B (1987) Eye migration and cranial development during flatfish metamorphosis: a reappraisal (Teleostei: Pleuronectiformes). *J Fish Biol* 31:805–833
- Chambers RC, Leggett WC (1992) Possible causes and consequences of variation in age and size at metamorphosis in flatfishes (Pleuronectiformes): an analysis at the individual, population, and species levels. *Neth J Sea Res* 29: 7–24
- Conway DVP, Tranter PRG, Coombs SH (1993) Digestion of natural food by larval and post-larval turbot *Scophthalmus maximus*. *Mar Ecol Prog Ser* 100:221–231
- Cooper D, Duffy-Anderson J, Norcross B, Holladay B, Stabeno P (2014) Nursery areas of juvenile northern rock

- sole (*Lepidopsetta polyxystra*) in the eastern Bering Sea in relation to hydrography and thermal regimes. *ICES J Mar Sci* 71:1683–1695
- Fernandez-Diaz C, Yufera M, Canavate JP, Moyano FJ, Alarcon FJ, Diaz M (2001) Growth and physiological changes during metamorphosis of Senegal sole reared in the laboratory. *J Fish Biol* 58:1086–1097
- Gebauer P, Paschke K, Anger K (2003) Delayed metamorphosis in decapod crustaceans: evidence and consequences. *Rev Chil Hist Nat* 76:169–175
- Geffen AJ, Van der Veer HW, Nash RDM (2007) The cost of metamorphosis in flatfishes. *J Sea Res* 58:35–45
- Gibson RN, Robb L, Wennhage H, Burrows MT (2002) Ontogenetic changes in depth distribution of juvenile flatfishes in relation to predation risk and temperature on a shallow-water nursery ground. *Mar Ecol Prog Ser* 229: 233–244
- Hamerlynck O, Janssen CR, Landschoote E (1989) Fasting and feeding in late larval and early post-larval plaice (*Pleuronectes platessa* L.). *Rapp P-V Réun Cons Int Explor Mer* 191:465
- Hurst TP, Ryer CH, Ramsey JA, Haines SA (2007) Divergent foraging strategies of three co-occurring north Pacific flatfishes. *Mar Biol* 151:1087–1098
- Hurst TP, Abookire AA, Knoth B (2010) Quantifying thermal effects on contemporary growth variability to predict responses to climate change in northern rock sole (*Lepidopsetta polyxystra*). *Can J Fish Aquat Sci* 67:97–107
- Kramer SH (1991) Growth, mortality and movements of juvenile California halibut *Paralichthys californicus* in shallow coastal and bay habitats of San Diego County, California. *Fish Bull* 89:195–207
- Lanksbury JA, Duffy-Anderson JT, Mier KL, Busby MS, Stabeno PJ (2007) Distribution and transport patterns of northern rock sole, *Lepidopsetta polyxystra*, larvae in the southeastern Bering Sea. *Prog Oceanogr* 72:39–62
- Laurel BJ, Stoner AW, Hurst TP (2007) Density-dependent habitat selection in marine flatfish: the dynamic role of ontogeny and temperature. *Mar Ecol Prog Ser* 338: 183–192
- Laurel BJ, Ryer CH, Spencer M, Iseri P, Knoth B, Stoner A (2012) Effects of natural and anthropogenic disturbance on polychaete worm tubes and age-0 flatfish distribution. *Mar Ecol Prog Ser* 466:193–203
- Laurel BJ, Danley C, Haines S (2014) The effects of temperature on growth, development and settlement of northern rock sole larvae (*Lepidopsetta polyxystra*). *Fish Oceanogr* 23:495–505
- McBride EF (1971) Mathematical treatment of size distribution data. In: Carver RE (ed) *Procedures in sedimentary petrology*. Wilson Interscience, New York, NY, p 95–114
- McCormick MI (1999) Delayed metamorphosis of a tropical reef fish (*Acanthurus triostegus*): a field experiment. *Mar Ecol Prog Ser* 176:25–38
- Morse DE, Hooker N, Duncan H, Jensen L (1979) Gamma-aminobutyric acid, a neurotransmitter, induces planktonic abalone to settle and begin metamorphosis. *Science* 204:407–410
- Norcross BL, Holladay BA, Mueter FJ (1995) Nursery area characteristics of pleuronectids in coastal Alaska, USA. *Neth J Sea Res* 34:161–175
- Norcross BL, Blanchard A, Holladay BA (1999) Comparison of models for defining nearshore flatfish nursery areas in Alaskan waters. *Fish Oceanogr* 8:50–67
- Orr JW, Matarese AC (2000) Revision of the genus *Lepidopsetta* Gill, 1862 (Teleostei: Pleuronectidae) based on larval and adult morphology, with a description of a new species from the North Pacific Ocean and Bering Sea. *Fish Bull* 98:539–582
- Osse JWM, Van den Boogaart JGM (1997) Size of flatfish larvae at transformation, functional demands and historical constraints. *J Sea Res* 37:229–239
- Pechenik JA (1990) Delayed metamorphosis by larvae of benthic marine invertebrates: Does it occur? Is there a price to pay? *Ophelia* 32:63–94
- Ryer CH, Hurst TP (2008) Indirect predator effects on age-0 northern rock sole *Lepidopsetta polyxystra*: growth suppression and temporal reallocation of feeding. *Mar Ecol Prog Ser* 357:207–212
- Ryer CH, Stoner AW, Titgen RH (2004) Behavioral mechanisms underlying the refuge value of benthic habitat structure for two flatfishes with differing anti-predator strategies. *Mar Ecol Prog Ser* 268:231–243
- Ryer CH, Laurel BJ, Stoner AW (2010) Testing the shallow water refuge hypothesis in flatfish nurseries. *Mar Ecol Prog Ser* 415:275–282
- Ryer CH, Spencer ML, Iseri P, Knoth BA, Laurel BJ, Stoner AW (2013) Polychaete worm tubes modify juvenile northern rock sole *Lepidopsetta polyxystra* depth distribution in Kodiak nurseries. *J Exp Mar Biol Ecol* 446:311–319
- Stabeno P, Moore S, Napp J, Sigler M, Zerbini A (2012) Comparison of warm and cold years on the southeastern Bering Sea shelf. *Deep-Sea Res II* 65–70:31–45
- Stoner AW, Ottmar ML (2003) Relationships between size-specific sediment preferences and burial capabilities in juveniles of two Alaska flatfishes. *J Exp Mar Biol Ecol* 282:85–101
- Stoner AW, Titgen RH (2003) Biological structures and bottom type influence habitat choices made by Alaska flatfishes. *J Exp Mar Biol Ecol* 292:43–59
- Stoner AW, Spencer ML, Ryer CH (2007) Flatfish-habitat associations in Alaska nursery grounds: use of continuous video records for multi-scale spatial analysis. *J Sea Res* 57:137–150
- Tanaka M, Kawai S, Seikai T, Burke JS (1996) Development of the digestive organ system in Japanese flounder in relation to metamorphosis and settlement. *Mar Freshw Behav Physiol* 28:19–31
- Toole CL (1980) Intertidal recruitment and feeding in relation to optimal utilization of nursery areas by juvenile English sole (*Parophrys vetulus*: Pleuronectidae). *Environ Biol Fishes* 5:383–390
- van der Veer HW, Bergman MJN (1986) Development of tidally related behaviour of a newly settled 0-group plaice (*Pleuronectes platessa*) population in the western Wadden Sea. *Mar Ecol Prog Ser* 31:121–129
- Victor BC (1986) Delayed metamorphosis with reduced larval growth in a coral reef fish (*Thalassoma bifasciatum*). *Can J Fish Aquat Sci* 43:1208–1213
- Wilderbuer TK, Hollowed AB, Ingraham WJ Jr, Spencer PD, Conners ME, Bond NA, Walters GE (2002) Flatfish recruitment response to decadal climatic variability and ocean conditions in the eastern Bering Sea. *Prog Oceanogr* 55:235–247
- Yufera M, Parra G, Santiago R, Carrascosa M (1999) Growth, carbon, nitrogen and caloric content of *Solea senegalensis* (Pisces: Soleidae) from egg fertilization to metamorphosis. *Mar Biol* 134:43–49