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The effects of temperature on growth, development and settlement of northern rock sole larvae (*Lepidopsetta polyxystra*)

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ABSTRACT

Northern rock sole (*Lepidopsetta polyxystra*) is a commercially important fish in the North Pacific and a focal species in understanding larval transport to nursery grounds in the Bering Sea. However, the temperature-dependent vital rates and settlement dynamics for this species have not been described in detail. We reared northern rock sole larvae in the laboratory to measure growth, condition, development and settlement parameters across four temperatures (2, 4, 7 and 10°C). Both length and mass-measured growth rates increased with temperature and were best described by non-linear regression. Residuals of the length–mass relationships were positively related to temperature, indicating larval condition also increased with temperature. Larval development and settlement were largely size dependent, resulting in reduced larval stage duration and earlier settlement at higher temperatures owing to more rapid growth at elevated temperatures. However, larvae at colder temperatures were less developed at a given size, but more likely to settle at smaller sizes than larvae reared in warmer conditions. These temperature–response parameters can be used to refine current and future transport models for northern rock sole larvae under changing environmental conditions in the North Pacific.

Key words: climate change, dispersal, inner front, larval transport, metamorphosis, settlement dynamics

INTRODUCTION

Temperature is considered to be one of the most important factors regulating the vital rates of marine fish during their early life history (Miller *et al.*, 1988; Bailey and Houde, 1989; Hare and Cowen, 1997). Small variations in temperature can have profound effects on growth rates of larvae, which in turn impact pelagic larval duration (O'Connor *et al.*, 2007), exposure times to size-dependent predation (Atkinson, 1996) and size at which larvae develop into juveniles (Green and Fisher, 2004). Similarly, temperature-dependent developmental changes during the larval growth period affect distribution by way of improved sensory and swimming performance (e.g., Hewitt, 1981). Through these mechanisms, temperature can impact marine fish annually at the population level and shape biogeographic patterns over longer time scales (Laurel and Bradbury, 2006; Bradbury *et al.*, 2008; Houde, 2008). However, temperature response data in marine fish are highly variable and often limited to growth for larval stages (Jobling, 1997).

Flatfish larvae undergo a distinct metamorphosis and transition to the benthos (i.e., settlement) at the end of the larval period, approximating the point in time where large-scale, passive dispersal processes end (e.g., wind, currents and oceanic fronts) and small-scale, active dispersal processes begin (e.g., horizontal swimming and habitat selection). While the morphological changes associated with this have been reviewed extensively (Chambers and Leggett, 1987; Fuiman, 1997), the environmental factors controlling the onset of metamorphosis and settlement are poorly understood. Field studies tend to sample at a resolution too coarse to capture these processes *in situ*, but laboratory experiments suggest size, growth conditions and energetic reserves can contribute to variable onset of metamorphosis and settlement in some flatfish species (Geffen *et al.*, 2007). Therefore, temperature-mediated vital rates have broad ecological consequences for flatfish larvae in terms of dispersal, survival and successful recruitment to juvenile nursery areas.

The Alaska flatfish fisheries are one of the most productive in the world, represented by >10 species of

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soles and flounders. Of these, the northern rock sole (*Lepidopsetta polyxystra*: hereon referred to as NRS) is considered to be one of the most abundant flatfish, primarily distributed on the continental shelf of the eastern Bering Sea and the Gulf of Alaska (Orr and Matarese, 2000). NRS is a pleuronectid flatfish that was recently classified as a distinct species from southern rock sole (*L. bilineata*) (Orr and Matarese, 2000) but continues to be managed as a single stock in Alaskan waters where both species share an overlapping distribution. NRS spawn on the shelf in early spring (Stark and Somerton, 2002) and are a highly abundant species alongside walleye pollock (*Gadus chalcogrammus*) and Pacific cod (*Gadus macrocephalus*) in icthyoplankton collections from the Gulf of Alaska and Bering Sea (Matarese *et al.*, 2003; Doyle *et al.*, 2009).

NRS is 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.*, 2012, 2014). NRS larvae can experience a range of temperatures (2–8°C) across their geographic distribution in the eastern Pacific (Matarese *et al.*, 2003; Lanksbury *et al.*, 2007), and are exposed to interannual temperature variability that alternates between warm and cold conditions every 4–6 yr (Stabeno *et al.*, 2012). Under cold conditions (2005 until present), winter ice coverage sets up a spatially variable 'cold pool' of <2°C bottom temperature in the spring and summer, coinciding with the time of settlement in the Bering Sea (Cooper *et al.*, 2012). The failure of habitat resource models to predict distributions of post-settled juvenile NRS are thought to be as a result of such oceanographic conditions (Cooper *et al.*, 2014). Consequently, there is increased demand to understand the thermal response of NRS larvae (e.g., growth, pelagic larval duration and settlement) beyond descriptive and distributional information from icthyoplankton and beam trawl surveys (Matarese *et al.*, 1989; Orr and Matarese, 2000; Cooper *et al.*, 2014). Current temperature response data for NRS are restricted to the demersal egg (Laurel and Blood, 2011) and post-settled juvenile stages (Hurst and Abookire, 2006; Laurel *et al.*, 2007; Hurst *et al.*, 2010) when individuals are not in the plankton. Applying temperature-response models across ontogenetic stages is difficult because marine fish larvae tend to be more stenothermic than juveniles and growth rates are highly sensitive to size (Pörtner and Peck, 2010). Therefore transport models for NRS larvae are currently limited by available biological data.

The purpose of this study was to measure and describe temperature-dependent growth, condition, development and settlement of NRS larvae in the laboratory. From these data we also develop and parameterize models of size-at-age, size-at-metamorphosis and pelagic larval duration. These temperature-dependent parameters are intended for future refinement of larval transport models for NRS larvae under changing thermal regimes in the North Pacific.

MATERIALS AND METHOD

Broodstock collections

Adult northern rock sole (n = 25; 32–40 cm TL) were collected at a 30-m depth by trawl vessels in Chiniak Bay, Kodiak, AK (57°46′N, 152°21′W) during late August in 2009. Adults were transported to the shore and held without food for a 48-h period at the Kodiak Fisheries Research Center to prepare them for shipment to the Hatfield Marine Science Center (HMSC) in Newport, OR. Fish were placed in 10-L plastic bags filled with pure oxygen and <500 mL of filtered seawater and then packed into chilled coolers for 24 h of transport to the HMSC. Upon arrival, rock sole were transferred to a 3-m diameter round holding tank with sand substrate and held under a temperature/photoperiod schedule simulating conditions in Chiniak Bay. Fish were fed a combination of gel food and chopped herring/squid three times weekly during holding.

In 2011, 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 from excess milt and tissue. The use of a single female and multiple males did not rule out possible parental contributions to eggs that would affect survival between different batches of eggs (*sensu* Chambers *et al.*, 1989). However, our goal was not to determine the range of variation in egg characteristics. Rather, the experiment was designed to isolate the effects of temperature on the vital rates of larvae without introducing variation from maternal effects e.g., egg size and size-at-hatch.

Fertilized eggs were thinly dispersed as a single layer in a series of 4-L containers with 220 μm mesh sides and solid bottoms. Incubating containers were suspended into 1 × 1 × 0.5 m square tanks and supplied with temperature-controlled seawater (6°C) at a rate of 2–3 L min^{-1}. Gently lifting and lowering containers in the seawater bath twice daily achieved seawater exchange within each container. The onset of hatch occurred after 16 days of incubation, with high daily hatching occurring for an additional 6 days.

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) for growth experiments. Round tanks were initially held at the same temperature as the egg incubation temperatures during the transfer of larvae. Tanks were kept at a 12 : 12 light/dark photoperiod to approximate day length conditions experienced by rock sole larvae in the Gulf of Alaska in March–April. Water was supplied at a rate of 250 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. A total of 1500 larvae were transferred to each tank by 22 days post-fertilization (dpf). At 23 dpf, tanks were gradually adjusted to a rearing temperature (2, 4, 7 and 10°C) at a rate of ~2°C per day. A total of three replicate tanks were used for each of the four temperature treatments.

Tanks were supplied with nutritionally enriched rotifers (*Brachionus plicatilis*). Rotifers were enriched with Algamac 2000, an enrichment containing high percentages of essential long-chained fatty acids known to be important for North Pacific fish larvae (Copeman and Laurel, 2010). Rotifers were supplied at a density of 5 prey mL^{-1} 2× daily starting at 22 dpf. These densities sustain equivalent-sized Pacific cod and walleye pollock larvae at 8°C (Laurel *et al.*, 2011). The Otohime microparticulate dry food (Marubeni Nisshin Feed Co., Tokoyo, Japan) was also added to tanks 2× daily at 0.3 g $tank^{-1}$ from the start to the end of the experiment. The microparticulate diet is larger in diameter than the mean rotifer length (220–350 μm) and was anticipated to be important in faster growing fish as they switched to preferentially larger prey sizes. Day '0' of the growth experiment was considered to be 26 dpf when temperatures were fully adjusted and the first signs of feeding on live prey were apparent.

Larvae were sampled for morphometric and dry weight measurements at Day 0 and again at 7- to 14-day intervals from each tank (n = 10 larvae $tank^{-1}$). Sampling for growth was terminated before the onset of settlement, at 56 dph in the 7 and 10°C treatments and 80 dph in the 2 and 4°C, to reduce possible growth-related shifts associated with metamorphosis (Geffen et al., 2007). Sampled larvae were anesthetized in a solution of Tricaine methanesulfonate (MS-222) and individually photographed under calibrated magnification using a digital camera attached to a stereo microscope. Morphometric measurements were obtained from digital images using IMAGEPRO® software (Media Cybernetics, Bethesda, MD, U.S.A.). Morphometric measures included standard length (SL) and body depth of the myotome at the anus (BD) to the nearest 0.1 mm. The degree of gut fullness was also quantified on a scale of 0–3, with 0 indicating empty and 3 indicating full. Larval development was scored by the degree of observed tail flexion using the criteria established by Hawkyard *et al.* (2014): Stage 0 – straight notochord (no flexion); Stage 1 – straight notochord with the appearance of a caudal peduncle 'node' near the posterior end; Stage 2 – bent notochord with caudal peduncle formation near the posterior end; Stage 3 – bent notochord and initial envelopment of the notochord by the caudal peduncle i.e., the notochord did not protrude beyond the caudal peduncle; and Stage 4 – full envelopment of the notochord by the caudal peduncle with only a remnant of the bent notochord still visible.

Larval dry mass (DM) was taken on the 10 larvae imaged from each tank by pooling them 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 (Columbus, OH, U.S.A.). The mean individual larval DM was calculated by subtracting the pre-weighed foil and dividing by the total number of larvae on the sample.

After sampling for growth ceased, larvae were maintained at their rearing temperatures to observe the onset and rate of settlement. Behavioral measures of settlement were used because the developmental milestone of metamorphosis (e.g., eye migration and pigmentation) can occur before and after NRS settle to the bottom (B. J. Laurel, A. Basilio and C.H. Ryer unpub. data). The number of settled fish was counted approximately twice a week in each tank in each temperature treatment. Settled fish were allowed to remain in the tanks between observational periods as mortality was very low after the onset of settlement. All observations were conducted between the morning and afternoon feeding times i.e., 13.00–14.00 h. Fish laying flat on the bottom or sides of the rearing tank were classified as 'settled'. The total population (both pelagic and settled larvae) was also counted by direct visual observation approximately once a week. The weekly counts utilized the tank's center drain structure to provide a starting and ending point for a counter clockwise scan of larvae in the tank. Only one scan was conducted to count settled larvae because individuals were sedentary and restricted to one focal plane. Two scans were used to count pelagic larvae, the first scan for larvae at or near the water surface (0–5 cm)

and the second for larvae within the remainder of the water column. Because pelagic larvae were surface oriented, the two scan method allowed the observer to count the majority of larvae in the tank without adjusting the focal distance.

Settlement observations were terminated in the 7 and 10°C treatment when ~100% of the larvae were settled. Observations in the colder temperature treatments were terminated before the entire population settled owing to logistical constraints of maintaining slow-growing larvae for additional months in the laboratory.

Statistical analysis

The General Linear Model (GLM) was used to determine statistical differences in size-at-age as determined by standard length (SL mm) and dry mass (M mg) as a function of temperature. Data were transformed (e.g., LOG, ln) in cases where the residuals failed to meet the assumptions of normality of the GLM.

Growth rates were calculated for each replicate tank using the mean size of larvae measured at each sampling period. Growth in length (g_L, mm day^{-1}) and mass (g_M, mg day^{-1}) was calculated from the linear regression of the mean length and log-transformed dry mass against sampling date, respectively. A quadratic equation was fitted to describe the relationship between temperature and growth rate (both g_L and g_M) measured in each replicate tank (n = 3 per temperature) as the level of observation. Additional models were also fit to data to describe length and mass as a function of degree-days of development (*DD*), where *DD* is defined as the product of larval age and the associated mean rearing temperature. Models were based on known relationships of larval growth and development and initially limited to two parameters. Models were reduced to linear relationships after log-transformation if the 2nd model term contributed <2% of the explained variance.

A second set of analyzes was used to determine if temperature had an effect on larval condition. Alongside growth, condition data were used to: (i) assess future survival probabilities for NRS larvae under varying thermal regimes; and (ii) provide a possible mechanistic interpretation (i.e., energetic content) to temperature-dependent settlement. Larval condition was calculated from the residuals of the linear regression of the mean dry mass (log-transformed) on the standard length collected from all sampled larvae. These residuals were then plotted against the corresponding treatment temperature to visually observe for directional change in condition with rearing temperature. Mean responses from each replicate tank (n = 3 per temperature) were analyzed by ANOVA to determine if there was an effect of 'temperature' on larval condition in the experiment, using 'gut fullness' as a covariate.

Piecewise regressions (SIGMAPLOT 11.0, Systat Software, Inc, San Jose, CA, USA) were used to examine the temperature-dependence of size at development. Separate piecewise regressions were performed for each temperature treatment, using size (SL) as the explanatory variable and development (flexion index) as the response variable. The breakpoint parameter of the piecewise regression (t_1) indicated whether the size-at-onset of flexion for each temperature differed. Differences in slopes following the breakpoint provided an indication whether temperature had an effect on size-at-development after the onset of flexion.

Piecewise regressions were also used to determine the onset of settlement in each of temperature treatments, and these data were further used to calculate temperature-dependent settlement parameters. The breakpoint parameter of the piecewise regression was used as the age of first settlement (SET_{t1}, dph). Length- and mass-at settlement was calculated by substituting SET_{t1} into the size-at-age models for each temperature. The settlement rate (SET_b, % cohort day^{-1}) was based on the slope coefficient (b) of the piecewise regression and was subsequently used to calculate the number of days over which settlement occurred.

RESULTS

Size-at-age and growth

Corrected for sampling, there was no effect of temperature on percent larval survival at the end of the growth experiment ($F_{[2,11]}$ = 0.013; P = 0.564), but growth rates of NRS larvae significantly increased with temperature, both in terms of standard length (Fig. 1a; g_L, $F_{[2,11]}$ = 36.3, P< 0.001, Fig. 2a) and dry mass (Fig. 1b; g_M, $F_{[2,11]}$ = 41.3, P < 0.001; Fig. 2b). Larvae sampled at 56 dph in the 10°C treatment were still 6.3 times larger in terms of mass than larvae sampled at 104 dph in the 2°C treatment (1.45 versus 0.23 mg). A quadratic equation explained the relationship between growth and temperature (g_L and g_M, Fig. 2a,b; Table 1) although there was only modest curvilinearity observed over the entire experimental temperature range. The quadratic equation best described growth (i.e., dry mass) as a function of degree-days ($F_{[2,63]}$ = 526.8, P < 0.001; Fig. 3; Table 1).

Condition

Nearly 90% of the variation in mass was explained by length (r^2 = 0.87, P < 0.001; Fig. 4a). Note, the

Table 1. Best fit regression models for relationships between size and temperature in northern rock sole (*Lepidopsetta polyxystra*) larvae reared at four temperatures (2, 4, 7 and 10°C). Analysis was performed on mean larval sizes per replicate tank (n = 3 tanks per temperature). Original observations of sizes drawn from 10 larvae per replicate at each sampling period. Model selection criteria are described in Materials and Methods.

Predicted response	Predictor variable	Model type	Model	R^2
Log-transformed dry mass (M_{LOG})[1]	Standard length (L)	Linear	$M_{LOG} = -2.5292 + 0.2233 \cdot L$	0.87
Dry mass (M) in mg	Degree-days (D)	Quadratic	$M = 0.0821 - 0.0004 \cdot D + 0.0000049 \cdot D^2$	0.94
Growth (g_L) in mm standard length per day[2]	Temperature (T)	Quadratic	$g_L = 0.0272 + 0.0112 \cdot T - 0.003 \cdot T^2$	0.89
Growth (g_M) in mg dry mass per day[2]	Temperature (T)	Quadratic	$g_M = 0.0035 + 0.0025 \cdot T - 0.000007 \cdot T^2$	0.90

[1]Mass is the individual estimated dry mass (mg) based on pooled larvae (n = 10) for the sample period during which individual length (mm) measures were taken.
[2]Data for the model are based on model fits for length (Fig. 2a) and mass (Fig. 2b).

Figure 1. Relationships between (a) standard length (SL mm) and (b) dry mass (DM mg) and age in northern rock sole (*Lepidopsetta polyxystra*) larvae reared at four temperatures. Line fits are based on linear regression of the mean size per replicate versus age at each temperature. All data are shown but statistical analysis was conducted on mean sizes at the age of tank replicates.

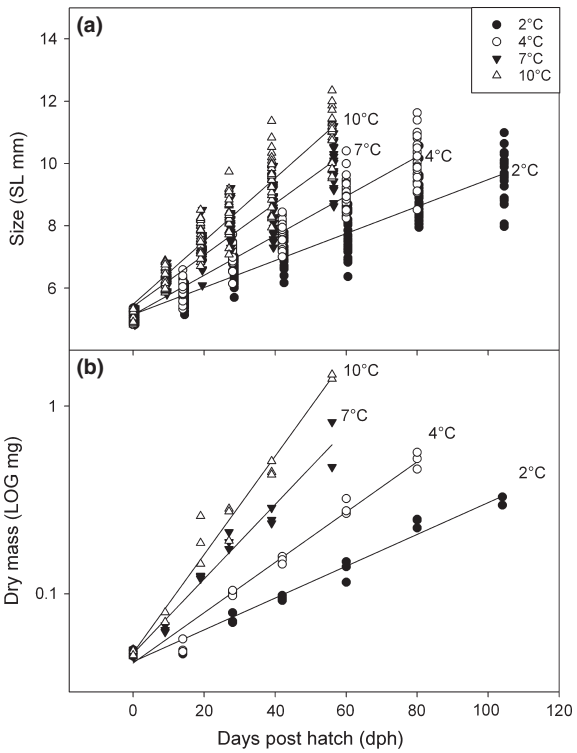
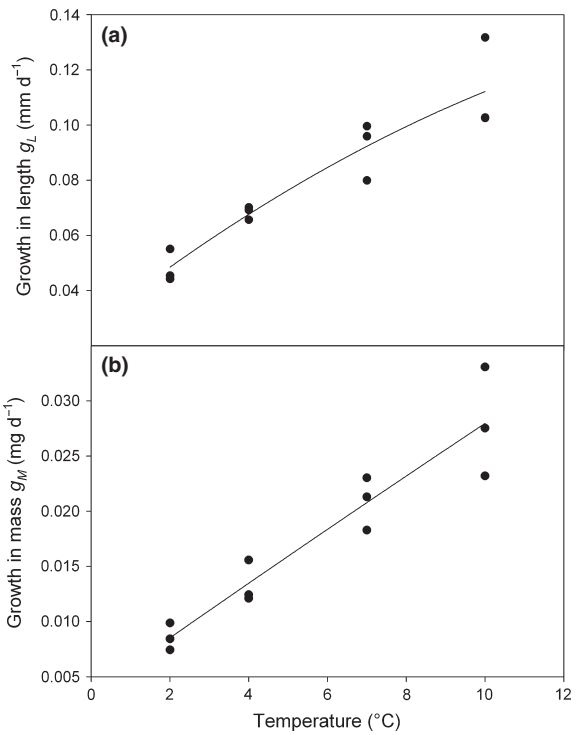
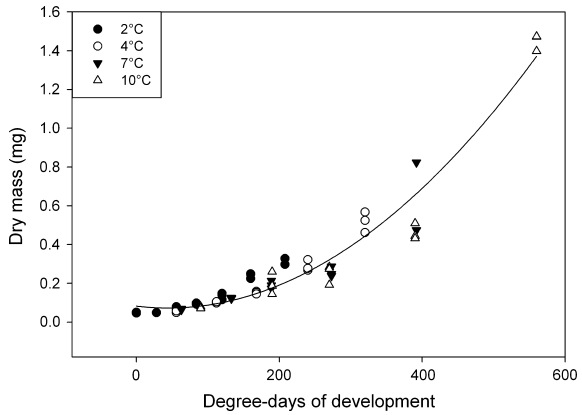


Figure 2. Predicted temperature-dependent growth of northern rock sole (*Lepidopsetta polyxystra*) larvae based on (a) standard length (SL mm) and (b) dry mass (DM mg). Data are mean larval growth rates per replicate tank at four temperatures, starting from hatch and ending before the onset of settlement.



residuals of the relationship were assumed to be solely owing to changes in fish condition rather than subtle differences in fish allometry. These residual were intended to be used as the dependent variable in a two-way ANOVA examining the effects of temperature and gut fullness on larval condition. However, gut fullness was dropped from the model because >95% of larvae had full stomachs after week 1. A direct effect of temperature on larval condition was detected (ANOVA, $F_{[3,11]} = 41.2$, $P < 0.001$; Fig. 4b).

Figure 3. The relationship between dry mass (DM) and 'degree-day' (rearing temperature × days post hatch) in northern rock sole (*Lepidopsetta polyxystra*) larvae. Data are mean larval mass per replicate tank at each temperature. Line fit is based on the quadratic equations (Table 1).



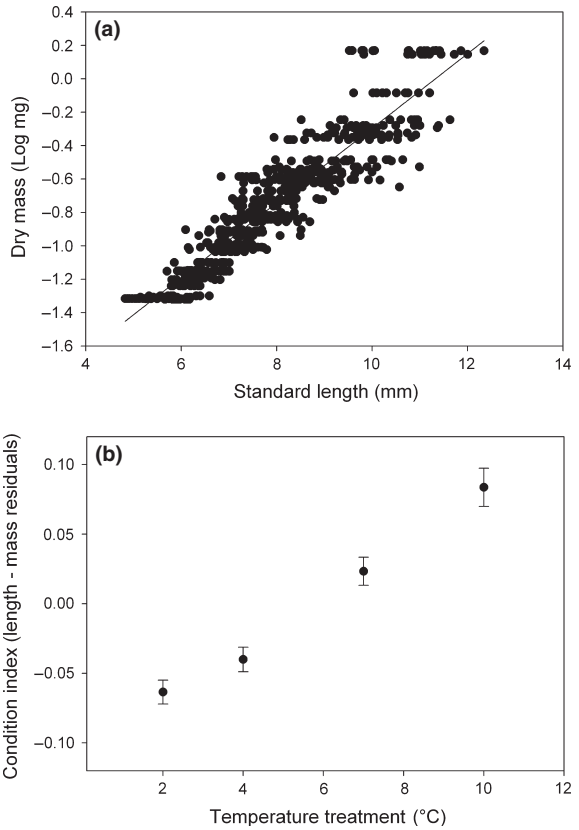
Development

Yolks were fully absorbed by the first sampling period in all temperature treatments i.e., 9 dph at 7 and 10°C; 14 dph at 2 and 4°C. Although temperature-dependent growth affected the day at which flexion began, the onset of flexion was predominantly size-dependent, occurring around 7 mm SL in all the temperature treatments (Fig. 5). However, after the onset of flexion, the piecewise regression indicated that size-dependent development was also a temperature-specific process (Fig. 5). Larvae in colder temperatures were less developed at a given size than warmer-reared larvae, as indicated by the shallower slopes after the breakpoint (Fig. 5). These trajectories indicate that NRS larvae metamorphose at a smaller size with increasing temperature. Note, onset of eye migration was only observed in the 7 and 10°C treatment, although these cases were limited to larger larvae (>10 mm SL) which, in spite of continued sampling to 80 dph, were not available for sampling from the 2 and 4°C treatments.

Settlement

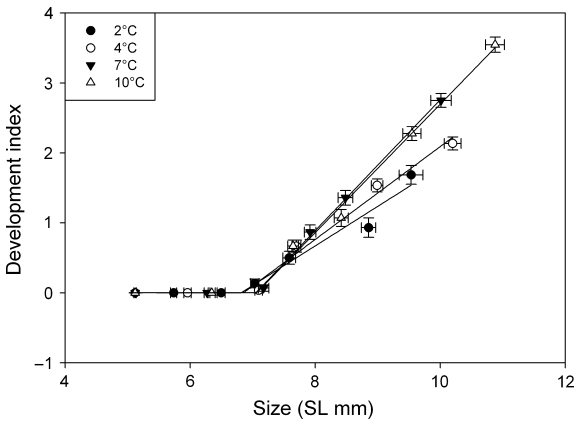
The timing, size and rate at which NRS larvae settled was temperature dependent, described by linear and non-linear models listed in Table 2. The experiment was terminated before the onset of settlement in the 2°C treatment, so this temperature treatment was not used to derive any of the settlement models. However, settlement parameter estimates for 2°C were estimated using the outputs from models derived from the remaining temperature treatments (Table 2). The breakpoint in the piecewise regression indicated

Figure 4. The relationship of (a) dry mass (DM mg) and standard length (SL mm) and the effect of (b) rearing temperature (°C) on condition for northern rock sole (*Lepidopsetta polyxystra*) larvae. The mass–length relationship in the top panel is based on all larvae sampled over the course of the experiment. Note, length data are presented as means (±1 SE) of the pooled larvae used in deriving dry mass data for each sample (See Materials and Methods). The condition index is based on the residuals of the mass–length relationship in (a). Data in (b) are tank means (n = 3) for each temperature ±1 SE.



settlement began at 67, 91 and 120 dph in the 10, 7 and 4°C treatment, respectively (Fig. 6). The model predicted settlement onset would delay to 136 dph at 2°C, and although no observations of settlement were observed before this period, the accuracy of this model prediction was not tested because the experiment was terminated at 138 dph. Size-at-settlement, both in terms of length and dry mass, was predicted to be smaller at colder temperatures based on growth trajectories and the predicted onset of settlement (Table 2). The rate of settlement (i.e., % of cohort settled day^{-1}) increased with increasing temperature (Table 2) and is visualized by the steeper slopes observed of the piecewise regression (Fig. 6). All NRS settled in the 10 and 7°C treatment over a period of 33 and 40 days,

Figure 5. The effects of temperature on development – size (SL mm) relationships in northern rock sole (*Lepidopsetta polyxystra*) larvae. The development index is based on the degree of observed tail flexion in individual larvae (See Materials and Methods). Lines represent piecewise regression fits for each temperature treatment. Larvae in colder temperatures were generally less developed at a given size than warmer-reared larvae, as indicated by the shallower slopes after the onset of flexion (denoted by the line breakpoint).



respectively. The experiment was terminated before all larvae settled in the 4°C treatments, but the slope of the piecewise regression indicated settlement occurs over a period 119 days at this temperature. Based on these outputs, an exponential decay model was derived and predicted a cohort of NRS larvae at 2°C temperature would need a period of 312 days to fully settle after the onset of settlement. However, we recognize that the settlement forecasts for 2 and 4°C are uncertain given they are beyond the observed data range from this study.

DISCUSSION

Growth, development and settlement rates of NRS larvae increased with temperature, typical of other cold-water marine fish species (Pepin, 1991; Jobling, 1997). Here we present the first description of these processes for NRS, an important commercial fish and focal species for larval transport in the Bering Sea. The additional effects of temperature on size-at-development, mass-length and size-at-settlement were unexpected, but potentially important components of survival and dispersal potential for this species under varying environments.

Growth and condition

Growth rates and condition of NRS larvae increased with temperature, and there were no sharp departures in either of the trajectories to suggest sub-optimal growth at the upper temperature range of the experiment (10°C). Therefore, it appears NRS larvae could potentially exploit the upper temperature ranges of the Gulf of Alaska and Bering Sea during the spring when average temperatures vary between 2 and 8°C (Matarese *et al.*, 2003; Lanksbury *et al.*, 2007). It is uncertain whether NRS larvae can efficiently grow at temperatures >10°C, as newly hatched larvae experience thermal stress at 12°C by way of higher rates of developmental deformations and inefficient conversion of yolk reserves to somatic growth (Laurel and Blood, 2011). Interestingly, the low mass-at-size of larvae in the colder treatments (this experiment) suggests that NRS larvae experience thermal stress at the lower end of their thermal range. Temperature-dependent condition data for fish larvae are rare (McCormick and Molony, 1995), so it is uncertain whether direct effects of temperature on condition are common in other species. One caveat to our data is that they reflect growth and condition under food unlimited scenarios. In food limited scenarios or prey 'mismatches', larval growth, condition and survival will likely be optimized at the lower or middle range of the species' thermal tolerance (Jobling, 1997). Many cold-water marine fish larvae have little to no measurable lipid reserves, and warm environments can increase starvation risk when prey availability is low during exogenous feeding (Pörtner and Peck, 2010; Laurel *et al.*, 2011). In the case of newly hatched NRS larvae, individuals can survive ~2 weeks longer in the absence of food at 2°C compared with 12°C owing to reduced metabolic demands on endogenous yolk reserves (Laurel and Blood, 2011).

Growth rates of NRS larvae appear to be less sensitive to temperature than other co-occurring Pacific species. Under identical rearing conditions, Pacific cod larvae grow similarly to NRS larvae at 2 degrees (~0.05 mm days^{-1}) but nearly twice as fast at higher temperatures (e.g., ~0.17 mm days^{-1} at 10°C versus ~0.11 mm days^{-1} for NRS); see Hurst *et al.* (2010) for Pacific cod growth rates. The growth rate of juvenile NRS is not as responsive to changes in temperature as observed in other Pacific flatfishes (Hurst and Abookire, 2006; Ryer *et al.*, 2012). Similarly, swimming and activity levels in juvenile NRS are less variable across temperature environments compared with Pacific halibut (Laurel *et al.*, 2007). These data suggest NRS have 'cold-adapted' physiology and may lose competitive advantages (e.g., outgrowing and evading predators) in warm environments.

Growth rates of larvae in this study were estimated by examining changes in the mean size-at-age of individuals sampled from a tank population over time. While this is a standard experimental approach in

Table 2. Best fit regression models for relationships between settlement parameters and temperature in northern rock sole (*Lepidopsetta polyxystra*) larvae reared at four temperatures (2, 4, 7 and 10°C). Settlement onset, rate and duration are based on the piecewise regression from Fig. 6. Length and dry mass estimates of larvae during the onset of settlement are based on model forecasts from the temperature-dependent size and mass relationships (Fig. 1) to the time of observed settlement.

Temperature (T) parameter	Onset of settlement (SET_{t1}) in days post hatch (dph)[1]	Size (SL mm) at settlement (SET_S)[2]	Mass (dry mg) at settlement (SET_M)3	Rate of settlement (SET_b) in % cohort day^{-1}4	No. of days over which cohort settlement occurs SET_{dur} as calculated by $100/SET_b$
2	136.0*	11.0**	0.605**	0.32*	312.5***
4	119.6	12.7	1.716	0.84	119.1
7	90.5	13.0	3.025	2.52	39.7
10	67.4	12.8	2.830	3.00	33.3
Model	$SET_{t1} = 153.4 - 8.7T$	$SET_S = -5.976 + 18.875\left(1 - e^{-1.1481T}\right)$	$SET_M = -2.198 + 5.341\left(1 - e^{-0.363T}\right)$	$SET_b = -0.4 + 0.36T$	$SET_{dur} = 27.668 + 902.676 \times e^{-0.576T}$

[1]Data calculated using the breakpoint parameter (t1) from the piecewise regression equation for each temperature from Fig. 6.
[2]Data calculated by substituting SET_{t1} for x in the regress equation for length-at-age for each temperature from Fig. 1a.
[3]Data calculated by substituting SET_{t1} for x in the regress equation for mass-at-age for each temperature from Fig. 1b.
[4]Expressed as (% cohort day^{-1}) based on the slope coefficient of the piecewise regression for each temperature from Fig. 6.
*estimated from the temperature model **based on the estimated SET_{ti} value ***based on the estimated SET_b value.

larviculture, growth rates can be biased upwards or downwards by way of size-selective mortality. Two lines of evidence suggest that this was not an issue in our experiment. First, although mortality of larvae was variable among tanks, there was very little variance in growth between tanks within the same temperature treatment. Second, the primary driver of size-selective predation (cannibalism) was unlikely in these reared populations of NRS as larval NRS have wide, laterally compressed bodies and relatively small mouths. We also observed no instances of cannibalism in the tank or from larvae examined during image analysis.

Development and metamorphosis

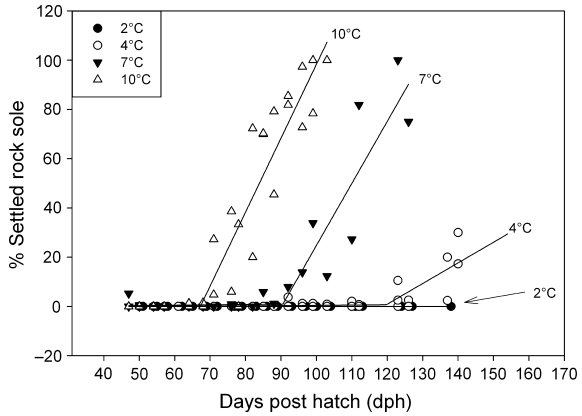
Growth and development were highly correlated in NRS and adhered to trends found in other species of larvae (e.g., Victor, 1986; Chambers and Leggett, 1987). However, NRS larvae were less developed at a given size under colder rearing conditions, indicating that temperature can influence development rates in this species by multiple mechanisms i.e., temperature-dependent growth and temperature-dependent size-at-development. Assuming larvae continued on their developmental trajectories, this would be the first temperature effect on size-at-metamorphosis described in a cold-water fish species (see Green and Fisher, 2004). However, NRS larvae will need to be sampled throughout the entire process of metamorphosis (e.g., complete eye migration) and after settlement to confirm these predictions.

The mechanism driving changes in size-at-development are uncertain. In aquaculture conditions, thyroid hormones can be administered to flatfish to decouple growth and development for the purposes of synchronizing metamorphosis and settlement (Schreiber, 2001; Gavlik *et al.*, 2002). However, the role of temperature on thyroid production has not been explored in larval flatfish (Power *et al.*, 2008). It is also possible the observed temperature effects are related to energetic reserves available to the larvae at the time of the earliest possible onset of metamorphosis. The residuals of the relationship between dry mass and length indicated rock sole larvae in the colder temperature treatments were in poorer condition and may not have had the energy to initiate such developmental changes at smaller sizes. Energetic constraints may explain the wide variation in size- and age-at-metamorphosis within flatfish species (Brewster, 1987). The role of temperature on the developmental processes associated with metamorphosis, either directly or indirectly by way of energetic reserves, growth rate and/or thyroid hormone, merits further study.

Settlement

Settlement is a critical period in the ontogeny of flatfish, as it marks a distinct transition between the pelagic (larval) and benthic (juvenile) phase. The processes controlling settlement are highly variable in flatfish, and are not necessarily fixed to one development, growth or size stage (Geffen et al., 2007). In our

Figure 6. The effects of rearing temperature on settlement rates in northern rock sole (*Lepidopsetta polyxystra*) larvae. Percentage settlement was defined as the percentage of larvae observed on the bottom or sides of the rearing tank for each sampling period. Data are fitted with a piecewise regression to denote onset of settlement (line breakpoint) and rate of settlement (slope) for each temperature treatment (exception 2°C where no settlement was observed throughout the experimental period).



study, settlement was both a temperature- and size-dependent process in NRS larvae. The size of first settling larvae did not vary considerably across the temperature treatments (11–13 mm SL), although the time needed to reach this size window was the lowest at high temperatures. These patterns adhere to size- and growth-dependent models for settlement of cold-water flatfish reared under a single temperature treatment (e.g., Chambers and Leggett, 1987; Chambers *et al.*, 1988; Gavlik *et al.*, 2002), and settlement sizes were similar to rock sole larvae observed in a separate study from the lab and field (B. J. Laurel, A. Basilio and C.H. Ryer unpublished data). However, the occasional catch of larger rock sole larvae in plankton nets (>20 mm SL; Orr and Matarese, 2000) indicate there is high variance around these mean sizes and suggest other factors (e.g., genetic, environmental) can shift size at settlement. Interestingly, we also detected a temperature effect on size at settlement. Based on growth trajectories, larvae settling in colder temperatures would be smaller than those reared in warmer conditions. In the case of the coldest temperature treatment (2°C), rock sole larvae were estimated to be ~2 mm smaller and ~20% the dry mass on average of larvae settling at 7–10°C. In addition, the size-at-development trajectories suggested cold-reared larvae settled at an earlier stage of metamorphosis than warm-reared larvae (see above). Follow-up studies will need to verify the model predictions of settlement, but such temperature effects raise several mechanistic and evolutionary questions. We speculate that energetic factors are an important contributing factor given the negative effect of temperature on larval condition. For example, settlement at a smaller size in cold conditions may be a means of increasing feeding opportunities and/or reducing physiological demands associated with swimming. Such trade-offs have been shown for invertebrate species and suggested for marine flatfish larvae (Kramer, 1991). In a separate study in which sediments were manipulated, rock sole larvae delayed settlement and settled at a larger size when preferred habitats were not available (B. J. Laurel, A. Basilio and C.H. Ryer unpublished data). These data suggest resources can be evaluated by pelagic NRS larvae, and size at settlement can vary across environmental conditions as a result of behavior.

The temperature effects on settlement, by way of regulating growth and development rates, have important bearing on successful delivery to nursery grounds and population connectivity. The temperature effects on pelagic larval duration and dispersal potential share similar directionality but are highly variable among marine species (O'Connor *et al.*, 2007; Bradbury *et al.*, 2008). The temperature response parameters from this study should therefore be useful in producing more accurate transport models for NRS larvae in the field. For example, NRS larvae in the Eastern Bering Sea can encounter a 'cold pool' of <2°C bottom temperature in years of expanded winter ice coverage (Stabeno *et al.*, 2012). Based on our temperature response data, larvae would not be able to grow fast enough in this cold pool to settle during their first summer, thereby supporting the hypothesis that the presence of this cold pool is an important factor regulating ingress of larval rock sole to coastal nursery areas (Cooper *et al.*, 2014). However, single parameter estimates of pelagic larval duration and settlement should be judiciously applied to oceanographic dispersal models for this species, especially at lower temperatures where variances around mean estimates were high. For example, settlement occurred over a period of ~30 days in the high temperature treatment whereas was predicted to occur over ~4 months at 4°C. Likely, this is as a result of variable growth and development within a temperature treatment (Burke *et al.*, 1999). Although our behavioral assessment of the tank population was a non-lethal means of examining settlement, it did not provide data on individual larvae. Complementary experiments will therefore be necessary to better resolve variance and address links between metamorphosis, behavior, growth and condition in this species.

Conclusion

Temperature impacted the early life history of NRS larvae in two ways. On one hand, temperature had a direct positive effect on growth and condition, thereby increasing size-at-age, which in turn decreased both the time to settlement and the developmental changes associated with metamorphosis. In contrast, temperature changed the size-dependency of development and settlement, such that larvae reared in colder environments would settle at smaller sizes and earlier stages of metamorphosis. Both effects of temperature should be considered in transport models as larval transport models may overestimate dispersal potential in this species by ignoring individual thermal histories and relying only on growth- and size-dependent effects of temperature.

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