

**Abstract**—Northern rock sole (*Lepidopsetta polyxystra*) is a commercially important flatfish in Alaska and was recently classified as a distinct species from southern rock sole (*L. bilineata*). Taxonomic and vital rate data for northern rock sole are still not fully described, notably at early egg and larval stages. In this study, we provide new taxonomic descriptions of late-stage eggs and newly hatched larvae, as well as temperature-response models of hatching (timing, duration, success), and larval size-at-hatch and posthatch survival at four temperatures (2°, 5°, 9°, and 12°C). Time-to-first-hatch, hatch cycle duration, and overall hatching success showed a negative relationship with temperature. Early hatching larvae within each temperature treatment were smaller and had larger yolk sacs, but larvae incubated at higher temperatures (9° and 12°C) had the largest yolk reserves overall. Despite having smaller yolks, size-at-hatch and the maximum size achieved during the hatching cycle was highest for larvae reared at cold temperatures (2° and 5°C), indicating that endogenous reserves are more efficiently used for growth at these temperatures. In addition, larvae reared at high temperatures died more rapidly in the absence of food despite having more yolk reserves than cold-incubated larvae. Overall, northern rock sole eggs and larvae display early life history traits consistent with cold-water adaptation for winter spawning in the North Pacific.

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## The effects of temperature on hatching and survival of northern rock sole larvae (*Lepidopsetta polyxystra*)

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Temperature is arguably the most important environmental influence driving development, growth, and survival of marine fish during their early life history (Pepin, 1991). In cold water marine systems, small fluctuations in temperature can have profound effects on an individual's vital rates, which at the population level can mediate connectivity patterns (Laurel and Bradbury, 2006; O'Connor et al., 2007), genetic structure (Bradbury et al., 2010), cohort survival, and eventual recruitment to the adult fish population (Houde, 2008). However, beyond the general positive relationships between temperature and poikilothermic metabolism (Jobling, 1997), the temperature response for fish is highly variable among species and populations, necessitating the measurement of temperature effects on a species-by-species basis. In commercially harvested species, this information is useful for estimating spawning stock biomass (using daily egg production methods; DEPM; Pena et al., 2010), measuring larval mortality (Pepin, 1991), and predicting recruitment (Houde, 2008).

Northern rock sole (*Lepidopsetta polyxystra*) is a commercially harvested marine fish in Alaskan waters and was recently classified as a distinct species from southern

rock sole (*L. bilineata*) (Orr and Matarese, 2000). Northern rock sole spawn earlier in the year (mid-winter) compared to southern rock sole (summer) in the region around Kodiak Island (Stark and Somerton, 2002). Pertseva-Ostroumova (1961) reported peak spawning of northern rock sole (as *Lepidopsetta bilineata bilineata*) off the east coast of Kamchatka from late March to early April and described egg and larval development at temperatures averaging 2.9–3.5°C. However, temperature effects on growth, mortality, and behavior have been principally restricted to the postsettlement phase (Hurst and Duffy, 2005; Laurel et al., 2007; Hurst et al., 2010). The egg and larval phases of eastern Bering Sea and Gulf of Alaska northern rock sole remain poorly understood, with the exception of descriptive studies on the taxonomy and distribution of *Lepidopsetta* spp. (Matarese et al., 1989, 2003; Orr and Matarese, 2000; Lanksbury et al., 2007).

The Gulf of Alaska and Bering Sea are experiencing extensive changes in environmental conditions, which in turn have affected seasonal temperature, ice extent, and larval prey production (Hunt et al., 2002). These changes raise concerns on how northern rock sole will respond directly to such environments. Developmental

data for northern rock sole are available for stocks in the western Bering Sea (Pertseva-Ostroumova, 1961), but these data, in addition to not being geographically representative of Alaskan waters, are incomplete to develop a full temperature-development model. Descriptions of late-stage eggs and newly hatched larvae are also absent for northern rock sole larvae in Alaskan waters despite their commercial importance. Therefore, the objectives of this study were 1) to provide a description of late-stage eggs and newly hatched northern rock sole larvae from laboratory reared specimens, and 2) to measure and model temperature effects on development rate, survival, and morphometric features (e.g., hatch length, condition, yolk volume) of eggs and newly hatched northern rock sole larvae from the eastern Pacific Ocean (Gulf of Alaska).

## Materials and methods

### Broodstock collections

Adult northern rock sole ( $n=25$ ; 32–40 cm total length [TL]) were collected at 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 shore and held without food for a 48-hour period at the Kodiak Fisheries Research Center (KFRC) 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 <500 mL of filtered seawater and saturated with pure oxygen, and then packed into chilled coolers for 24 hours during transport to the HMSC. Upon arrival, northern rock sole were transferred to a 3-m round holding tank with sand substrate and held under a temperature and photoperiod schedule simulating conditions in Chiniak Bay. Fish were fed a gelatinized combination of herring, capelin, and squid three times weekly during the holding period.

Males and females showed signs of sexual maturation starting in February that were similar to reported maturity schedules for adults sampled near our collection sites (Stark and Somerton, 2002). Mature males and females were injected with a luteinizing-hormone-releasing hormone (LNHRa) and strip-spawned 24 hours later by gentle squeezing of the abdomen. The gametes of ripe males ( $n=3$ ) and a female ( $n=1$ ) were combined in a clean, dry container for a 1-minute period before the addition of ambient seawater. Seawater was added repeatedly and decanted from the egg batches to rinse away 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 eggs and larvae. Fertilized eggs were held for a 6-hour period at 4°C and gradually adjusted over 24 hours to

temperature treatments of the experimental apparatus to initiate the experiments.

### Egg incubation

The rearing of northern rock sole eggs was conducted in a temperature-controlled, flow-through system consisting of four temperature treatments: 2°, 5°, 9°, and 12°C. Temperature-adjusted seawater was fed to a series of seawater baths (i.e., 1×1×0.5 m square tanks): four replicates of temperature treatments at 2°, 5°, and 9°C and three replicates at 12°C;  $n=15$  total tanks. Temperature-controlled seawater was supplied to each of the seawater baths at a rate of 2–3 L/min. A 4-L plastic egg incubation basket with 220- $\mu$ m mesh sides and solid bottom was placed within each temperature bath. Eggs were scattered in a thin layer over the bottom of each basket (2 mL of eggs per basket). Based on counts from 2-mL egg volumes ( $n=5$ ), the number of eggs equaled  $1701 \pm 42$  eggs (mean  $\pm 1$  standard deviation [SD]) per egg basket. An air stone was placed in each basket to increase water flow over eggs during the incubation period. In addition to the egg baskets, water baths were outfitted with 1-L mesh-bottomed containers to hold newly hatched larvae for observation beyond the hatching period. Gently lifting and lowering containers in the seawater bath twice daily achieved seawater exchange within each container. Before hatch, 50 eggs were preserved in a 5% buffered formalin solution, and measured to the nearest 0.01 mm with a dissecting microscope with transmitted light. All taxonomic descriptions egg and larval stages were based upon individual samples from the 2°C treatment.

### Experimental design

Egg baskets were checked daily during the course of the experiment for any signs of hatch. At the onset of hatch, larvae were counted daily and removed from the basket, a subsample of which was taken for morphometric measurements ( $n=10$ –18 larvae). However, to quantify “hatch quality,” all removed larvae were inspected for malformations (i.e., curved or twisted shape) before being discarded. Subsampled larvae were anesthetized with a 0.0005 ppm solution of tricaine methanesulfonate (MS-222) for measurement under a dissecting microscope. The following morphometric measurements were recorded: 1) standard length (SL); 2) myotome height at the anus (MH); 3) eye diameter (ED); and 4) yolk area. Precise length measurements (to the nearest 0.01 mm) were obtained with an image analysis system (Image-Pro Plus, Media Cybernetics, Bethesda, MD) connected to the microscope. Yolk area was determined by using the tracing tools of the image analysis software.

To determine mortality rates of unfed larvae, 100 larvae were transferred by pipette from each replicate egg basket into a corresponding 1-L plastic container with mesh bottom. Each 1-L container was suspended in a water bath, the temperature of which corresponded with that of the incubation baskets. This was done 1–2 days after the beginning of the hatch cycle to ensure

sufficient larvae were available for the containers and morphometric measurements. Daily mortality was measured by counting and removing dead larvae from each container bottom with a pipette.

### Data analysis

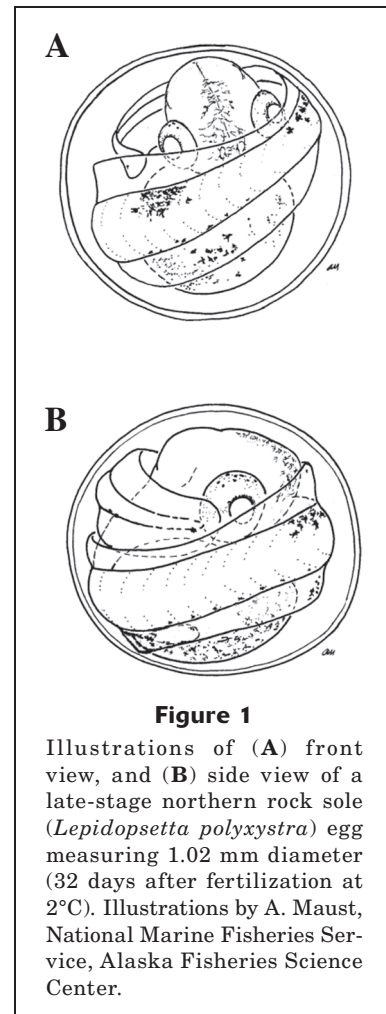
The general linear model (GLM) was used to determine statistical differences in size (SL, mm), yolk area (YA, mm<sup>2</sup>) and body depth (BD, mm) as a function of temperature and hatch rank. Hatch rank (HR) was calculated for each temperature treatment by dividing the hatching day by the total number of hatching days observed at that temperature. All analyses were performed on tank averages and residuals were checked to ensure the data met the assumptions of normality of the GLM. Data were plotted in three dimensions to capture the nature of significant trends and interactions.

Hatch characteristics (days to first hatch, hatch cycle duration, days to peak hatch, hatch quality, and hatch success), posthatch survival (i.e., time 50% mortality [ $M_{50}$ ]) and maximum size achieved by yolk sac larvae ( $S_{max}$ ) were all analyzed by using linear and nonlinear regression tools in SigmaPlot, vers. 10.1 (Systat Software, Inc., San Jose, CA). Model types (e.g., exponential, Gaussian, etc.) were initially selected on the basis of equivalent temperature relationships between eggs and larvae of other cold-water marine fish species (Jordaan and Kling, 2003; Laurel et al., 2008). The number of parameters in the statistical models were then chosen by using the Akaike information criterion (AIC; Akaike, 1974). The AIC value provides a relative goodness-of-fit to a range of models but penalizes models that use additional parameters to explain small amounts of variance. Models were ultimately selected if the correlation coefficient ( $R^2$ ) values were greater than 90% and their AIC values were within 2 of the minimum in the range of compared models. All model fits were performed on raw data.

## Results

### Taxonomic description of eggs and newly hatched larvae

Egg development from fertilization to middle-late stage (tail  $1\frac{1}{4}$ – $1\frac{1}{2}$  of the way around the yolk) has been described by Pertseva-Ostroumova (1961). Late-stage eggs from our study are 0.96–1.10 mm in diameter and overlap with reported egg sizes for the genus *Lepidopsetta* (0.86–1.08 mm; Orr and Matarese 2000). Just before hatching, the tail of the embryo is  $1\frac{1}{4}$  of the way around the yolk and the tail tip is even with the posterior margin of the eye. Pigment on the head is restricted to the snout, extending from just below the nares to the midbrain (Fig. 1A). The eyes are partially pigmented; a patch of larger melanophores is present on the anterodorsal quadrant and smaller fine melanophores are scattered over the dorsal half of the eye and concentrated along the upper rim of the lens (Fig. 1B).

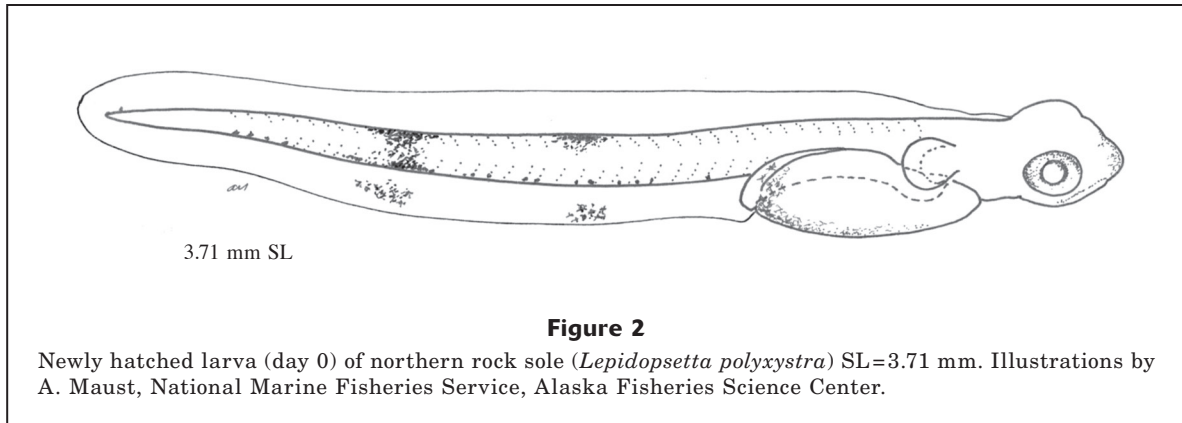


**Figure 1**

Illustrations of (A) front view, and (B) side view of a late-stage northern rock sole (*Lepidopsetta polyxystra*) egg measuring 1.02 mm diameter (32 days after fertilization at 2°C). Illustrations by A. Maust, National Marine Fisheries Service, Alaska Fisheries Science Center.

Dendritic pigment is present along the posterior dorsal gut margin, vent margin, and on the ventral yolk sac. A dorsal patch of pigment is located at 50% body length (BL) and a band of pigment is present at 75% BL; below both the patch and band is corresponding pigment on the anal fin. A row of postanal ventral melanophores (PVMs) extends from the anus to the band at 75% BL. A small group of spots is present on the ventral body margin near the last 2 or 3 myomeres and an additional 3 or 4 spots are located along the ventral margin of the notochord beyond the last myomere. The most posterior spot is near the end of the notochord.

Newly hatched larvae (Fig. 2) have light dendritic pigment on the snout from the midbrain to the edge of the lower jaw. Pigment outlines the lower jaw, which is not yet open. Eyes are moderately pigmented; pigment around the lens, in the dorsoanterior quadrant, and at the posterior edge of the eye is darker. The lower lateral and ventral yolk sac is lightly pigmented with dendritic melanophores. The same type of pigment is present on the posterior edge of the yolk sac and on the dorsal area of the hindgut close to the body. A



**Figure 2**

Newly hatched larva (day 0) of northern rock sole (*Lepidopsetta polyxystra*) SL=3.71 mm. Illustrations by A. Maust, National Marine Fisheries Service, Alaska Fisheries Science Center.

pigment patch on the dorsal half of the body is at 50% SL and a band is present at 75% SL. Below the patch and band are corresponding patches of pigment just above the ventral edge of the anal fin; anterior anal-fin pigment spots are more closely spaced than those within the posterior patch. A single row of PVMs is present starting at three myomeres posterior to the anus. These PVMs are spaced at approximately one per myomere and stop just beyond the pigment band at 75% SL. There are 4 or 5 PVMs along the last 3 or 4 myomeres. Pigment may be present on the notochord and is variable; there may be 1 or 2 spots on the ventral margin, 1 spot on the dorsal margin near the tip of the notochord, pigment only on the upper or lower margin, or no pigment may be present. Overall, northern rock sole larvae have less pigment on the postanal portion of the body than southern rock sole and can easily be differentiated. Southern rock sole larvae have an additional dorsal pigment patch 1–5 myomeres after the anus and a dorsal patch or caudal bar at the posteriormost myomere. Subsequent descriptions of preflexion, flexion, and postflexion stages of both rock sole species are found in Orr and Matarese (2000).

### Hatching patterns

Successful hatching was observed in all of the temperature treatments, but hatch patterns (time to first hatch, peak hatch, and hatch duration) were negatively related to temperature as indicated in the series of exponential decay, two-parameter models shown in Figure 3 (see Table 1 for model parameter estimates). Hatch quality and hatching success were also negatively associated with temperature (Fig. 4; Table 1), largely driven by the high numbers of malformed larvae (>50%) observed in the 12°C treatment. Malformed larvae were alive but curved in appearance and had poor swimming capabilities shortly after hatching. A subset of malformed larvae held over the course of the hatch cycle continued to swim poorly and did not appear to straighten out during the entire yolk sac period. Despite the malformation, these larvae sur-

vived approximately the same length of time in the absence of food as normally formed larvae held at the same temperature (~7 days; see below).

Size-at-hatch and yolk reserves (yolk area; [YA]) were a function of both temperature and timing in the hatch cycle. Overall, larval size-at-hatch ranged from 2.95 to 5.43 mm SL among all temperature treatments, but larvae were larger if they were late hatching or were incubated in colder water (Fig. 5). The maximum size-at-hatch achieved over the course of the hatch cycle occurred at 5°C (Fig. 6) and was best described by a Gaussian model (Table 1). However, the temperature effect was more apparent in late-hatching larvae as indicated by the significant interaction term (Table 2).

Yolk area also significantly varied as a function of temperature and hatch timing (Fig. 7), although the patterns were more variable than hatch size and there was no significant interaction between the two model terms (Table 2). Yolk reserves were larger in the early part of the hatch cycle and at warm incubation temperatures. However, although not statistically described in the model, larvae hatching on the second day of the hatch cycle in the 12°C treatment had larger yolk reserves than those hatching on day 1. In all other temperature treatments, larvae hatching on successive days had reduced yolk reserves.

Eye diameter varied between 0.22 and 0.33 mm and did not vary as a function of temperature or hatch rank (Table 2). However, larvae tended to have larger eyes later in the hatch cycle across all temperatures, although this was not statistically significant ( $P=0.066$ ). Larger and late-hatching larvae also appeared to have more eye pigmentation than small, early-hatching larvae.

The posthatch survival time of larvae was negatively temperature dependent (Fig. 8), ranging from 12 to 34 days among temperature treatments. Survival patterns followed a type-III functional response for each temperature treatment, and there was little variability among replicates. Plots of  $M_{50}$  (point of 50% mortality) with temperature were described with an exponential decay model with an  $R^2=0.99$  (Table 1).

**Table 1**

Types of models and estimated parameters for hatching patterns and posthatch survival of northern rock sole (*Lepidopsetta polyxystra*) larvae as a function of temperature (2°, 5°, 9°, and 12°C). Analysis was performed on tank means ( $n=3-4$  tanks per temperature) of 15–20 larvae for each tank during each sampling period ( $n=4-10$  sampling periods). Criteria for model selection are found in the *Material and Methods* section.  $R^2$ =correlation coefficient.

Variable	Model type	Equation and parameters	df	F	$R^2$	P
Days to first hatch	Exponential decay	$f(x)=37.516e^{-0.133x}$	1	353.010	0.97	<0.001
Hatch duration	Exponential decay	$f(x)=17.556e^{-0.119x}$	1	451.941	0.97	<0.001
Days to peak hatch	Exponential decay	$f(x)=46.499e^{-0.136x}$	1	593.968	0.98	<0.001
Hatch quality	Gaussian	$f(x)=104.872e^{-0.5\left(\frac{x-4.366}{6.023}\right)^2}$	2	55.873	0.90	<0.001
Hatch success	Gaussian	$f(x)=72.454e^{-0.5\left(\frac{x-2.194}{6.313}\right)^2}$	2	10.587	0.66	0.003
50% mortality ( $M_{50}$ )	Exponential decay	$f(x)=9.951 + 39.970e^{-0.391x}$	2	747.608	0.99	<0.001
Maximum size-at-hatch	Gaussian	$f(x)=4.377 + 0.826e^{-0.5\left(\frac{x-3.782}{3.916}\right)^2}$	3	29.336	0.89	<0.001

## Discussion

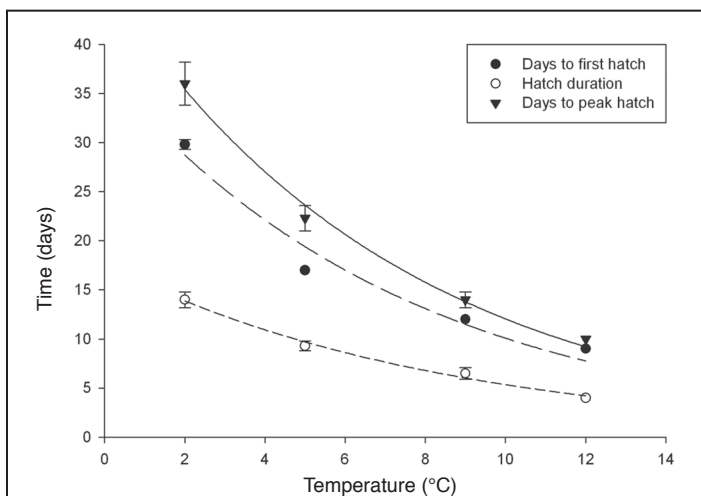
The temperature effects on hatching patterns (i.e., time to first hatch, hatch duration, and time to peak hatch) followed an expected negative relationship. Development rates among teleost fish are highly variable and largely a function of initial egg size (Pauly and Pullin, 1988), but temperature ultimately controls the development response curve within each fish species (Pepin, 1991; Jobling, 1997). Hatch synchrony in fish generally decreases with temperature (e.g., Ims, 1990), although it can be dependent to some extent on other environ-

mental variables (e.g., predation; Bradbury et al., 2004). Northern rock sole appear to follow this pattern, but comparisons of our laboratory data to field data were not possible because northern rock sole eggs are demersal and have not been collected from the wild. Larvae have been captured at the surface during April–August in the Bering Sea and Gulf of Alaska when surface temperatures can vary from  $-1^{\circ}$  to  $10^{\circ}\text{C}$  (Matarese et al., 2003).

Northern rock sole eggs are difficult to distinguish from co-occurring Pacific cod (*Gadus macrocephalus*) because they are similar in size (0.96–1.10 mm vs. 0.98–1.08 mm, respectively), demersal, semi-adhesive, and have a thick chorion. However, late-stage eggs of these species differ in several ways: yolk pigment is present in northern rock sole, but absent in Pacific cod, and postanal pigment on northern rock sole embryos consists of a dorsal patch at 50% SL and a band at 75% SL, whereas Pacific cod embryos have pigment bands at both 50% and 75% SL. Anal finfold pigment is present on northern rock sole embryos below the patch and band, but there is no anal finfold pigment on Pacific cod embryos.

Cold temperatures (2–5°C) produced larger larvae at time of hatching, but the effects of egg incubation temperature on hatch size in other fish species appear to be species-specific. Atlantic herring (*Clupea harengus*) and Pacific cod also produce larger larvae at low temperatures across a similar thermal range (Alderdice and Velsen, 1971; Laurel et al., 2008), whereas walleye pollock (*Theragra chalcogramma*), Atlantic silverside (*Menidia menidia*) and yellowtail flounder (*Pleuroctes ferrugineus*) tend to produce larger larvae at warmer temperatures (Bengston et al., 1987; Blood et al., 1994; Benoit and Pepin, 1999).

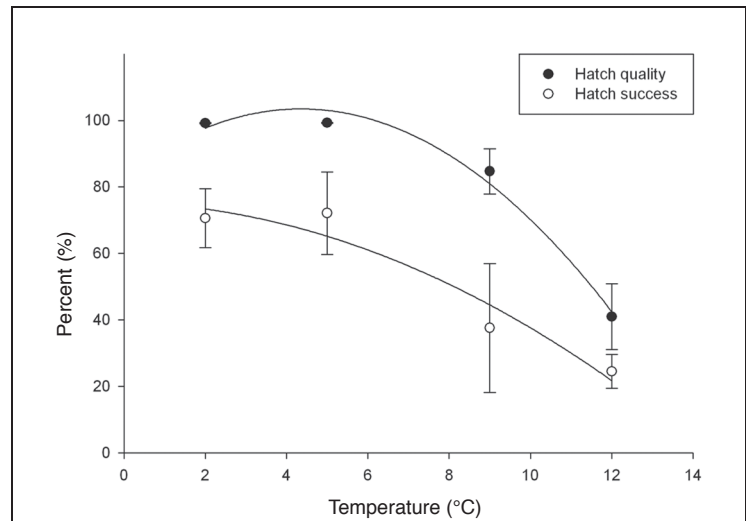
The effects of hatch rank (i.e., an individual's day-of-hatch within a batch of eggs) on size-at-hatch and yolk reserves have been measured in

**Figure 3**

The effects of temperature on the number of days to first hatching (closed circles), hatch duration (open circles), and timing of peak hatching (closed triangles) in northern rock sole (*Lepidopsetta polyxystra*) eggs. Data are means ( $\pm 1$  standard error [SE]) based on 2 mL of eggs in three replicate tanks at each of the following temperatures: 2°, 5°, 9°, and 12°C.

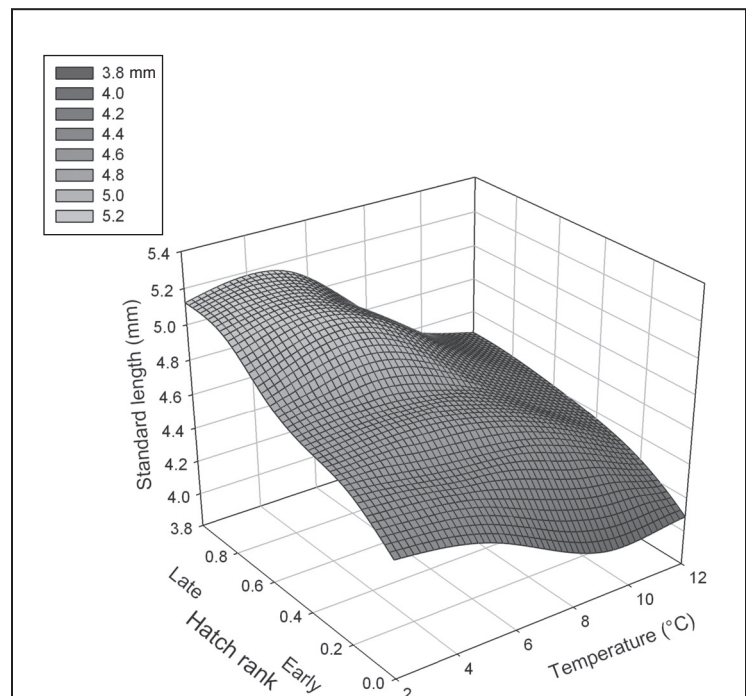
a number of species, but the ecological significance is poorly understood. Like northern rock sole, late-hatching larvae in other marine species are generally larger and have smaller yolk sacs (e.g., capelin [*Mallotus villosus*], Chambers et al., 1989; wolffish [*Anarhichas lupus*], Ringo et al., 1987). Methven and Brown (1991) also showed similar effects on hatch rank for ocean pout (*Macrozoarces americanus*), but this pattern was only observed at low temperatures when the hatching period was extended. Alternatively, late-hatching Atlantic silverside larvae are smaller than earlier hatching larvae (Bengston et al., 1987), although this occurrence appears to be a rare exception. Interestingly, despite the increased attention on parental effects on offspring size variation in marine fish (e.g., ~1 mm size range variation [3.8–4.8 mm SL] in Atlantic cod [*Gadus morhua*] larvae, Paulsen et al. 2009), the role of hatch rank and temperature cannot be ignored because they appear to account for an equivalent (if not more) amount of variability in offspring size from a single parent (~2.5 mm size range variation [2.95–5.43 mm SL], this study).

From an evolutionary perspective, the production of offspring of variable size and yolk reserves may be a bet-hedging strategy in a variable environment of temperature, food availability, and predator risk. However, in fish larvae, the survival benefits have seldom been tested explicitly beyond a few case studies. In walleye pollock, early-hatching larvae had higher growth potential than late-hatching larvae (Porter and Bailey, 2007). Similar results have been reported for early-hatching Atlantic herring larvae (Geffen, 2002), although this effect is short-lived because early- and late-hatching Atlantic herring larvae have overlapping size-at-age and growth trajectories shortly after the onset of exogenous feeding (Panagiotaki and Geffen, 1992). In general, smaller early-hatching larvae likely have under-developed sensory organs, swim capabilities, and digestive enzymes to immediately handle feeding exogenously (Porter and Bailey, 2007). As a tradeoff, these larvae may have an extended capability for surviving in the absence of food given their larger yolk reserves (Laurel et al., 2008). The latter feature also appears to be true for northern rock sole. Although eye diameter was not significantly larger in late-hatching larvae, there was a weak trend in increased eye diameter and increased eye pigmentation to indicate increased visual development with late hatching. More importantly, as has been shown with several gadid species, yolk reserves in northern rock sole were larger in early hatching larvae. Increased yolk reserves in early-hatching Pacific cod larvae allowed individuals to live 3–8



**Figure 4**

The effects of temperature on hatch quality (dark circles) and hatch success (open circles) of northern rock sole (*Lepidopsetta polyxystra*) eggs. Data are means ( $\pm 1$  standard error [SE]) based on 2 mL of eggs in three replicate tanks at each of the following temperatures: 2°, 5°, 9°, and 12°C.



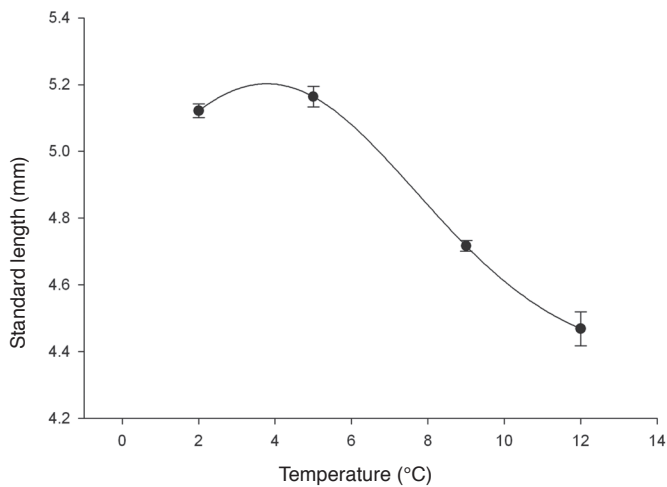
**Figure 5**

The effects of temperature and hatch time (days into hatch cycle) on northern rock sole (*Lepidopsetta polyxystra*) larval size-at-hatch (standard length [SL] mm). Values for SL are means ( $\pm 1$  standard error [SE]) based on image analysis of larvae taken from three replicate tanks (10–15 larvae sampled per tank).

**Table 2**

Results of the general linear model (GLM) on the effects of temperature and hatch rank on standard length (SL), yolk area (YA, mm<sup>2</sup>) and eye diameter (ED, mm) in newly hatched northern rock sole (*Lepidopsetta polyxystra*). Analysis was performed on tank means ( $n=3-4$  tanks per temperature) of 15–20 larvae for each tank during each sampling period ( $n=4-10$  sampling periods).

Source	df	F	P
<b>Standard length (SL mm)</b>			
Temperature	1	76.26	<0.001
Hatch rank	15	25.87	<0.001
Temperature×hatch rank	1	4.08	0.048
Error	63		
<b>Yolk area (YA mm<sup>2</sup>)</b>			
Temperature	1	4.49	0.038
Hatch rank	15	23.81	<0.001
Temperature×hatch rank	1	2.67	0.108
Error	63		
<b>Eye diameter (ED mm)</b>			
Temperature	1	0.14	0.710
Hatch rank	15	1.74	0.066
Temperature×hatch rank	1	0.56	0.457
Error	63		

**Figure 6**

Maximum size-at-hatch achieved by northern rock sole (*Lepidopsetta polyxystra*) larvae as a function of temperature (2°, 5°, 9°, and 12°C). Size data are based on means ( $\pm 1$  standard error) of maximum size observed among replicate tanks ( $n=3$ ) for each temperature of early and late-hatching larvae. Data for each hatch period were fitted with a peak, Gaussian, 4-parameter nonlinear regression shown in the figure panel.

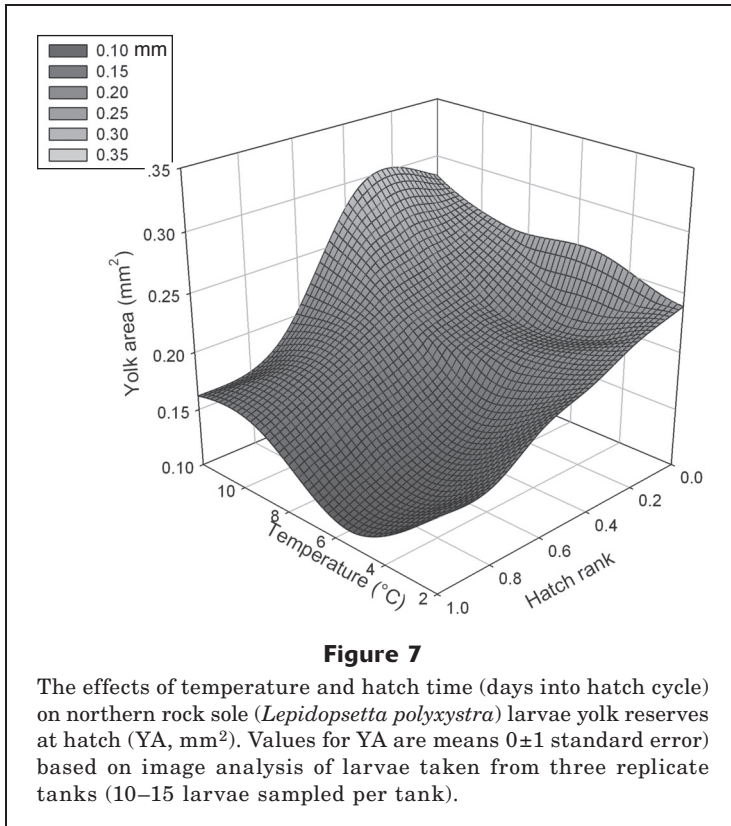
days longer than late-hatching larvae depending on the incubation temperature (Laurel et al., 2008).

In Pacific cod, early-hatching eggs survived longer as free-swimming larvae in the absence of food than did late hatching eggs, but hatch rank had no overall effect on time-to-starvation from the point of fertilization (Laurel et al., 2008). In other words, early hatching larvae survived longer as larvae, whereas late-hatching larvae survived longer as eggs. Early hatching larvae may gain more experience handling and ingesting prey before they need to feed, or may experience higher growth potential than late-hatching larvae (Porter and Bailey, 2007).

The upper range of thermal tolerance of northern rock sole larvae appears to be around 12°C as evidenced by the precipitous decline in hatch quality at this temperature. In the wild, northern rock sole larvae seldom experience temperatures  $>6^{\circ}\text{C}$  because spawning occurs in mid-winter and early spring in Alaskan waters (Stark and Somerton, 2002). Northern rock sole adults are also generally restricted to higher latitudes, from the northern coast of Hokkaido to the Okhotsk Sea in the western North Pacific Ocean, the Bering Sea near St. Lawrence Island and south to the shelf areas of the Gulf of Alaska (Mecklenburg et al., 2002). Collections of larvae have extended as far north as the Chukchi Sea and outer shelf areas of the Bering Sea where water temperatures can be below 0°C (Matarese<sup>1</sup>). In contrast, southern rock sole adults are distributed further south (southeastern Bering Sea along the Alaska Peninsula and throughout the shelf areas of the Gulf of Alaska to Baja California), with the most northernmost extent (rare) being documented at around 59°N (just south of Nunivak Island). Where northern and southern rock sole overlap in Alaska, southern rock sole spawning generally occurs later in the warmer summer months (Stark and Somerton, 2002). The poor hatching performance of northern rock sole at 12°C, along with the contrasting spatial and temporal distribution of northern and southern rock sole in the field, suggest temperature tolerance may be an important environmental variable reducing gene flow between these closely related pleuronectids.

It was interesting to note that despite having larger yolks, larvae in the warm water treatments starved more quickly and were not able to mobilize yolk reserves as efficiently into growth as larvae in cold temperature treatments. The growth performance in poikilotherms is generally optimized at the lower range of the organism's thermal tolerance under low-food situations (Jobling, 1997), but there is a lack of such studies at life stages when organisms are dependent on endogenous resources. In a review of more than 100 marine fish species, Pepin (1991) found that time to starvation decreases with temperature,

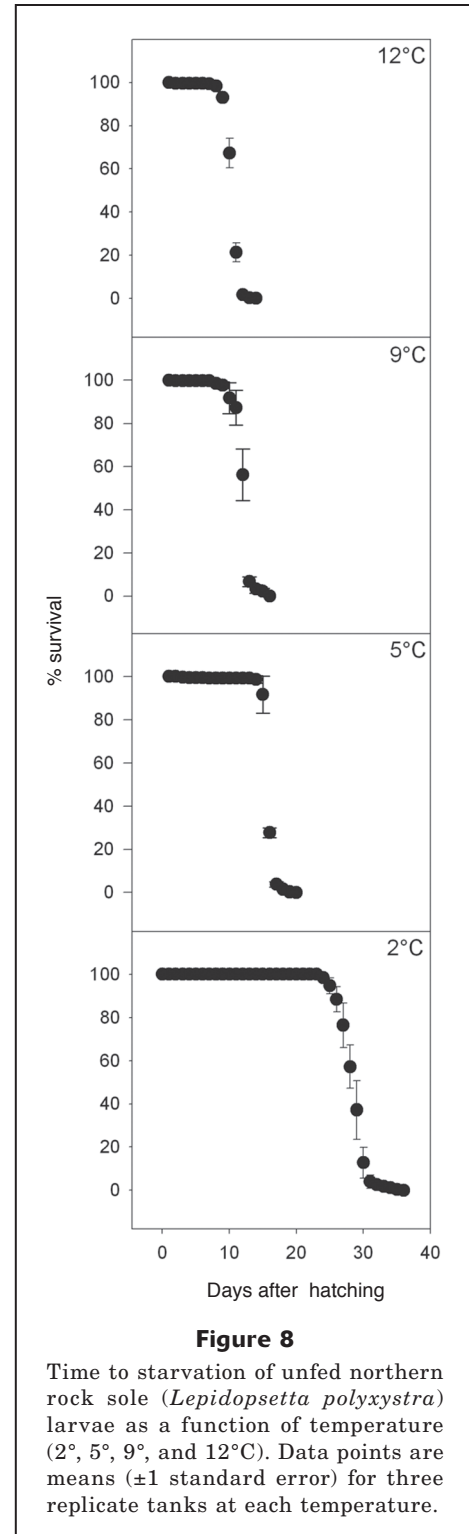
<sup>1</sup> Matarese, A. 2010. Unpubl. data. Alaska Fisheries Science Center, Seattle, WA 98115



but the incubation temperature, by way of mediating yolk reserves, may be an important factor in this relationship. Regardless, it appears that northern rock sole larvae have physiological adaptations to maximize the use of endogenous resources under cold conditions (i.e.,  $\leq 9^\circ\text{C}$ ). Similarly, the growth rates and swimming performance of juvenile northern rock sole remain relatively high at cold temperatures compared to other Alaskan flatfish species (Hurst and Abookire, 2006; Laurel et al., 2007).

**Conclusion**

Temperature and hatch rank had distinct effects on size-at-hatch and yolk reserves, but the effects on post-hatch survival were not fully explored. Temperature had multiple influences on northern rock sole larvae by affecting developmental rates, size-at-hatch, and metabolic demands on yolk reserves. In the absence of predators, optimal incubation temperatures for northern rock sole eggs and prefeeding larvae appear to be  $\sim 2\text{--}5^\circ\text{C}$ . However, the benefits of successful hatching, increased size-at-hatching, and reduced risks of starvation need to be weighed against possible increased predation risk (e.g., stage dependent mortality; Houde, 2008) and dispersal potential (O'Connor et al., 2007) at low temperatures. In addition, it will be interesting to compare these data with those of southern rock sole, especially since differing thermal responses may be an



important regulator of adult latitudinal distributions and seasonal spawning patterns between these species. Collectively, these questions will be important areas of research in the light of changing environmental conditions in the North Pacific.



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