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# The effect of early and late hatching on the escape response of walleye pollock (*Theragra chalcogramma*) larvae

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*Hatching of fish eggs fertilized at the same time occurs over a period of several days. Differences in the escape response of fish larvae during the hatching period have not hitherto been studied. In this study, the escape response of walleye pollock (*Theragra chalcogramma*) larvae over the hatching period was examined. Escape speed, response to multiple touches with a fine probe, response to water currents generated by a predator and predation by euphausiids (*Thysanoessa inermis*) and amphipods (*Pleusirus secorrus*) were measured in the laboratory. Otolith measurements of field-collected larvae support a broad hatching period for walleye pollock eggs in the sea similar to that observed in the laboratory. The escape response of walleye pollock larvae was affected by rank in the order of hatching, thus with respect to predation, hatching order may affect the survival of larvae in the sea. Early hatching larvae were smaller, less sensitive to tactile stimulation, had a slower, weaker escape response and higher laboratory rates of predation mortality than those that hatched later.*

## INTRODUCTION

The hatching period of fish eggs fertilized at the same time spans several days, which may have some important implications for the survival rates of the larvae. Fish larvae that hatch earlier are smaller and have larger yolks than larvae from the same cohort that hatch later (Methven and Brown, 1991; Geffen, 2002). In laboratory studies, late-hatching ocean pout, *Macrozoarces americanus*, experienced less mortality and grew more slowly after hatching than those that had hatched earlier (Methven and Brown, 1991). In the case of herring, *Clupea harengus*, embryo growth in the egg was decreased when compared with embryos that were intentionally released early from eggs (Geffen, 2002), but there was no size disadvantage to hatching early because by the end of the hatching period, the larvae that were intentionally released were of similar size as those that hatched late (Geffen, 2002).

Predation is an important source of mortality for marine fish eggs and larvae. Predation mortality is inversely related to larval size (Bailey and Houde, 1989), and early developmental stages of fish larvae are more vulnerable to predation than later stages (Bailey and Batty, 1984; Sugisaki *et al.*, 2001). Development of larval sensory mechanisms plays an important role in detecting and escaping predators. Many teleosts lack eye pigmentation at hatching and the eyes are probably nonfunctional at this time (Blaxter, 1986), including those of walleye pollock, *Theragra chalcogramma*, larvae (Porter and Theilacker, 1999). Thus, for fish larvae at hatching, the mechano-sensory system most likely plays the major role in eliciting the escape response. The most important mechano-receptors for eliciting an escape response are thought to be the otolithic receptors of the ear, naked neuromasts, the lateral line, and the Rohon–Beard cells (Eaton and DiDomenico, 1986). At hatching, free neuromasts are located on the head and trunk in varying numbers, depending on the species (Blaxter and Fuiman, 1989). In Pacific cod, *Gadus macrocephalus*, some neuromasts may be functional before hatching and others may not be functional until some

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time after hatching (Otsuka and Nagai, 1997). The neuromasts on the trunk of Pacific cod embryos are functional earlier than those on the head (Otsuka and Nagai, 1997), and the number of free neuromasts increases as the larvae grow (Blaxter, 1984; Otsuka and Nagai, 1997). There is evidence that the free neuromasts may have a connection to the Mauthner cells or other reticulospinal cells to induce a C-start escape response (Blaxter and Fuiman, 1989). In the embryonic and early stages of amphibian and fish larvae development, Rohon–Beard cells are the first sensory neurons to innervate the skin (Roberts and Hayes, 1977; Whiting *et al.*, 1992). They make up an important tactile sensory network located under the skin, and this network is later replaced by neurons from the dorsal root ganglia as larvae develop (Roberts and Hayes, 1977).

We hypothesized that the escape response of early hatching fish larvae is not as well developed as those that hatch later and that they may thus be more vulnerable to predation. We chose walleye pollock (*T. chalcogramma*) for this study because of accumulated information about its development and early life history. Walleye pollock is a commercially important fish species in the Bering Sea and Gulf of Alaska. Walleye pollock eggs are pelagic and the time it takes for a single cohort of eggs to completely hatch is strongly dependent on temperature. Duration of the hatching period ranges from 240 h at 2.8°C, to 60 h at 5.7°C (Blood *et al.*, 1994; Blood, 2002). Walleye pollock larvae from the same group of fertilized eggs that hatched later were larger than those that had hatched earlier (Blood, 2002). In the sea, just-hatched walleye pollock larvae are exposed to many invertebrate predators, and euphausiids and amphipods have been shown to be major predators of yolksac walleye pollock larvae in the Gulf of Alaska (Bailey *et al.*, 1993). The escape response of walleye pollock larvae to attacks by predators rapidly improves after hatching (Sugisaki *et al.*, 2001). However, differences in the escape response of walleye pollock larvae (or other species) during the hatching period have not been examined. In this study, we examined the differential escape responses of walleye pollock larvae from a single cohort of eggs over the hatching period. We also present otolith measurements from wild and laboratory-reared larvae to support the observation that the broad hatching period in the laboratory occurs in wild fish.

# METHOD

## Egg collection and rearing

Adult walleye pollock were collected by trawl in Shelikof Strait, Gulf of Alaska, by the NOAA ship Miller Freeman during the spawning season in March 2002, 2004 and 2005. In 2002, eggs from two females were fertilized with sperm from three males. In 2004 and 2005, single parent fertilizations (eggs from one female were fertilized with sperm from one male) were used to eliminate possible maternal effects that may be present when eggs from different females are mixed together. The purpose of using both single and multi-female egg groups was not to test for differences between them, but to confirm that results were similar between a mixture of eggs and eggs from single parentage. Fertilized eggs were maintained aboard ship in the dark at 3 ± 0.3°C for a few days before being transported to the Alaska Fisheries Science Center, Seattle, WA, USA in chilled thermos bottles. For the rest of the egg stage, they were incubated at 6 ± 0.2°C in the dark in 4 L glass jars filled with 3 L of filtered seawater (33 PSU).

## Experiments

One set of experiments was conducted in 2002, and two sets of experiments were done in 2004 (2004A and 2004B, using two different single parent fertilizations). Larvae reared in 2005 were utilized to increase the number of replicates used in the euphausiid predation experiments (see Euphausiid and Amphipod Predation). All experiments were conducted at 6 ± 0.2°C in a temperature-controlled room. To track the progress of hatching of each group, 100 eggs were placed into each of 3, 1 L jars, the jars were checked every 24 h and the number of larvae that had hatched was recorded. The remaining eggs were kept in 4 L jars (approximately 1000 eggs per jar) covered with black plastic and larvae used in the experiments were obtained from these stocks. At the end of each day, all larvae were removed from the jars with a pipette so that only eggs remained; for the following day's experiment, larvae that had hatched during the night were used or they were placed into separate 4 L jars for retesting at a later time; 5 to 10 larvae were measured from each hatching group to determine standard length (SL). ANOVA and Tukey or Student–Newman–Keuls (SNK) multiple comparison tests were used to compare sizes among hatch days.

## Video-taped observations of escape speed and reaction to water current

Ten to 20 larvae were placed in a 9.0 cm diameter by 1.8 cm deep Petri dish filled 1 cm deep with filtered (pore size 1 μm) seawater to record escape speed on videotape. Larvae were transferred using a wide bore pipette to avoid injuring them. A video camera (COHO 4810 series monochrome frame transfer CCD) was mounted above the Petri dish containing the larvae and

an infrared light source was mounted below the dish. An overhead fluorescent light fixture provided visible light (0.9 μmol photon $m^{-2}$ $s^{-1}$ light level using full spectrum light bulbs). Larvae were touched on the side of the trunk/tail region with a fine (0.5 mm) probe to elicit an escape response. An escape response was defined as the larva swimming away from the probe when touched. A total of 50 larvae were tested in each experiment. The larvae were tested on the day of hatching and later at 1, 2 and 4 days after hatching in 2002 to examine developmental changes of the escape response. In 2004, observations were recorded on the day of hatching and 3 and 4 days after hatching. The fish tested after hatching were not the same individuals tested on the day of hatching.

We measured the escape speed of walleye pollock larvae during the first 200 ms after contact with the probe. Williams and Brown (Williams and Brown, 1992) showed that for winter flounder larvae (age 1 and 10 days), maximum escape speed occurred during the first 200 ms after contact with an amphipod. Although the maximum escape speed for walleye pollock larvae may not occur during this time, this time frame would be expected to provide an escape speed near maximum. To calculate the escape speed of walleye pollock larvae, escape response sequences from videotape were converted to digital files and Image-Pro® Plus image analysis software (Media Cybernetics, Inc.) was used to measure the distance a larva swam during the first six frames of the escape response (each frame is 1/30 of a second). For each experiment, 9 to 10 individual escape speeds were calculated. Only escape sequences where the larvae did not swim into the edge of the Petri dish were used. Differences in escape speeds were examined using linear regression, ANCOVA, the two-sample *t*-test, ANOVA and the Tukey multiple comparison test.

After recording escape speeds, the same individuals were tested for response to water currents created by a tethered euphausiid (*Thysanoessa inermis*, length = 21 mm, collected from Shelikof Strait, Gulf of Alaska; 2002) or small mysiid (length = 23 mm, collected from Puget Sound, WA, USA). In 2004, euphausiids were not available during these experiments, so small mysiids were used in their place. A fine wire was glued to the carapace of an individual euphausiid or mysiid and the animal was turned on its side so that its beating pleopods could be seen when viewed from above. The animal was slowly moved toward a larva and the larva's response to the water current created by the animal's beating pleopods was recorded. From each recording, 25 different larvae were observed and it was determined whether they reacted to water flow. A response to the water flow was defined as the larva swimming away from the tethered animal before being touched. Contingency tables ($\chi^2$ test) were used to compare results.

## Reaction to probe touch

Larvae were tested to determine their response to repeated touches on the head and trunk/tail region with a fine probe. The same individuals that had been used for the escape speed and water current experiments were used in this experiment as well. The number of times a larva responded to five touches on the side of the head and five touches on the side of the body was recorded. For each hatching day, a total of 50 larvae were tested and each larva was tested only once. Responses on the day of hatching and after hatching were examined. Nonparametric tests were used to examine changes in tactile response because histograms showed that the data were highly skewed (i.e. not normally distributed). The Kruskal–Wallis (KW) test and Dunn multiple comparison test were used to compare hatching groups (Zar, 1996). The Mann–Whitney test was used to examine response after hatching. The percentage of larvae responding to at least one touch of either type was examined using linear regression and ANCOVA, after the data were arcsin ($x^{0.5}$) transformed (Zar, 1996).

## Euphausiid and amphipod predation

In 2002 and 2005, euphausiids were used, and in 2004, gammarid amphipods (*Pleusirus secorrus*, size = 13 mm, collected from Puget Sound, WA, USA) were used as predators of walleye pollock eggs and larvae. These predators were chosen because both animals prey upon yolksac walleye pollock larvae in Alaska waters (Bailey *et al.*, 1993), and they have somewhat contrasting modes of feeding. Euphausiids are filter feeders (with eggs and larvae, they appear to set up currents with their pleopods and then grasp larvae as they approach the feeding basket) and amphipods approach and grasp their prey. Predators were acclimated to preying on walleye pollock larvae for 1 week prior to beginning the experiments and they were starved for 24 h before an experiment was started. For each hatching day, 20 larvae or eggs (on the first hatching day) and 2 predators were placed into a 1 L glass beaker and the number of eggs or larvae remaining after 24 h was recorded. For each experiment, there were three replicate controls (larvae or eggs and no predators) and three replicate experimental treatments (containers with predators). A 16/8 h light/dark cycle was used. The euphausiid predation experiments conducted in 2002 showed that there was high variability among replicates; this gave low statistical power to detect differences among hatching groups. To increase the power of our

statistical tests, another series of euphausiid predation experiments were conducted in 2005 to increase the number of replicates on each hatching day. The replicates from 2002 and 2005 were pooled to increase the number of replicates to between four and six for each hatching day. Mortality rates of the controls (number per day) were calculated using: $M_c = -\ln(N_{1c}/N_{0c})$, where $N_{0c}$ is the number of larvae at the start of the experiment, $N_{1c}$ is the number of larvae remaining at the end of the experiment. Mortality rates of the experimental treatments (number per day) were calculated using: $M_e = -\ln(N_{1e}/N_{0e}) - M_c$; since the ln(0) is undefined, 1 was substituted for the replicates where $N_{1e} = 0$ (i.e. when all the larvae had been consumed during the experiment). Linear, and nonlinear regression models were calculated to examine the changes in the mortality rate during the egg stage and hatching period. The model that best fit the data was determined by the $P$ and $r^2$ values. For ANOVA, the fourth root data transformation was applied to make the mortality rate data more homoscedastic, and then the test was used to compare the mortality rates of hatching days to egg mortality.

### Otolith measurements

For each hatching day, 10 to 20 larvae were preserved in 95% ethanol for otolith diameter measurement. Otoliths from 10 larvae from each hatching day (2004B group) were dissected and mounted on microscope slides, and the maximum diameter of the lapilli otoliths was measured at 1000× to the nearest micron using an ocular micrometer. The lapilli otoliths from day of hatching walleye pollock larvae collected from the Gulf of Alaska in 1991 were also measured. The mean otolith size for each larva was calculated and the Kolmogorov–Smirnov two-sample test was used to compare the otolith size distribution of the laboratory-reared and field-collected larvae. Walleye pollock larvae were reared in the laboratory under environmental conditions (similar temperature and photo period) that typically occur in the Gulf of Alaska during the springtime when they are present, thus we feel that comparing the otolith size of laboratory-reared and field-collected larvae is justified.

## RESULTS

### Day of hatching

All three egg groups hatched over a 5-day period, and most hatching occurred during the last 3 days of this period (Table I). For all groups, larvae that hatched early were significantly smaller than those that hatched last (ANOVA; $F_{4,20} = 29.97$; $F_{3,36} = 103.24$; $F_{4,45} = 46.46$ for 2002, 2004A and 2004B, respectively, and in all cases $P < 0.001$; Fig. 1). Mean hatching size ranged from 4.19 mm on day 1 (2004B) to 5.14 mm on day 5 (2002, Fig. 1). Throughout the Results section, hatching days 1 through 5 refers to a specific cohort of larvae that hatched on that day.

*Table I: Cumulative percentage of walleye pollock (T. chalcogramma) eggs hatching for experiments conducted in 2002 and 2004*

| Hatching day | 2002 cumulative % hatch | 2004A cumulative % hatch | 2004B cumulative % hatch |
|---|---|---|---|
| 1 | 0.33 | < 0.4 | 0.7 |
| 2 | 0.67 | 0.4 | 5 |
| 3 | 23 | 9 | 40 |
| 4 | 65 | 27 | 77 |
| 5 | 100 | 100 | 100 |

As hatching progressed, the escape speed (mm $s^{-1}$) and responsiveness to touch on both the head and trunk/tail region of the larvae from all three egg groups increased (the slope of linear regression lines for escape speed were positive and significantly different from 0 for each group, $P < 0.05$, Fig. 2; Table II, Fig. 3A and B). The increase in the escape speed during the hatching period was similar for all three groups (ANCOVA, $F_{2,128} = 1.78$, $P = 0.17$; Fig. 2). No escape speed measurements were made on the first day of hatching for 2004A because of low numbers of larvae. When escape speed was examined as body lengths per second, results for 2002 and 2004B were the same as those for mm $s^{-1}$. The slope of the line for 2004A using body lengths per second was not significantly different from

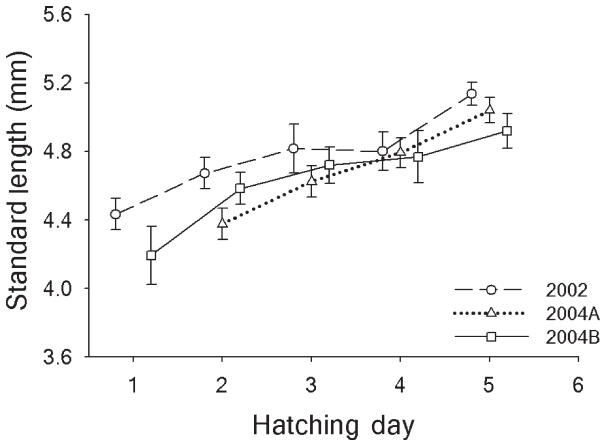


**Fig. 1.** The mean size (SL, mm ± standard deviation) at hatching for walleye pollock (*T. chalcogramma*) larvae for experimental groups 2002, 2004A and 2004B. No length measurements were taken on day 1 for group 2004A.

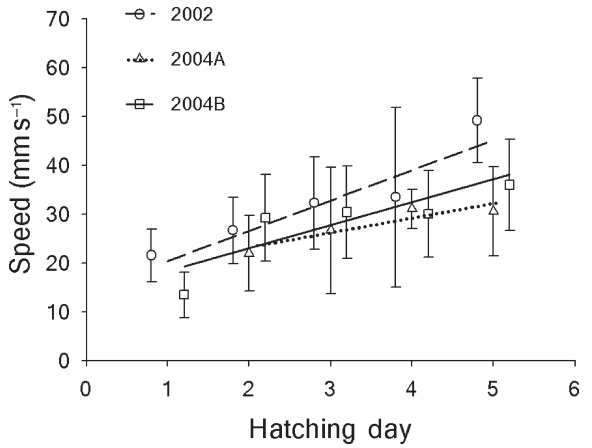


**Fig. 2.** Escape speed (mm s$^{-1}$; mean ± standard deviation) and linear regression lines for 2002, 2004A and 2004B experimental groups of walleye pollock (*T. chalcogramma*) larvae during the hatching period.

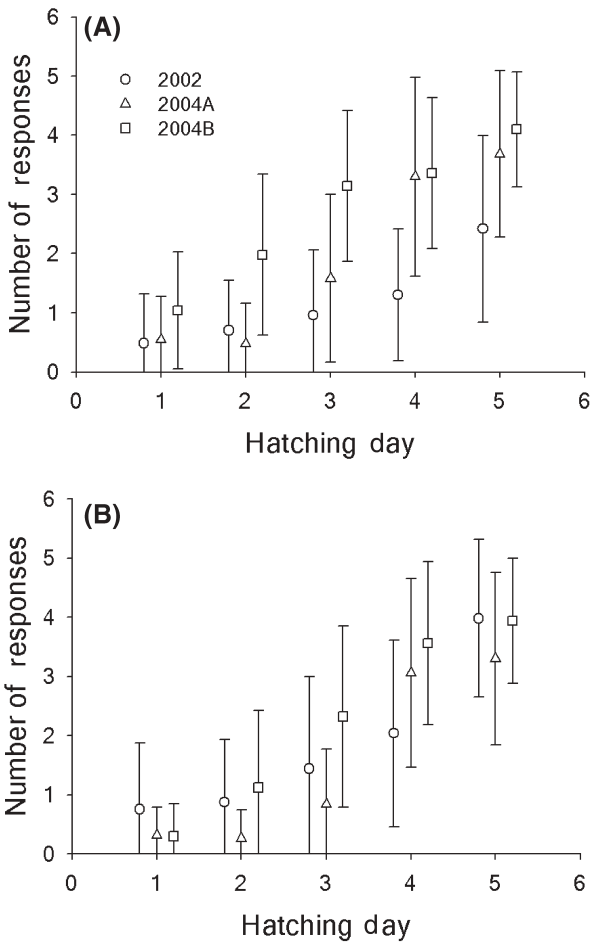


**Fig. 3.** The number of responses (mean ± standard deviation) out of five touches with a probe to the head (**A**) and tail (**B**) of walleye pollock (*T. chalcogramma*) larvae for 2002, 2004A and 2004B experimental groups during the hatching period.

0 ($P = 0.16$), but the absence of measurements on day 1 may have contributed to this result. The percentage of larvae responding to at least one touch of either type (on the head or trunk/tail region) increased from the first to the last day of hatching for all three experimental groups (the slope of the linear regression line for each experimental group was positive and significantly different from 0, $P < 0.05$; Fig. 4). The slopes of three lines representing each experimental group were not significantly different from each other (ANCOVA, $F_{2,9} = 0.13$, $P = 0.88$, Fig. 4), thus the percentage of larvae responding to touch increased in a similar manner for each experimental group of eggs.

The early hatching larvae were the least sensitive to water currents generated by euphausiid pleopods. No larvae were observed responding to water currents created by euphausiid pleopods on the first day of hatching. On the last day of hatching (day 5), 36% of larvae tested responded to water flow, these larvae were significantly more responsive than those that hatched on the first day ($\chi^2 = 8.67$, df = 1, $P = 0.003$). This was the only significant difference in the response to water current among hatching groups on the day of hatching. Unlike the tethered euphausiid experiments, the earliest hatched larvae responded to currents created by a tethered mysiid (11–20%). This may be due to stronger water flow being generated by this animal. For both 2004 experimental groups (2004A and 2004B), there was no significant change in the percentage of larvae responding to water current on the day of hatching ($\chi^2 = 1.72$, df = 3, $P = 0.63$ and $\chi^2 = 2.41$, df = 4, $P = 0.68$, respectively).

*Table II. Results of the KW test for changes in response to touch with a probe for walleye pollock (T. chalcogramma) larvae over the course of the hatching period for 2002, 2004A and 2004B egg groups*

| | | Kruskal–Wallis | | |
|---|---|---|---|---|
| Year | Touch response | Test statistic | Degrees of freedom | *P*-value |
| 2002 | Head | 53.47 | 4 | <0.001 |
| 2002 | Tail | 94.18 | 4 | <0.001 |
| 2004A | Head | 129.26 | 4 | <0.001 |
| 2004A | Tail | 144.00 | 4 | <0.001 |
| 2004B | Head | 113.53 | 4 | <0.001 |
| 2004B | Tail | 143.52 | 4 | <0.001 |

The Dunn multiple comparison test was used to examine differences among hatching days within the same year.

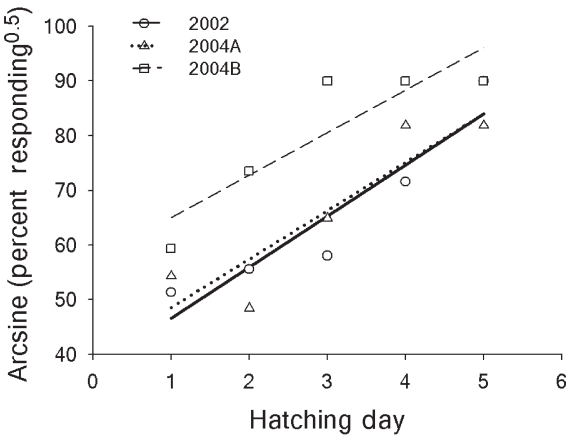


**Fig. 4.** Percentage of walleye pollock (*T. chalcogramma*) larvae responding to at least one touch with a probe on the head or tail for 2002, 2004A and 2004B experimental groups during the hatching period. Data are arcsine transformed, and linear regression lines for each year are shown.

Euphausiid predation on walleye pollock larvae was highly variable and this may have contributed to the low $r^2$ and nonsignificant *P*-values for both the regressions ($r^2 = 0.15$, $P = 0.13$ for the nonlinear regression model and $r^2 = 0.00$, $P = 0.79$ for the linear regression model). For example, on day 2, the number of larvae preyed upon ranged from 4 to 20. Even though the regressions were not significant, the trend of the data suggests that the mortality rate due to euphausiid predation may have declined over the course of the hatching period (Fig. 5A). The mortality rate on eggs was significantly lower than the rates for all hatching days except for day 4 (ANOVA, $F_{5,22} = 2.87$, $P = 0.04$, Dunnett's test; Fig. 5A). For amphipod predation, results of 2004A experiments were not included in the analysis because the amphipods were acclimated to walleye pollock larvae for <1 week and this may have affected their ability to prey on them. For the 2004B experimental group, no predation trials were conducted on day 5 beacause of low numbers of larvae on this day. Amphipod predation was less variable than euphausiid predation, the greatest difference in the number of larvae preyed upon between replicates was seven. The nonlinear regression model was the best fit for amphipod predation ($r^2 = 0.70$, $P = 0.001$; $r^2 = 0.16$, $P = 0.15$ for the linear model; Fig. 5B). This model showed low egg mortality and decreasing larval mortality rates over the hatching period (Fig. 5B). When the larval mortality rate for each hatching day was compared with the egg mortality rate, all hatching days had a significantly higher mortality rate than the eggs (ANOVA, $F_{4,9} = 17.92$, $P < 0.01$, Dunnett's test, Fig. 5B).

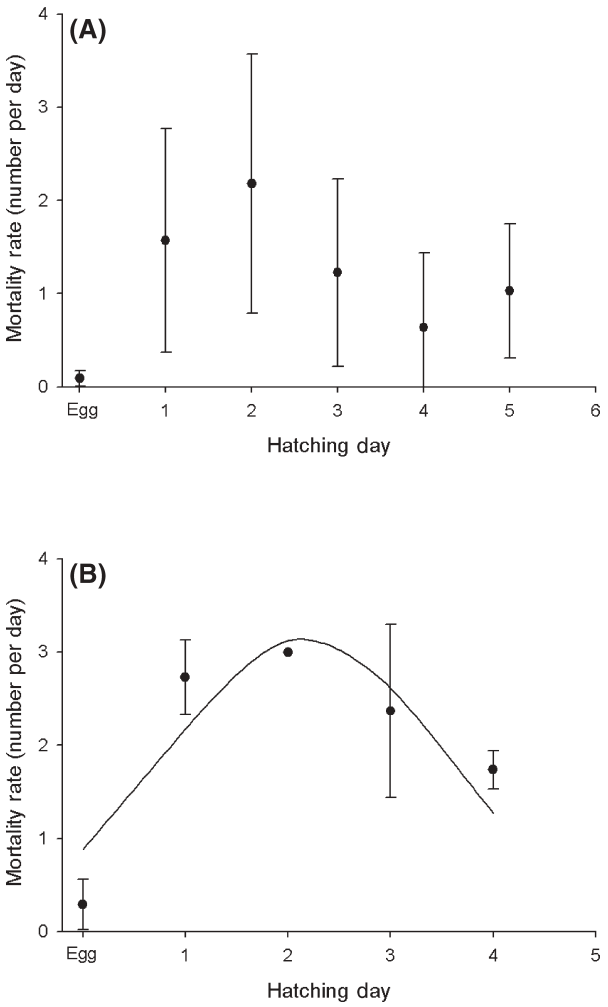


**Fig. 5.** Mortality rates (mean number per day ± standard deviation) of walleye pollock (*T. chalcogramma*) eggs and larvae from euphausiid (**A**) and amphipod (**B**) predation as a function of hatching day.

## Continued development of the early hatched larvae

At the end of all hatching, larvae that hatched early were larger and their escape speed was as quick as those that hatched last. Larvae that hatched early grew faster during the hatching period than those that remained in the egg and hatched later. When tested on the day after all hatching was completed in 2002 (day 6), larvae that had hatched on day 2 were significantly larger than either of those that had hatched on days 4 and 5 (ANOVA, $F_{2,12} = 4.42$, $P = 0.04$, SNK test, Table III). In addition, for 2004B on hatching day 5, the SL of larvae from hatching day 1 was significantly larger than that of the larvae that hatched on this day (two-sample *t*-test, $T = 2.86$, df = 18, $P = 0.01$, Table III), a growth pattern similar to results from

*Table III: SL, escape speed and total tactile response (combined response of head and tail probe touch) for early and late-hatched walleye pollock (T. chalcogramma) larvae at the end of all hatching for 2002, 2004A and 2004B egg groups*

| Year | Hatching day | Testing day | SL (mm ± SD)[a] | Escape speed (mm $s^{-1}$ ± SD)[b] | Total tactile response (no. ± SD)[c] |
|---|---|---|---|---|---|
| 2002 | 2 | 6 | 5.44 ± 0.06 | 42.8 ± 9.2 | 5.28 ± 2.47 |
| | 4 | 6 | 5.23 ± 0.15 | 48.7 ± 9.1 | 4.02 ± 2.90 |
| | 5 | 6 | 5.25 ± 0.15 | 48.4 ± 17.2 | 5.52 ± 2.41 |
| 2004A | 2 | 5 | 5.00 ± 0.09 | 38.1 ± 12.3 | 3.10 ± 3.13 |
| | 5 | 5 | 5.04 ± 0.08 | 30.6 ± 9.1 | 6.98 ± 2.28 |
| 2004B | 1 | 5 | 5.10 ± 0.18 | 38.7 ± 7.6 | 6.74 ± 2.11 |
| | 5 | 5 | 4.92 ± 0.10 | 36.0 ± 9.3 | 8.04 ± 1.52 |

[a]Mean ± standard deviation; $n = 5–10$.
[b]$n = 9–10$.
[c]Head and tail responses combined; $n = 50$.

2002. There were no SL measurements taken on hatching day 1 for group 2004A. For 2002, the escape speeds (mm $s^{-1}$) of larvae from hatching days 2, 4 and 5 were all measured 1 day after all hatching was completed (day 6) and there were no significant differences among them (ANOVA, $F_{2,26} = 0.68$, $P = 0.52$, Table III). For both the 2004A and 2004B groups on hatching day 5, the escape speed between larvae that hatched on this day and those that hatched early (day 1 or 2) was not significantly different (two-sample *t*-tests, $T = 1.55$, df = 18, $P = 0.14$ and $T = 0.70$, df = 18, $P = 0.49$, respectively, Table III).

The overall responsiveness to touch (number of head and tail responses combined) of the early hatched larvae from all egg groups increased from hatching to the last day of hatching (Mann–Whitney test; Mann–Whitney *U*-test statistic = 2555, df = 1, $P < 0.001$, Mann–Whitney *U*-test statistic = 755.50, df = 1, $P < 0.001$, and Mann–Whitney *U*-test statistic = 43.50, df = 1, $P < 0.001$ for 2002, 2004 A and 2004B, respectively). For the total tactile response at the end of all hatching, early hatched larvae from only one out of the three egg groups were as sensitive to touch as those that hatched at the end. The total tactile response of early hatched larvae in 2002 developed at a rate similar to those that hatched at the end, that is, both groups of larvae responded in a manner similar to touch at the end of all hatching. When measured 1 day after all hatching was completed (at day 6), the total response to touch of larvae from hatching group day 2 was not significantly different from either hatching group days 4 or 5 (KW test, KW test statistic = 8.81, $P = 0.01$, Dunn multiple comparison test, Table III). The early hatched larvae of both 2004 groups were not as responsive as those larvae that hatched on the last day (Mann–Whitney tests, Mann–Whitney *U*-test statistic = 2066.00, df = 1, $P < 0.001$, and Mann–Whitney U-test statistic = 784.00, df = 1, $P = 0.001$, respectively; Table III). For 2004A, the early hatched group may have been in poor condition, because they were dying within 1–2 min after being transferred into a Petri dish.

### Size of lapillus otolith at hatching

The diameter of the lapillus otolith from laboratory-reared larvae at hatching increased during the hatching period ($n = 53$ larvae, linear regression, slope was positive and significantly different from 0, $P < 0.01$; Fig 6A). The size distribution of these otoliths was not significantly different from the size distribution of larval walleye pollock otoliths collected from the Gulf of Alaska in 1991 determined to be the day of hatching ($n = 12$ larvae; A. Dougherty and K. Bailey unpublished results; Kolmogorov–Smirnov two-sample test, $P = 0.71$; Fig. 6B), indicating that the time span over which both laboratory and *in situ* eggs hatched was similar.

## DISCUSSION

Predation is a major source of mortality for fish larvae, and yolksac larvae are vulnerable to a wide variety of predators (Bailey and Houde, 1989). Euphausiids and amphipods are major invertebrate predators of yolksac walleye pollock larvae in the Gulf of Alaska (Bailey *et al.*, 1993). Our laboratory study showed that the ability of walleye pollock larvae to escape predators was affected by rank in the order of hatching. Measurements of lapilli otolith diameters indicate that the duration of the hatching period of walleye pollock eggs in the laboratory is similar to that occurring in the Gulf of Alaska. In laboratory-reared fish, there is a positive relationship between the lapillus diameter and the day of hatching, and in wild fish, there is a wide range of diameters from just-hatched larvae that overlap with the laboratory measurements. Thus, it appears likely that the early

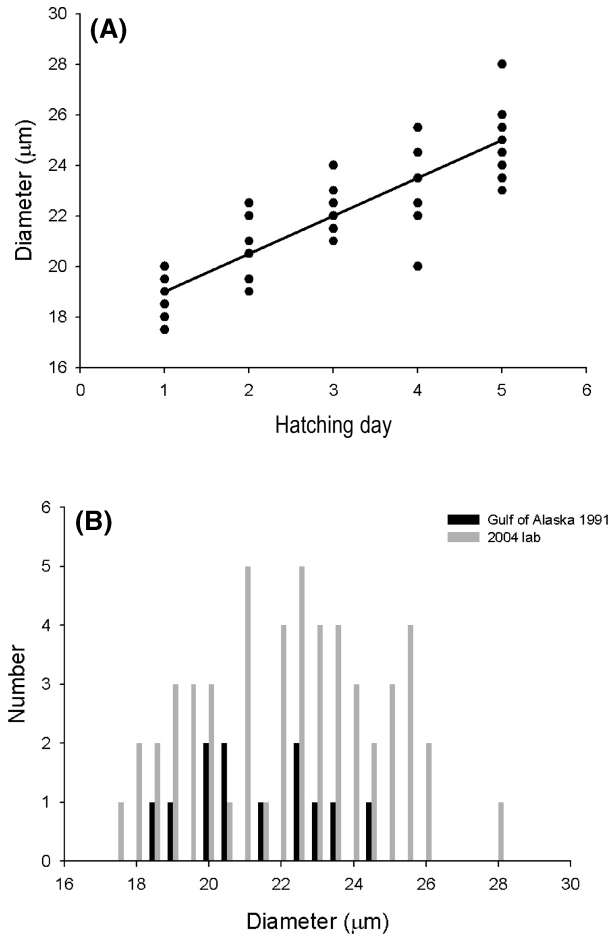


**Fig. 6.** Diameter of lapilli otoliths of walleye pollock (*T. chalcogramma*) larvae at hatching. (**A**) Increase in size of otoliths during the hatching period for laboratory-reared larvae (2004B; $n = 53$). (**B**) Histogram of the range of sizes of otoliths at hatching for both laboratory-reared larvae (2004B; $n = 53$) and larvae collected in the Gulf of Alaska in 1991 ($n = 12$).

hatching of walleye pollock larvae occurs in nature, as has been observed for other species (Mashiko, 1976).

The laboratory-measured predation mortality rates of just-hatched walleye pollock declined as hatching progressed. The earliest hatching walleye pollock larvae may be more vulnerable to predation than later hatching larvae because their escape response is relatively undeveloped, i.e. they had the slowest escape speed and were the least responsive to both direct and indirect contact compared to later hatching larvae used in our experiments. When the larval size was taken into account, escape speed (i.e. body lengths per second) increased over the course of the hatching period, indicating that this increase in speed was not entirely due to differences in the larval size. After hatching, the vulnerability of early hatched larvae to predation may decrease as their escape response develops similarly to that for late-hatching larvae and thus they are better able to detect and escape from predators. However, in the sea, the relationship may be more complex, as vulnerability to predation by mechano-receptive and visual predators is mitigated by lower activity of the early hatching larvae. Walleye pollock larvae increase their swimming activity after hatching (Spring, 1996), so a consequence of early hatching could be that they are less active than late-hatched larvae, making them more difficult to detect by some predators. Although our experiments were not designed to measure spontaneous swimming activity, we examined activity on our video recordings and we did not detect a significant difference in the swimming activity between the earliest hatching larvae and those that hatched last (2002 egg group, two-sample *t*-test, $T = -0.33$, df $= 28$, $P = 0.75$).

There are advantages to delayed hatching, as shown by our study, including lower predation mortality of eggs than larvae, improved escape response (escape speed and tactile sensitivity) and larger size at hatching. All these factors may decrease predation mortality on later-hatching walleye pollock larvae. The chorion may provide a refuge from small grasping predators through the egg stage, thus conferring some survival advantage to late-hatching individuals. For the Australian lungfish, development of the sensory and motor neurons continues after hatching (Whiting *et al.*, 1992). Thus, as development proceeds, the escape response is expected to improve (i.e. the larvae become more responsive to tactile stimulation and their swimming escape speed increases). Similar development may be occurring during the prolonged egg stage of the late-hatching larvae.

Tactile stimuli are the first to elicit an escape response in fish larvae, and as the larvae develop, the number of different stimuli that they respond to increases (Eaton and DiDomenico, 1986). Herring larvae 10–12 mm in length only responded to tactile stimuli; later in development, they had a fast startle response to acoustic stimuli (Blaxter and Batty, 1985). Blaxter and Batty (1985) also showed that herring larvae did not distinctly respond to the inward flow of water caused by suction from a pipette. The responsiveness of walleye pollock larvae to direct touch improved as hatching progressed, this may be due to late-hatching larvae having more functional neuromasts and a more extensive Rohon–Beard network than early hatching walleye pollock. The walleye pollock larvae used in the present study did not respond as frequently to water currents as they did to direct touch, and by the end of hatching, nearly all larvae responded when touched by a probe, but only about one-third responded to water currents generated by a predator. This suggests that there is differential vulnerability to predators;

just-hatched walleye pollock larvae may be vulnerable to predators that use suction or feeding currents.

For herring larvae, the potential for growth is higher for larvae that hatch earlier when compared with those that remain in the egg; this may be due to constrained growth in the egg during the later part of the incubation period (Geffen, 2002). Results from the present study support constrained growth in late-hatching walleye pollock larvae. Walleye pollock larvae that hatched first were smaller than the larvae that hatched at the end of the hatching period, but they grew to be significantly larger than the later hatching fish by the end of the hatching period. This observation suggests that there is a wide spectrum of development strategies to maximize the survival rates. In a situation where predators are sparse, early hatching may benefit survival due to enhanced growth, whereas when predators are abundant, a longer egg stage is beneficial because the egg chorion provides some protection from small invertebrates and newly hatched larvae are more developed and better able to escape predators. Over a broad range of species, planktonic marine animals apparently have evolved to shorten egg hatch times in response to predation (Hirst and López-Urrutia, 2006), although this association may not be simple because of the enhanced vulnerability of newly hatched larvae to some predators.

Late-hatching walleye pollock larvae may be able to better escape predators because they are larger in size (predation mortality is inversely related to size) (Bailey and Houde, 1989), more sensitive to touch and have a faster escape speed at hatching than those that hatch early. For early hatched larvae (approximately the first 25% to hatch), there appears to be a 2–3-day period when the larvae are at a distinct disadvantage in their escape response when compared with those that hatch later and this can potentially affect their survival. Initially, the escape response of these larvae is less developed, but by the end of the hatching period, their escape speed is equal to that of larvae that hatch last, although their sensitivity to touch may not be as well developed. These results indicate the potential importance of early hatching in nature and of defining the day of hatching for laboratory studies, because laboratory results could be affected by variability in development at hatching.

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## REFERENCES

Bailey, K. M. and Batty, R. S. (1984) Laboratory study of predation by *Aurelia aurita* on larvae of cod, flounder, plaice and herring: development and vulnerability to capture. *Mar. Biol.*, **83**, 287–291.

Bailey, K. M. and Houde, E. D. (1989) Predation on eggs and larvae of marine fishes and the recruitment problem. *Adv. Mar. Biol.*, **25**, 1–83.

Bailey, K. M., Brodeur, R. D., Merati, N. and Yoklavich, M. M. (1993) Predation on walleye pollock (*Theragra chalcogramma*) eggs and yolk-sac larvae by pelagic crustacean invertebrates in the western Gulf of Alaska. *Fish. Oceanogr.*, **2**, 30–39.

Blaxter, J. H. S. (1984) Neuromasts and cupular growth of cod larvae. In Dahl, E., Danielssen, D. S., Moksness, E. and Solemdal, P. (eds), *The Propagation of Cod Gadus Morhua L. Flødevigen rapportser 1*, pp. 183–188.

Blaxter, J. H. S. (1986) Development of sense organs and behavior of teleost larvae with special reference to feeding and predator avoidance. *Trans. Am. Fish. Soc.*, **115**, 98–114.

Blaxter, J. H. S. and Batty, R. S. (1985) The development of startle responses in herring larvae. *J. Mar. Biol. Assoc. UK*, **65**, 737–750.

Blaxter, J. H. S. and Fuiman, L. A. (1989) Function of the free neuromasts of marine teleost larvae. In Coombs, S., Görner, P. and Münz, H. (eds), *The Mechanosensory Lateral Line: Neurobiology and Evolution*. Springer-Verlag, New York, 481–499.

Blood, D. M. (2002) Low-temperature incubation of walleye pollock (*Theragra chalcogramma*) eggs from the southeast Bering Sea shelf and Shelikof Strait, Gulf of Alaska. *Deep-Sea Res. II*, **49**, 6095–6108.

Blood, D. M., Matarese, A. C. and Yoklavich, M. M. (1994) Embryonic development of walleye pollock, *Theragra chalcogramma*, from Shelikof Strait, Gulf of Alaska. *Fish. Bull.*, **92**, 207–222.

Eaton, R. C. and DiDomenico, R. (1986) Role of the teleost escape response during development. *Trans. Am. Fish. Soc.*, **115**, 128–142.

Geffen, A. J. (2002) Length of herring in relation to age and time of hatching. *J. Fish Biol.*, **60**, 479–485.

Hirst, A. and López-Urrutia, A. (2006) Effects of evolution on egg development time. *Mar. Ecol. Prog. Ser.*, **326**, 29–35.

Mashiko, K. (1976) Ecological study on breeding of an eleotrid goby, *Odontobutis obscurus* (Temminck et Schlegel), under rearing conditions. *Jap. J. Ecol.*, **26**, 91–100.

Methven, D. A. and Brown, J. A. (1991) Time of hatching affects development, size, yolk volume, and mortality of newly hatched *Macrozoarces americanus* (Pices: Zoarcidae). *Can. J. Zool.*, **69**, 2161–2167.

Otsuka, M. and Nagai, S. (1997) Neuromast formation in the prehatching embryos of the cod fish, *Gadus macrocephalus* Tilesius. *Zool. Sci.*, **14**, 475–481.

Porter, S. M. and Theilacker, G. H. (1999) The development of the digestive tract and eye in larval walleye pollock, *Theragra chalcogramma*. *Fish. Bull.*, **97**, 722–729.

Roberts, A. and Hayes, B. P. (1977) The anatomy and function of 'free' nerve endings in an amphibian skin sensory system. *Proc. R. Soc. Lond. B.*, **196**, 415–429.

Spring, S. (1996) Swimming behavior of walleye pollock *Theragra chalcogramma* in relation to food. Masters Thesis. University of Washington, Seattle, USA, pp. 1–123.

Sugisaki, H., Bailey, K. M. and Brodeur, R. D. (2001) Development of the escape response in larval walleye pollock (*Theragra chalcogramma*). *Mar. Biol.*, **139**, 19–24.

Whiting, H. P., Bannister, L. H., Barwick, R. E. and Bone, Q. (1992) Early locomotor behaviour and the structure of the nervous system in embryos and larvae of the Australian lungfish, *Neoceratodus forsteri*. *Zool. Soc. Lond.*, **226**, 175–198.

Williams, P. J. and Brown, J. A. (1992) Development changes in the escape response of larval winter flounder *Pleuronectes americanus* from hatch through metamorphosis. *Mar. Ecol. Prog. Ser.*, **88**, 185–193.

Zar, J. H. (1996) *Biostatistical Analysis*, 3rd edn. Prentice Hall, Upper Saddle River, New Jersey, pp. 1–662.