Abstract.—Condition of field-caught walleye pollock, *Theragra chalcogramma*, larvae was assessed by using a measurement of midgut cell height that reliably diagnosed the nutritional status of laboratory-reared walleye pollock. The midgut cell height was simple to measure on histological sections. Several correction factors were developed for applying the midgut measurement to a field study. These included regressions to characterize change in larval length associated with net collections of various elapsed times and with fixation in several types of preservatives. The response of midgut cell height to field collection procedures also was tested. The field study indicated larval walleye pollock were starving in the Shelikof Strait, Gulf of Alaska, in 1991. At some stations up to 40% of the larvae were in poor condition. Larvae were most vulnerable to starvation for 2 weeks following the day of first-feeding.

Condition of larval walleye pollock, *Theragra chalcogramma*, in the western Gulf of Alaska assessed with histological and shrinkage indices*

Gail H. Theilacker
Steven M. Porter

Alaska Fisheries Science Center
National Marine Fisheries Service, NOAA
7600 Sand Point Way NE, Seattle, WA 98115

In 1986 the Fisheries-Oceanography Coordinated Investigations (FOCI) program of the Alaska Fisheries Science Center and the Pacific Marine Environmental Laboratory began studying the biological and physical processes controlling variability in recruitment of walleye pollock, *Theragra chalcogramma*, in Shelikof Strait, Gulf of Alaska. Each year, large concentrations of adult walleye pollock aggregate in the Strait and spawn in late March and early April, producing dense patches of eggs at a depth of about 200 m; there is little variation in the timing or location of the spawning (Kim and Kendall, 1989; Kendall and Picquelle, 1990; Schumacher and Kendall, 1991). After hatching, larvae rise to the upper waters where they may be transported along the Alaska Peninsula, off the shelf to the southeast, or be retained in eddies (Vastano et al., 1992). It is believed that the area young pollock occupy during the larval stage is important for their survival (Schumacher and Kendall, 1991). Assessing the nutritional condition of larval pollock collected from different areas in Shelikof Strait should aid in determining whether food availability is one of the factors influencing survival and recruitment.

A variety of indices have been applied to examine the nutritional condition of larval fish. However, for most of the indices, the response rate of the variable to changes in feeding is unknown. Thus, it is difficult to apply the index to estimate mortality rates in the field and subsequent recruitment variability. Histological analyses have yielded valuable information on larval nutritional condition (O'Connell, 1976; Theilacker, 1978; O'Connell and Paloma, 1981; Sieg, 1992). Furthermore, starvation-induced mortality rates for histological studies have been estimated by combining the results from histological condition assessments with information on growth and starvation rates (Theilacker, 1986; Theilacker and Watanabe, 1989). Using the estimate of starvation-induced mortality rates, attempts have been made to calculate the proportion of natural mortality due to starvation mortality (Hewitt et al., 1985; Owen et al., 1989).

Preliminary studies on larval walleye pollock have revealed that the height of the midgut mucosal cells are sensitive to starvation, decreasing in height measurably over time

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as food is withheld. A decrease in the thickness of the intestine during starvation has been noted for other fishes (Kostomarova, 1962; Nakai et al., 1969; Umeda and Ochiai, 1975; Ehrlich et al., 1976; O'Connell, 1976; Theilacker, 1978, 1986; Kashuba and Matthews, 1984; Boulhic and Gabaudan, 1989; Oozeki et al., 1989; Theilacker and Watanabe, 1989; McFadzen et al., 1994), including the Altantic cod, *Gadus morhua* L. (Yin and Blaxter, 1987), which is closely related to walleye pollock. In this study we describe a midgut cell height index for laboratory-reared walleye pollock.

Shrinkage of larval fish captured in a net and preserved aboard ship differs from that observed in the laboratory (Blaxter, 1971; Theilacker, 1980, 1986; Hay, 1981; McGurk, 1985; Jennings, 1991). The amount a larva shrinks may be dependent on size, the duration of handling (time in the net and how soon after death it is preserved), and the type of preservative used. Preservative and gear-related (net) shrinkage have been examined for larvae of a variety of fish species (Theilacker, 1980, 1986; Hay, 1981, 1982; Fowler and Smith, 1983; Tucker and Chester, 1984; McGurk, 1985; Radtke, 1989; Kruse and Dalley, 1990; Jennings, 1991; Hjorleifsson and Klein-MacPhee, 1992). Jennings (1991) found that the magnitude of shrinkage differed among species and concluded that a correction factor for each species must be determined. To relate our laboratory observations to the field, we derived shrinkage indices for larval walleye pollock subject to net treatment and several preservatives. To determine the utility of the midgut cell height index in the field, larval walleye pollock were collected in Shelikof Strait, and their nutritional condition was assessed from experimental results.

Methods

Laboratory rearing

Adult walleye pollock were collected from Shelikof Strait, Gulf of Alaska, in April of 1990 to 1993. The fish were spawned aboard ship and the fertilized eggs flown to Friday Harbor Laboratories, University of Washington, in 1991 and to the Alaska Fisheries Science Center in 1990, 1992, and 1993. We raised the larvae in 120-L black fiberglass circular tanks with clear plastic covers and used a 16-h daylight cycle. Seawater temperatures were maintained at 6°C which are typical in May in Shelikof Strait when larvae initiate feeding (Kendall et al., 1987). Each year there were two treatments: one tank contained larvae into which prey were added (fed tank), the second contained larvae that were never offered prey (starved tank). Prey consisted of rotifers, *Brachionus*

plicatilis, at 10/mL and copepod nauplii, *Acartia* sp., at a minimum of 1–2/mL. Rotifers were raised on algal diets of *Isochrysis galbana* and *Pavlova lutheri*, which are high in unsaturated fatty acids (Nichols et al., 1989). The dinoflagellate, *Katodinium rotundatum*, was also added as prey for the rotifers and copepods. Ammonia levels were low for both treatments (<0.4 ppm). We sampled larvae from both the fed and starved tanks every day or every other day.

Calibration of midgut cell height

For the histological analysis, walleye pollock larvae were preserved in either Bouin's solution which was replaced with 70% ethanol 24 to 48 h later or in Z-Fix (solution of 10% formalin with zinc and buffers added[1]). Larvae were processed with standard histological procedures; they were dehydrated in a butyl alcohol series, embedded in paraffin wax, serially sectioned at 6 µm in the sagittal plane, and stained with hematoxylin and eosin. We measured the mucosal cell height of the anterior dorsal portion of the midgut at 400× magnification (Fig. 1). This area was chosen because it exhibits little gut folding which can make the midgut cell height measurement too variable to be useful. We measured three to six neighboring cells (with clearly defined nuclei, basement membrane, and microvilli) from the top of the basement membrane to the top of the microvilli and recorded the average height.

Fixative effects on larval length

To determine the shrinkage of larvae placed directly into preservative (laboratory shrinkage), we measured the standard length (SL, tip of upper jaw to end of notochord, to nearest 0.08 mm) of live larvae sampled from the fed tank and placed them individually into Bouin's solution, 5% formalin, Z-Fix, or 95% ethanol. Bouin's solution was changed to 70% ethanol 24 to 48 h later. Final size of larvae was determined one year after preservation. Final size of ethanol-fixed larvae was measured in distilled water; larvae preserved in the other fixatives were measured in the fixative.

Net-treatment and subsequent fixative effects on larval length

To examine the effect of net treatment on larval length, a larva was sampled from the fed tank and its standard length was measured. It was then placed in a small net and submerged in a tank of 6°C seawater that recirculated through the net to simulate a towed sam-
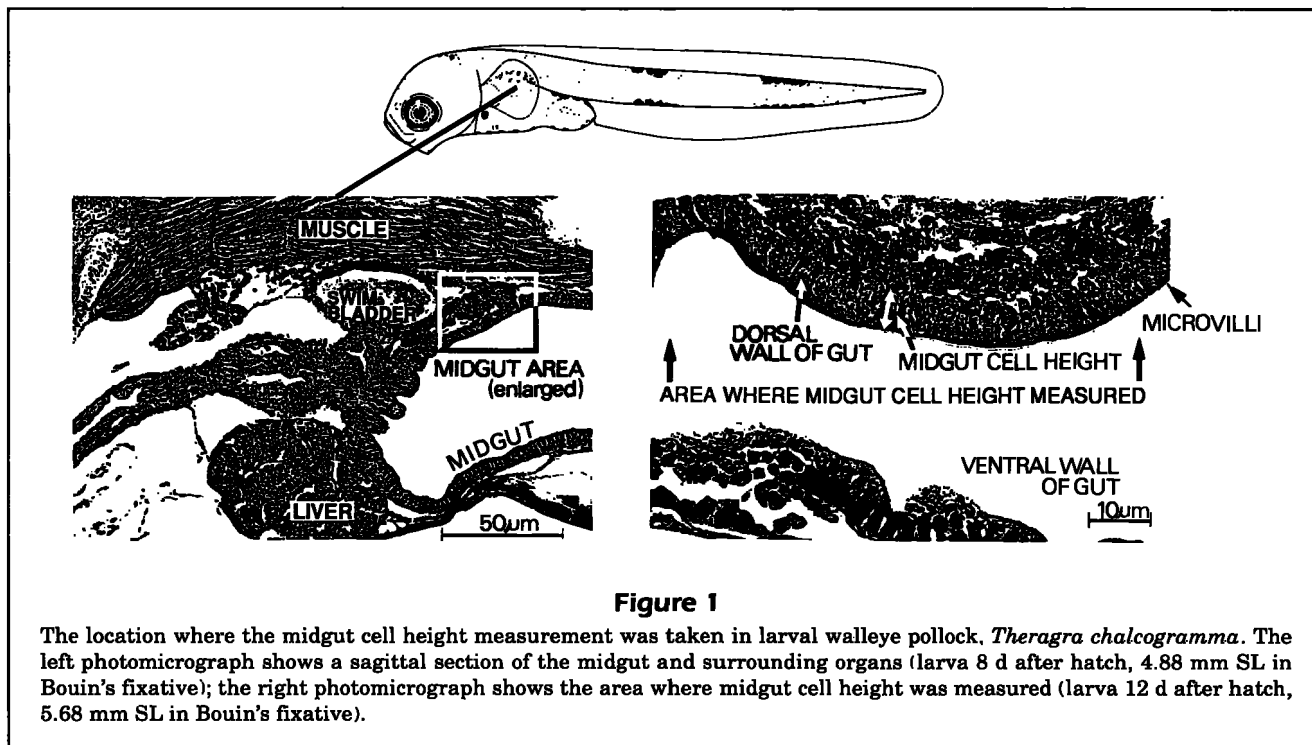

Figure 1

The location where the midgut cell height measurement was taken in larval walleye pollock, *Theragra chalcogramma*. The left photomicrograph shows a sagittal section of the midgut and surrounding organs (larva 8 d after hatch, 4.88 mm SL in Bouin's fixative); the right photomicrograph shows the area where midgut cell height was measured (larva 12 d after hatch, 5.68 mm SL in Bouin's fixative).

pling net (Theilacker, 1980). The larva was remeasured after 5 minutes. The final measurement was taken at 10, 15, or 20 min, and then the larva was placed into Bouin's, Z-Fix, or 5% formalin. For additional shrinkage due to preservation, larvae were remeasured from 1 month to 1 year after they were preserved.

Change in midgut cell height due to fixative and net treatment

Two fixatives used to preserve field-collected larvae were compared to determine their effect on the height of the midgut cells. The effect of Bouin's solution and Z-Fix was compared by sampling 10 fish daily from the fed tank and preserving 5 in Bouin's solution and 5 in Z-Fix. To determine whether the height of the midgut cells changed as larval length decreased during the net shrinkage experiment, one group of 20 larvae was measured and placed directly into Z-Fix while a second group of 20 was measured, net-treated, and then preserved in Z-Fix.
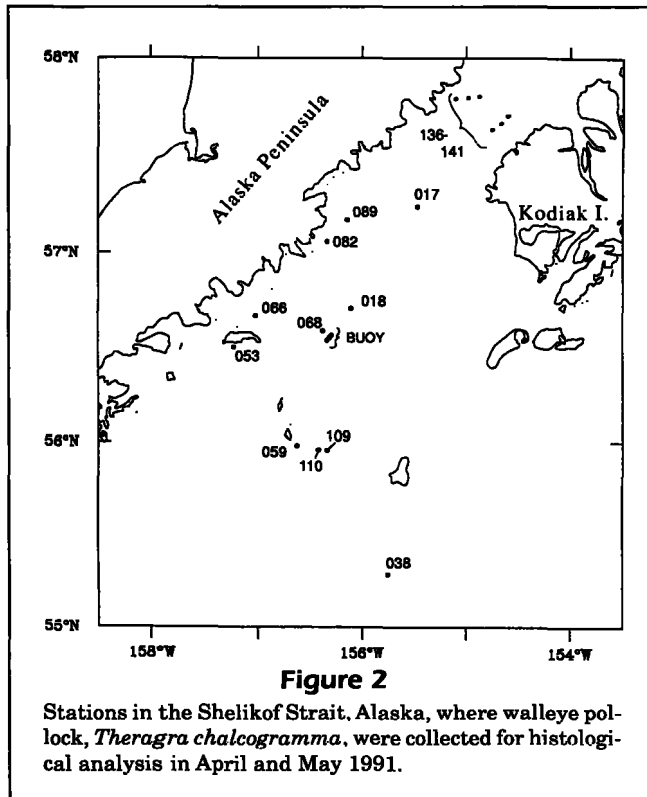
Data analysis

Data were analyzed by using SAS (SAS, Inc. 1988), SYSTAT (Wilkinson, 1988), and Minitab (Ryan et al., 1985) computer software. Simple linear regression analysis was used to derive equations to adjust larval size for shrinkage. ANOVA was used to compare

the midgut cell height of starved and fed larvae of the same size. Midgut cell height of starved larvae reared in 1991 and 1993 was compared with midgut cell height of fed larvae reared in 1991 and 1992 (in 1992, no starved larvae were sampled for midgut measurement, and in 1993, midgut was not measured for fed larvae). Years were used as independent treatments to avoid pseudoreplication (Hurlbert, 1984).

Field collections

Larval walleye pollock were collected for histology from stations located throughout the Shelikof Strait, Gulf of Alaska, during two cruises in the spring of 1991 (Fig. 2). The area sampled covered the entire spawning area in the Strait (Kendall and Picquelle, 1990). Collections were made with a 60-cm bongo net equipped with a 333-µm mesh and solid cod end, which were retrieved vertically from 70 m in about 7 minutes. Because larval fish tissues deteriorate quickly owing to autolysis (Theilacker, 1978), the net was not washed following a tow, and larvae were quickly sorted on sea water ice and preserved in Bouin's fixative. Earlier laboratory studies conducted with larval walleye pollock indicated they must be preserved within 12 min at 6°C in order to retain cellular integrity.[2]

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[2] Theilacker, G. H., S. M. Porter. U.S. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Alaska Fisheries Science Center, 7600 Sand Point Way NE, Seattle, WA 98115. Unpubl. data.

Figure 2

Stations in the Shelikof Strait, Alaska, where walleye pollock, *Theragra chalcogramma*, were collected for histological analysis in April and May 1991.

Table 1

Size of fed and starved treatments of 1991 laboratory-reared larval walleye pollock, *Theragra chalcogramma*. Larvae were reared at 6°C and preserved in Bouin's fixative. Means and standard deviations (SD) are for 5–10 larvae.

| | Laboratory-preserved standard length (mm) | | | |
| | Fed | | Starved | |
Days after hatching	mean	SD	mean	SD
8[1]	5.01	0.18	5.01	0.07
9	5.12	0.16	4.93	0.12
10	5.10	0.16	4.98	0.12
11	5.22	0.16	4.93	0.09
12	5.32	0.29	4.91	0.17
13	5.40	0.20	4.90	0.07
14	5.31	0.23	4.93	0.18
15	5.30	0.30	4.80	0.11
16	5.34	0.20	4.91	0.07
17	5.44	0.34	4.82	0.14
19			4.66	0.13
20			4.67	0.09

[1] Day of first feeding.

The standard length of all field larvae was measured before processing for histology. We processed all larvae from each field sample that were preserved within 12 minutes. Larvae were processed in the same manner as the laboratory-raised pollock, and midgut cell height was measured in the same area to determine their past feeding history.

The vertical depth inhabited by most walleye pollock larvae less than 10 mm is between 25 and 37 m (Kendall et al., 1994). Thus we estimated that the elapsed handling time from capture to placement into fixative for the average larva was from 6 to 9 min (3 to 4 min in the net retrieved at 10 m/min plus 3 to 5 min to sort and preserve), or an average of 7.5 minutes. We used 7.5 min as the average handling time to calculate field-collected size.

Results

Laboratory rearing 1991

Walleye pollock hatched at a mean SL of 3.5 mm (laboratory-preserved size), grew at an average rate of 0.12 mm/d, and at 17 d after hatching averaged 5.4 mm SL (Table 1). Larvae started feeding on average ($\bar{x}\pm SD$) 8 d after hatching (range 7–9 d) at

5.01 ±0.15 mm SL (range 4.56–5.20 mm SL, n=15), and the yolk was completely absorbed about 5 d later. The growth rate from first-feeding on day 8 to 13 d after hatching averaged 0.078 mm/day.

Starved larvae decreased in size as food was withheld for 11 d after first feeding (Table 1). The length of starved larvae began to decrease slowly on day 9 after food was withheld for only 1 day. After 8 d of starvation (16 d after hatching), larvae rapidly decreased in size.

Calibration of midgut cell height

The height of the midgut cells of larvae sampled from the starved and fed tanks and preserved in Bouin's solution ranged from 5 to 34 μm (Figs. 3 and 4). Cells were largest in prefeeding yolk-sac larvae before midgut differentiation was complete and the lumen fully formed. Starving the larvae caused the midgut cells to decrease slowly in height from about 13 μm at first feeding to about 9 μm after starving for 4 days; the average height remained at about 8 μm as food was withheld for an additional 5 days (Fig. 3). We arbitrarily set 11 μm to delimit the fed and starved groups because it gave the best division; this cutoff separated 87.2% of the fed larvae and 80.4% of the starved larvae.

The height of the mucosal cells of the anterior dorsal portion of the midgut increased slightly as fed

larvae grew (Fig. 4). Midgut cell heights of fed larvae were significantly larger (ANOVA, $P=0.006$, df=1) than those of starved larvae of equal length (4.50–5.49 mm SL), and the same treatment was not significantly different between years (ANOVA, $P=0.533$, df=2; Table 2). Moreover, the difference in midgut cell heights was attained after food was withheld for 1 day (t-test; $P<0.01$, df=8; mean fed midgut cell height after 1 d of feeding=12.96 ±1.31 µm, $n=5$; mean starved midgut cell height after 1 d of starvation = 9.12 ±0.99 µm, $n=5$; 1991 rearing data).
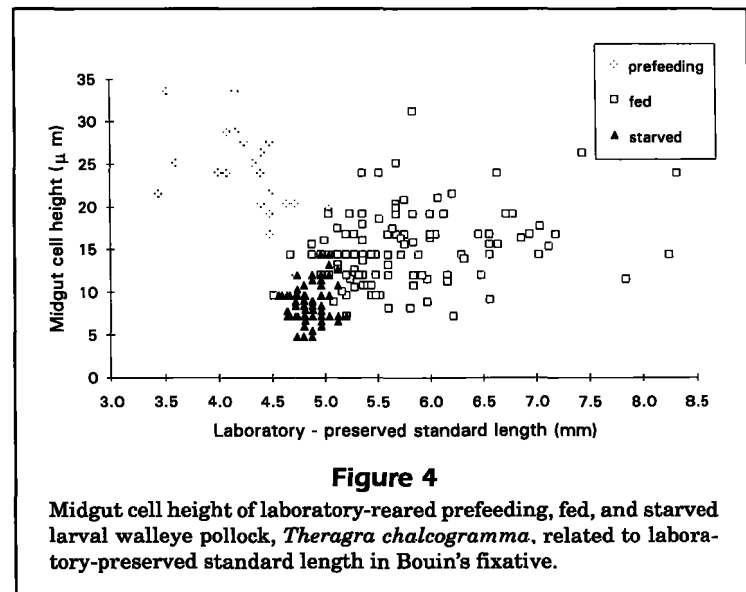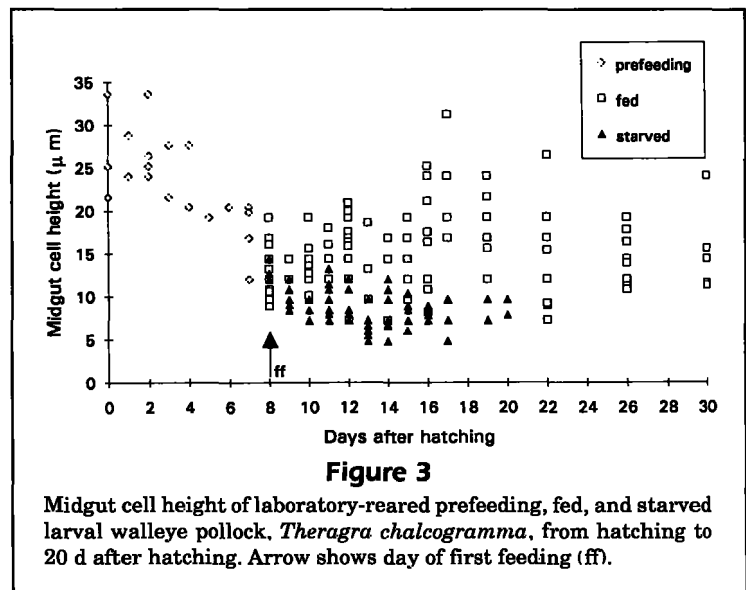
Midgut cell height was not affected by a net treatment of 7.5 min, the estimated time a larva is handled during field collection. The cell height of fish that were net-treated before being placed into preservative was not significantly different from those placed directly into preservative (t-test; $P=0.12$; df=30). Additionally, midgut cell height was not influenced by preservative type when Bouin's solution was compared with Z-Fix (t-test; $P=0.17$; df=80).

Fixative effects

Larval walleye pollock shrank an average of 4.7% in Z-Fix, 13% in Bouin's, and 8% in 5% formalin. There was no measurable shrinkage in 95% ethanol. For live larvae placed directly in Z-Fix, shrinkage was a constant proportion of size (Table 3). Laboratory shrinkage in Bouin's and 5% formalin was not a constant proportion of size; in each case the y-intercept of the regression differed significantly from zero ($P=0.04$ for Bouin's, and $P=0.001$ for 5% formalin). Smaller larvae shrank proportionately more than larger larvae in both preservatives (Fig. 5A; Table 3). For example, in using the regression equations in Table 3, the decrease in size of 5-mm-SL larvae preserved in Bouin's was 15% and in 5% formalin was 9%, whereas the decrease in size of 7-mm-SL larvae in Bouin's was 12% and in formalin was 7%. However, there was no observed shrinkage of larvae preserved in 95% ethanol. In fact, the average larva increased slightly in size, about 1% for 5-mm-SL larvae and 3% for 7-mm-SL larvae (Table 3).

Net-shrinkage effects

Larvae shrank an average of 7% after a 5-min net treatment, 14% after 10 min, 17% after 15 min, and 22% after 20 min; initial sizes ranged from 4.48 to

Figure 3

Midgut cell height of laboratory-reared prefeeding, fed, and starved larval walleye pollock, *Theragra chalcogramma*, from hatching to 20 d after hatching. Arrow shows day of first feeding (ff).

Figure 4

Midgut cell height of laboratory-reared prefeeding, fed, and starved larval walleye pollock, *Theragra chalcogramma*, related to laboratory-preserved standard length in Bouin's fixative.

9.90 mm SL (Table 3), and sizes after net treatment ranged from 3.76 to 9.48 mm SL. Length after net treatment was linearly related to initial standard length (net time=0) for each treatment period (Fig. 5B; Table 3). Analysis of covariance (ANCOVA) showed that it was possible to interpolate a 7.5-min regression (needed for field procedures; see Field Collections in Methods section; Table 3) between the 5- and 10-min net-shrinkage regressions; the slopes of the two regressions were not significantly different ($P=0.785$) and the lines were not coincident ($P<0.01$).

Table 2

Midgut cell height of fed and starved laboratory-reared larval walleye pollock, *Theragra chalcogramma*, at 6°C.

| Size class (mm)[1] | year | Fed | | Starved | | P |
		n	mean (SD)	n	mean (SD)	
4.50–5.49	1991	34	14.06 (3.62)	57	8.89 (2.23)	0.006
	1992	31	14.53 (4.01)			
	1993			20	8.16 (1.76)	
5.50–5.99	1991	13	18.78 (5.68)			

[1] Adjusted to equal Bouin's laboratory preserved size.

Table 3

Regression equations for adjusting the size of larval walleye pollock, *Theragra chalcogramma*, exposed to various conditions that cause shrinkage.

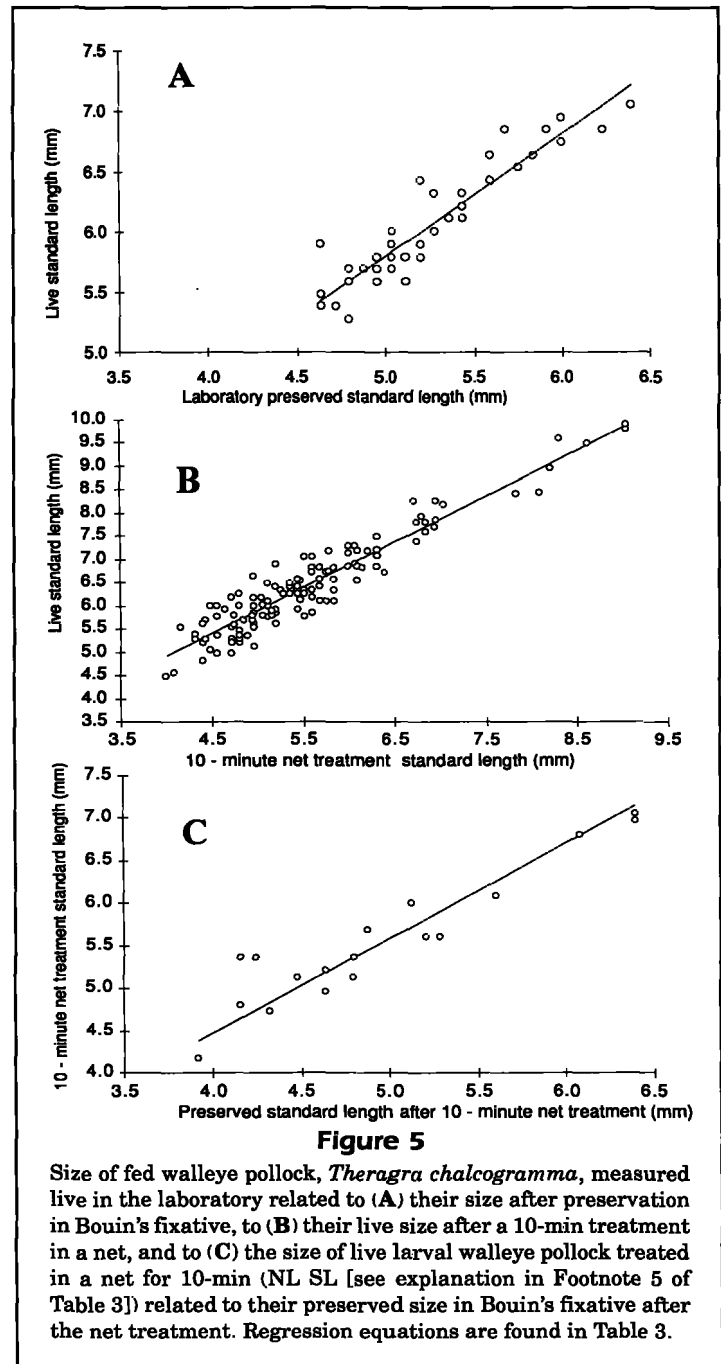| Shrinkage type	Live SL range (mm)	n	Regression equation	r^2
1a) Laboratory shrinkage in Bouin's[1]	5.27–7.06	47	live SL = 0.617+1.033(laboratory preserved SL)	0.88
1b) Laboratory shrinkage in Z-Fix[2,3]	5.20–7.60	56	live SL = 1.047(laboratory preserved SL)	0.99
1c) Laboratory shrinkage in 5% formalin[4]	4.20–20.00	42	live SL = 0.344+1.021(laboratory preserved SL)	0.98
1d) Laboratory shrinkage in ethanol (95%)[4]	5.80–18.90	42	live SL = 0.296+0.929(laboratory preserved SL)	0.99
2) Net treatment, 5 min	4.48–9.90	135	live SL=0.509+0.994(NL SL[5])	0.93
3) Net treatment, 7.5 min (interpolated)			live SL=0.703+0.988(NL SL)	
4) Net treatment, 10 min	4.48–9.90	134	live SL=0.974+0.983(NL SL)	0.90
5) Net treatment, 15 min	4.48–9.90	97	live SL=1.26+0.961(NL SL)	0.87
6) Net treatment, 20 min	4.96–9.90	71	live SL=2.26+0.841(NL SL)	0.84
7a) Shrinkage in Bouin's after net treatment[3]	5.52–8.24	18	NL SL = 1.118(preserved SL)	0.99
7b) Shrinkage in Z-Fix after net treatment[3]	5.36–7.84	35	NL SL= 1.036(preserved SL)	0.99
7c) Shrinkage in 5% formalin after net treatment[3]	4.48–8.40	50	NL SL = 1.097(preserved SL)	0.99

[1] Laboratory shrinkage = live larva placed directly into preservative.
[2] Z-Fix histological preservative, 10% formalin with aqueous zinc and buffers (see text).
[3] In some cases the constant was not significant and was removed from the regression.
[4] Data provided by K. M. Bailey, Alaska Fisheries Science Center.
[5] NL SL = net live standard length (size after net treatment before preservation).

Figure 5

Size of fed walleye pollock, *Theragra chalcogramma*, measured live in the laboratory related to (A) their size after preservation in Bouin's fixative, to (B) their live size after a 10-min treatment in a net, and to (C) the size of live larval walleye pollock treated in a net for 10-min (NL SL [see explanation in Footnote 5 of Table 3]) related to their preserved size in Bouin's fixative after the net treatment. Regression equations are found in Table 3.

Shrinkage in preservative after net treatment

Net-treated larvae shrank an additional 11.8% after being preserved in Bouin's preservative (Table 3). Shrinkage was constant over the size range treated (5.52–8.24 mm SL, $n=18$; Fig. 5C). Additional shrinkage of net-treated larvae in Z-Fix was 3.6% (5.36–7.84 mm SL, $n=35$; Table 3) and in 5% formalin was 9.7% (4.48–8.40 mm SL, $n=50$; Table 3).

Correction for field-captured size

The elapsed time from larval collection to preservation and the preservative type determine which two equations in Table 3 are needed to adjust field-collected and preserved size to the equivalent live size. To examine the accuracy of the regression equations for estimating the live length of field-collected larvae (Table 3), we measured the live size of 20 larvae,

net-treated them for 7.5 min to simulate field collection, preserved them in Z-Fix, and remeasured them several months later. The mean net-treated and preserved SL (4.58 mm ±0.25) was estimated to be 5.39 mm live size from Equations 3 and 7b in Table 3. The observed mean live standard length of the same 20 larvae was 5.46 mm (± 0.21), a difference of 1%.

Field distribution of larvae

Yolk-sac and first-feeding walleye pollock larvae were collected in the eastern Shelikof Strait in April 1991 (Fig. 2; stations 136–141). The abundance of first-feeding larvae increased from April to May. In May, larvae ranging in size from first-feeding to 7 mm were fairly evenly distributed along the coast, within the Strait, and out the sea valley.[3] Some yolk-sac larvae were also found near the Alaska Peninsula and at the exit of Shelikof Strait.

Condition of field-captured walleye pollock

Larvae were sampled from six stations in late April and from 15 stations in early May (Table 4). The level of starvation ranged from 10 to 17% at 3 of the 6 stations in April and at 7 of the 15 stations in May. However, in May, at 3 stations along the sea valley (018, 059, and 068), the percent starvation ranged from 30 to 40% (Fig. 2; Table 4).

Thirty-two percent (n=17/54) of larvae <5.00 mm SL were classified as starving in the combined April and May samples (Table 5). This category included larvae that were smaller than average at first feeding and those that had shrunk from starvation. The percent starving in the first-feeding size category (5.0 to <5.5 mm SL) was 27% (n=12/45), and the number decreased to 12% (n=5/41) for 5.5 to <6.0 mm SL group and finally to zero for larvae >6.0 mm (Table 5).

Discussion

The histological analysis showed that the height of the midgut mucosal cells of laboratory-reared walleye pollock corresponded to their feeding condition and that significant changes in cell height could be detected after food was withheld for one day. Thus, a simple measurement of midgut cell height discerned larval nutritional condition. To confirm the useful-

ness of the measurement for assessing the nutritional condition of field-collected larvae, it was necessary to determine the effects of field collection procedures on the midgut measurement. Although one might expect some compression of the fish body and perhaps a change in gut morphology as a larva shrinks, our experiments with walleye pollock showed no change in size of the midgut cells during the net shrinkage experiments. Thus, we felt confident in applying the laboratory calibration to assess the condition of sea-caught larvae. Theilacker and Watanabe (1989) also demonstrated that there was no change in the midgut cell height for northern anchovy, *Engraulis mordax*, held for periods up to 25 min in the net. Additionally, although preservative type affected the final size of pollock larvae, the midgut cell height did not differ between two preservative types tested, Bouin's and Z-Fix. Therefore field-collected larval pollock that are to be analyzed for condition may be preserved in either Bouin's or Z-Fix.

Since Farris (1963) first noted that larval sardine, *Sardinops sagax*, shrink when preserved in formalin, and Ryland (1966) observed that larval plaice, *Pleuronectes platessa*, collected in the field were smaller at comparable stages of development than were their laboratory-raised counterparts, information has accumulated on shrinkage of larval fish placed directly into preservatives and during the process of field collection and preservation (Blaxter, 1971; Theilacker, 1980, 1986; Hay, 1981; Tucker and Chester, 1984; Fowler and Smith, 1983; McGurk, 1985; Radke, 1989). The cause of larvae shrinkage is related to the loss of osmoregulatory ability when they die (Parker, 1963). Fish larvae also shrink while they are alive, and this shrinkage may be due to damage by the net to the integument, the main osmoregulatory system in larvae before the gills develop (Holliday and Blaxter, 1960). The amount of shrinkage is directly related to the interval that larvae remain in a collecting net. Shrinkage differs among fixatives within species because of the ionic strengths of the preserving fluids (Parker, 1963; Hay, 1982; Tucker and Chester, 1984). Why shrinkage is species specific may be related to differences in osmolarity (Theilacker, 1980; Jennings, 1991) or to thickness of the integument and mucous layer.

The shrinkage values found for walleye pollock larvae placed directly into preservative are similar to those in previous studies (reviewed by Jennings, 1991). Shrinkage of walleye pollock in 5% formalin was greater (as a percentage of length) for smaller larvae (9% for 5 mm SL) than for larger larvae (7% for 7 mm SL). These values are within the range of shrinkage values (3–15%) for other fish larvae summarized by Hjorleifsson and Klein-MacPhee (1992).

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[3] Bailey, K. M., M. F. Canino, J. M. Napp, S. M. Spring, and A. L. Brown. Two contrasting years of prey levels, feeding condition and mortality of larval walleye pollock (*Theragra chalcogramma*) in the western Gulf of Alaska. U.S. Dep. Commer., Natl. Mar. Fish. Serv., Alaska Fisheries Science Center, 7600 Sand Point Way NE, Seattle, WA 98115. Manuscr. in review.

Table 4

Number of starving walleye pollock, *Theragra chalcogramma*, larvae assayed for midgut cell height (MGCH) and prey concentrations in Shelikof Strait, Alaska, in April and May 1991. n = number larvae assayed or number starving (STV).

Station	Date 1991	Time PST[1]	Temp °C[2]	MGCH (n)	SL (mm)[3]	STV (n)	STV (%)[4]	Prey[5]
April 1991								
136	26 April	0428	3.93	2	4.70	2	—	—
137	26 April	1535	3.81	9	4.53–5.30	2	22	—
138	26 April	1706	4.08	8	4.27–5.22	1	13	—
139	26 April	2342	3.92	4	4.10–5.22	0	—	—
140	27 April	0047	3.96	7	4.10–5.47	1	14	—
141	27 April	0157	3.71	6	4.10–5.04	1	17	—
May 1991								
017	2 May	1417	—	14	4.70–5.47	2	14	—
018	2 May	1839	4.15	27	3.93–6.76	8	30	5.00
038	4 May	1309	3.45	6	4.45–5.81	1	17	1.97
053	5 May	1230	—	1	4.19	0	—	—
059	5 May	2153	3.78	5	4.70–5.64	2	40	—
066	6 May	1547	—	2	5.30–5.81	1	—	—
068	6 May	2006	3.86	10	4.62–5.90	4	40	3.93
082	7 May	1835	4.12	3	4.87–5.04	2	—	3.07
089	8 May	0507	4.21	3	5.73–6.07	0	—	3.67
109	10 May	1349	4.26	14	5.13–6.93	2	14	—
110	10 May	1525	4.28	14	4.36–6.76	2	14	—
Buoy 3	12 May	1045	4.14	15	4.79–6.58	0	0	—
Buoy 4	12 May	1147	4.15	9	4.96–6.67	1	11	8.53
Buoy 5	12 May	1256	4.10	10	5.56–6.76	1	10	10.54
Buoy 8	12 May	1555	4.27	7	5.22–6.33	1	14	—

[1] Pacific standard time.
[2] Average temperature between 21 and 40 m depth where a majority of walleye pollock larvae are found (Kendall et al., 1994) (— = no measurements taken).
[3] Preserved standard length (SL) of larvae assayed (SL adjusted to equal Bouin's laboratory preserved size, Table 3).
[4] Percent of larvae starving at stations where at least 5 larvae were assayed.
[5] Number of copepod eggs and nauplii > 150 µm/L averaged over 60 m depth (Bailey et al.[3]).

Walleye pollock preserved in 95% ethanol and measured in water showed a slight increase in size, between 1 and 3%. Values in the literature for ethanol shrinkage vary greatly from 0% (Theilacker, 1980) to 14% (Radtke and Waiwood, 1980), possibly because of concentration differences or because fish are often transferred from ethanol into water for measurement. The transfer permits rehydration which usually negates the original shrinkage.[4]

[4] Theilacker, G. H. U.S. Dep. Commer., NOAA, Natl. Mar. Fish. Serv., Alaska Fisheries Science Center, 7600 Sand Point Way NE, Seattle, Wa 98115. Unpubl. data.

Table 5

Histological condition of walleye pollock, *Theragra chalcogramma*, larvae collected in the Shelikof Strait, Alaska in April and May 1991.

	Midgut cell height			
	Starving	Healthy		
Size class	$\leq 11\ \mu m$	$>11\ \mu m$	No. of	Percent
SL (mm)[1]	n	n	larvae	starving
April 1991				
<5.00	6	20	26	23
5.00–5.49	1	9	10	10
May 1991				
<5.00	11	17	28	39
5.00–5.49	11	24	35	31
5.50–5.99	5	36	41	12
6.00–6.49	0	19	19	0
6.50–6.99	0	17	17	0
			Total	176

[1] Standard length (SL) adjusted to equal Bouin's laboratory preserved size.

It is not clear why the amount of shrinkage is related to larval size for some fixatives and not for others. In this study for example, shrinkage was a constant proportion of size for larvae preserved in Z-Fix. However, in Bouin's solution and 5% formalin, smaller larvae shrank more than larger ones.

The net-treatment experiment showed that shrinkage increased with elapsed time in the net, as in other studies addressing net-capture shrinkage of larval fish (Theilacker, 1980; Hay, 1981; McGurk, 1985). Although our field collections averaged 7.5 min, we included net shrinkage values for up to 20 min in a net for adjusting the size of larvae collected in standard bongo net hauls and MOCNESS tows.

The histological criteria demonstrated that a significant number of larval walleye pollock were starving in the Shelikof Strait, Alaska, in April and May, 1991. These results agree with the 1991 field study of Bailey et al.[3] who used biochemical criteria to determine condition of walleye pollock collected in the same area of Shelikof Strait. They found that more walleye pollock were in poor condition in 1991 than in 1990. Subsequent larval mortality, determined independently by ageing larvae from sequential cruises spanning 2 to 3 weeks, was also higher in 1991 than in 1990.[3] Concentrations of copepod nauplii and invertebrate eggs, the main prey eaten by walleye pollock (Canino et al., 1991), were anomalously low throughout Shelikof Strait in 1991, aver-

aging 6 prey/L, as compared with 38 prey/L in 1990[3] and >20 prey/L in earlier years (Incze et al., 1990; Canino et al., 1991). Others have shown that condition of wild larvae is associated with food availability. In particular, Canino et al. (1991) using a biochemical index showed that larval walleye pollock in Shelikof Strait inhabiting areas of sparse prey were in poorer condition and had fewer prey in their guts than their counterparts inhabiting areas of high prey density. Likewise, larval haddock, *Melanogrammus aeglefinus*, and cod, *Gadus morhua*, a close relative of walleye pollock, were shown to be in poorer condition in well-mixed areas on Georges Bank than in stratified sites where prey levels were higher (Buckley and Lough, 1987). Kashuba and Mathews (1984) showed that poor histological condition of larval shad, *Dorosoma* spp., correlated with low prey levels and with a subsequent abrupt decline in the population.

Our results for walleye pollock indicate that the youngest larvae are most vulnerable to starvation. While 29% of the first-feeding walleye pollock (combined <5.50 mm SL groups; $n=29/99$; Table 5) were classified as starving, one week later the number was reduced to 12%, and 2 weeks later it was zero (Table 5). Others also have found that starvation of larval fishes in the sea decreases quickly, usually within 1 or 2 weeks, as fish larvae mature (O'Connell, 1980; Theilacker, 1986; Robinson and Ware, 1988). Resistance to starvation increases after larval fish first feed (Hunter, 1972; Blaxter and Staines, 1971), acquire the ability to eat more varied prey (Hunter, 1972; Arthur, 1976) and are able to store energy reserves (Ehrlich, 1974; Fraser, 1989; Hakanson, 1989). We also found patchy areas along the Shelikof Strait sea valley in early May 1991 with large numbers of starving larvae, two to three times the background level. Whether small areas of high mortality affect total recruitment is unknown.

Despite arguments to the contrary (Sissenwine, 1984; Peterman et al., 1988) and a general belief that starvation is not a widespread occurrence in the sea (Heath, 1992), evidence from this study shows that starvation does occur and that it is the young stages of walleye pollock that are vulnerable. The advantages of the midgut histological assay, rather than one requiring grading of several tissues, is that it takes less time than does an extensive histological background and is a quantitative rather than a qualitative measure (Theilacker and Watanabe, 1989). Additionally, rates of starvation-induced mortality may be estimated by using the assay and employing laboratory-determined growth rates to determine size and stage durations. Currently, studies are underway to correlate changes in the physical environment

with larval histological condition, feeding, growth, prey availability, and independently determined mortality.

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