



**NOAA Professional Paper NMFS 7**

**U.S. Department  
of Commerce**

**May 2007**

**Spawning, egg development,  
and early life history dynamics  
of arrowtooth flounder  
(*Atheresthes stomias*)  
in the Gulf of Alaska**

**Deborah M. Blood  
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The NOAA Professional Paper NMFS (ISSN 1931-4590) series is published by the Scientific Publications Office, National Marine Fisheries Service, NOAA, 7600 Sand Point Way NE, Seattle, WA 98115.

The Secretary of Commerce has determined that the publication of this series is necessary in the transaction of the public business required by law of this Department. Use of funds for printing of this series has been approved by the Director of the Office of Management and Budget.

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Seattle, Washington

**Suggested reference**

Blood, Deborah M., Ann C. Matarese, and Morgan S. Busby. 2007. Spawning, egg development, and early life history dynamics of arrowtooth flounder (*Atheresthes stomias*) in the Gulf of Alaska. NOAA Professional Paper NMFS 7, 28 p.

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**Abstract**—Arrowtooth flounder (*Atheresthes stomias*) has the highest biomass of any groundfish species in the Gulf of Alaska, is a voracious predator of age 1 walleye pollock (*Theragra chalcogramma*), and is a major component in the diet of Steller sea lions (*Eumetopias jubatus*). Owing to its ecological importance in the Gulf of Alaska and the limited information available on its reproduction, interest has intensified in describing its spawning and early life history.

A study was undertaken in late January–February 2001–2003 in the Gulf of Alaska to obtain information on adult spawning location, depth distribution, and sexual maturity, and to obtain fertilized eggs for laboratory studies. Adults were found 200–600 m deep east of Kodiak Island over the outer continental shelf and upper slope, and southwest along the shelf break to the Shumagin Islands. Most ripe females (oocytes extruded with light pressure) were found at 400 m and most ripe males (milt extruded with light pressure) were found at depths  $\geq 450$  m. Eggs were fertilized and incubated in the laboratory at 3.0°, 4.5°, and 6.0°C. Eggs were reared to hatching, but larvae did not survive long enough to complete yolk absorption and develop pigment. Eggs were staged according to morphological hallmarks and incubation data were used to produce a stage duration table and a regression model to estimate egg age based on water temperature and developmental stage.

Arrowtooth flounder eggs (1.58–1.98 mm in diameter) were collected in ichthyoplankton surveys along the continental shelf edge, primarily at depths  $\geq 400$  m. Early-stage eggs were found in tows that sampled to depths of  $\geq 450$  m. Larvae, which hatch between 3.9 and 4.8 mm standard length, increased in abundance with depth. Observations on arrowtooth flounder eggs and early-stage larvae were used to complete the description of the published partial developmental series.

## Spawning, egg development, and early life history dynamics of arrowtooth flounder (*Atheresthes stomias*) in the Gulf of Alaska

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### Introduction

The worldwide commercial, recreational, and ecological importance of flatfishes warrants our efforts to understand factors that determine recruitment success or failure. The early life history of these fishes is primary to that understanding. Knowing whether eggs are demersal or pelagic helps us to know if human activities such as trawling or dredging will affect a species' reproduction. Knowledge of the timing of spawning and duration of incubation are necessary in the assessment of prey availability (Wilderbuer et al., 2002). Locations and depths where eggs are spawned determines when and where they will hatch, and to where the larvae will disperse prior to assuming a benthic lifestyle (Bailey and Picquelle, 2002). Learning about the early life history of a fish species could be considered the first step to discerning its role in its environment.

Arrowtooth flounder, *Atheresthes stomias* (Jordan and Gilbert, 1880), a large, piscivorous member of the right-eyed flounder family Pleuronectidae, has dramatically increased in biomass since the mid-1970s and, at present, is the most abundant groundfish in the Gulf of Alaska (GOA) (Mundy and Hollowed, 2005; Turnock et al., 2005). Estimated biomass of arrow-

tooth flounder has increased six-fold since 1970 and the projected biomass of age 3+ fish for 2006 is 2,140,170 metric tons (Turnock et al., 2005). Larval abundance also increased notably in the 1990s relative to the previous decade (Matarese et al., 2003; Doyle, 2004). Despite the abundance of arrowtooth flounder, it has limited commercial value due to poor quality of the flesh and the Pacific halibut (*Hippoglossus stenolepis*) bycatch that occurs with bottom trawling (Cullenberg, 1995). However, recent processing discoveries have allowed incidentally-caught arrowtooth flounder to be used for marketable products, including surimi and frozen fillets (Greene and Babbitt, 1990; Porter et al., 1993).

Although of limited commercial importance, arrowtooth flounder is ecologically important at higher trophic levels in the GOA food web. For example, it has been identified as a significant food source for Steller sea lions (*Eumetopias jubatus*), occurring in their diet 21–35% of the time in the area around Kodiak Island (Sinclair and Zeppelin, 2002). Whereas walleye pollock (*Theragra chalcogramma*) has consistently been the greatest component of the Steller sea lion diet in the area around Kodiak Island, arrowtooth flounder may be replacing other secondary food sources. Also,

an increasing trend in the natural mortality of walleye pollock at young ages has been attributed to the growing impact of arrowtooth flounder predation (Bailey, 2000; Hollowed et al., 2000).

Arrowtooth flounder ranges from Cape Navarin, Russia, to the Aleutian Islands in the eastern Bering Sea, throughout the Gulf of Alaska and Northeast Pacific Ocean southward to central California off San Simeon, and along the east coast of Kamchatka and the Commander Islands. It is found on soft, muddy bottom 12–900 m deep, usually offshore at depths of 50–300 m, and has been reported to attain lengths of 86 cm and weights of 7.7 kg (Mecklenburg et al., 2002). At present, most life history information is restricted to adults. Hirschberger and Smith (1983) collected arrowtooth flounder in spawning condition along the outer continental shelf east of Kodiak Island from March to August, but others have concluded that spawning occurs during fall and winter (Pertseva-Ostroumova, 1961; Novikov, 1974; Rickey, 1995). Rickey (1995) determined that arrowtooth flounder are synchronous batch spawners off the Washington coast. Zimmermann (1997) examined maturity and fecundity of arrowtooth flounder in the GOA and described maturity states from macro- and microscopic (histological) observations. Details of spawning behavior, timing, and depth distribution in the GOA are unknown.

Information on the early life history of arrowtooth flounder in the GOA is incomplete. Eggs and early-stage larvae are unknown. Larvae >6 mm standard length (SL) are easily distinguished from other flatfish larvae in the GOA by morphological characters and pigment patterns; in addition, arrowtooth flounder larvae possess preopercular and supraocular spines that are unique among pleuronectids in the GOA (Matarese et al., 1989). Arrowtooth flounder larvae cannot currently be distinguished from its congener Kamchatka flounder (*Atheresthes evermanni*; Jordan and Starks, 1904) in areas where adults co-occur (Bering Sea and Aleutian Islands). Kamchatka flounder is rare in the GOA; only 25 adults have been identified in Alaska Fisheries Science Center (AFSC) GOA surveys since 1990<sup>1</sup>, and there is no data to support that it spawns in the area. Arrowtooth flounder larvae have been collected in the GOA February through June (Matarese et al., 2003); the smallest larvae identified thus far (6–7 mm SL, reflexion) have been collected in greatest numbers over the outer continental shelf and upper slope during February–April, suggesting mid- to late winter deep water spawning. These small larvae have been collected in lesser numbers through May. From analysis of juvenile otoliths and

AFSC ichthyoplankton data, Bouwens et al. (1999) estimated mean length at hatch to be 8–9 mm SL, 15 April as the mean date of hatch, and 8 September as the mean date of settlement for arrowtooth flounder in the GOA; however, their mean length at hatch was based on the most common length class collected during standard ichthyoplankton tows ( $\leq 200$  m depth) and larval stage of development was not determined. Sea valleys and troughs in the GOA appear to be transport pathways for onshore movement of arrowtooth flounder and Pacific halibut larvae (Bailey and Picquelle, 2002). They found higher abundances of larger larvae in troughs and sea valleys and in nearshore shelf areas, particularly when El Niño/Southern Oscillation (ENSO) events occurred in the equatorial Pacific. Survival of larvae is often a recruitment bottleneck for commercial species; perhaps the nature of the transport pathways for arrowtooth flounder larvae enables them to more successfully negotiate this critical stage than other species. The distribution and abundance of eggs, larvae, and juveniles, and transport mechanisms to nursery areas, are largely unknown. Identifying areas where eggs and newly hatched larvae are found is important to verify the hypothesis of Bailey and Picquelle (2002) and may aid in understanding why this species dominates the GOA groundfish assemblage.

More complete life history data are needed to assess the impact of arrowtooth flounder on the GOA ecosystem. The outlook for commercial and forage fish that make up its diet, and for Steller sea lions that prey on them, could be significantly altered if arrowtooth flounder continues to increase in abundance or experiences a drastic decline. More early life history information is also needed to assess the potential impacts of climate and ecosystem shifts. For example, Anderson and Piatt (1999) suggest the 1977 shift to a warm regime has favored earlier-spawning fishes due to earlier zooplankton availability. Information on the timing, location, and dynamics of arrowtooth flounder spawning and early life history is needed to assess this hypothesis and to understand how factors affecting arrowtooth flounder might impact the GOA trophic web.

The primary objective of this study included identifying timing, duration, and location of arrowtooth flounder spawning in the GOA, and collecting spawning adults to obtain fertilized eggs for incubation studies. The morphological development of arrowtooth flounder eggs was described and temperature-specific equations were generated to estimate duration of development time to specific egg stages. Data were also collected on adult maturity states, sex ratios, mean length and percent maturity for each sex at different depths, and physical characteristics of the water column where spawning adults were collected. Other objectives included locating yolk-sac larvae to help describe their

<sup>1</sup> Orr, James. Personal commun. 2006. National Marine Fisheries Service, Alaska Fisheries Science Center, 7600 Sand Point Way NE, Seattle, WA 98115-6439.

onshore transport pathways. The ichthyoplankton surveys also provided preliminary data on abundance and distribution (geographic and vertical) of eggs and larvae. Yolk-sac and transforming stages, not previously described, were illustrated to complete the developmental series.

## Methods

Historical ichthyoplankton data, used to determine times and locations where the smallest arrowtooth flounder larvae (6–7 mm SL) have been collected, were obtained from surveys conducted by the AFSC Recruitment Processes Program. Data for cruises prior to 1988 were found in Dunn and Rugen (1989<sup>2</sup>), and those from 1989 to 2003 in the AFSC ichthyoplankton cruise database (Rugen 2000<sup>3</sup>). Ichthyoplankton data are accessible in the AFSC larval fish database (ICHBASE).

## Surveys

The first cruise was conducted 28 January–5 February 2001 aboard the NOAA ship *Miller Freeman* in the GOA from just southeast of Kodiak Island to Sanak Island (Fig. 1A). Both bottom trawl and oblique bongo sampling were conducted day and night over the outer continental shelf and upper slope to depths of approximately 200, 400, and 600 m; an additional trawl at 500 m was added to locate more ripe fish. The upper slope was steep and the locations where gear could be towed at the three target depths were very close to one another, so most stations were clustered in groups of three. Opportunistic sampling occurred in shallow water (64–137 m) closer to Kodiak and Sanak Islands when conditions were too rough to sample further offshore.

A second survey was conducted 3–9 February 2002, aboard the *Miller Freeman*, in the same general area as in 2001 (Fig. 1B). This cruise was restricted to bottom trawls (day and night) to collect adult arrowtooth flounder at locations where high abundances of eggs and larvae were found in 2001. Based on preliminary results of the 2001 cruise, stations were added at 500 m to maximize opportunities to collect equal mixes of mature and ripe males and females.

The third cruise was conducted 13–22 February 2003. Designed primarily to survey a grid of stations to assess ichthyoplankton east and south of Kodiak Island

(Fig. 2), the station plan allowed for opportunistic deep water sampling (day and night oblique bongo tows to depths of 200, 400, and 600 m) to collect arrowtooth flounder eggs and early-stage larvae. Once the survey grid was completed, bottom trawls were conducted, beginning southwest of Kodiak Island along the upper slope where we had collected spawning adult arrowtooth flounder during the previous two winter cruises. Trawl depths were 200, 300, 400, and 500 m.

Adult arrowtooth flounder were collected with a nylon Nor'eastern bottom trawl (Stark and Clausen, 1995). A sample of up to 40 fish from each trawl (519 total fish) in 2001 was sexed and measured to the nearest 1.0 cm fork length (FL); gonads were examined for macroscopic maturity states. Maturity categories of immature, mature, ripe, or spent were assigned to each individual examined. Maturity categories and criteria follow Zimmermann (1997) with some additions: 1) mature category for males further defined by being able to extrude only a drop of milt, 2) addition of ripe category for males defined as being able to extrude milt with light pressure, and 3) addition of mature category for females defined as ovaries large and filled with small eggs, but none able to be extruded. Up to four males and four females in each maturity category (18 total fish) were weighed individually, and their gonads were removed, weighed to the nearest 0.01 g, and preserved for histological examination. Microscopic examinations were used to verify visually-assigned maturity states, following Zimmermann (1997). In 2002, up to 181 fish from each trawl (599 total fish) were sexed, measured to the nearest 1.0 cm FL, weighed to the nearest 0.1 g, and examined macroscopically for maturity state. Adult data recorded in 2003 were numbers and lengths of fish used for spawning. At trawl stations where spawning arrowtooth flounder were found in 2001–2003, a Sea-Bird SBE-19 Seacat system was attached to the tow cable above the bongo net frame or a Sea-Bird 911 plus CTD system was deployed separately to collect conductivity, temperature, and depth (CTD) profiles.

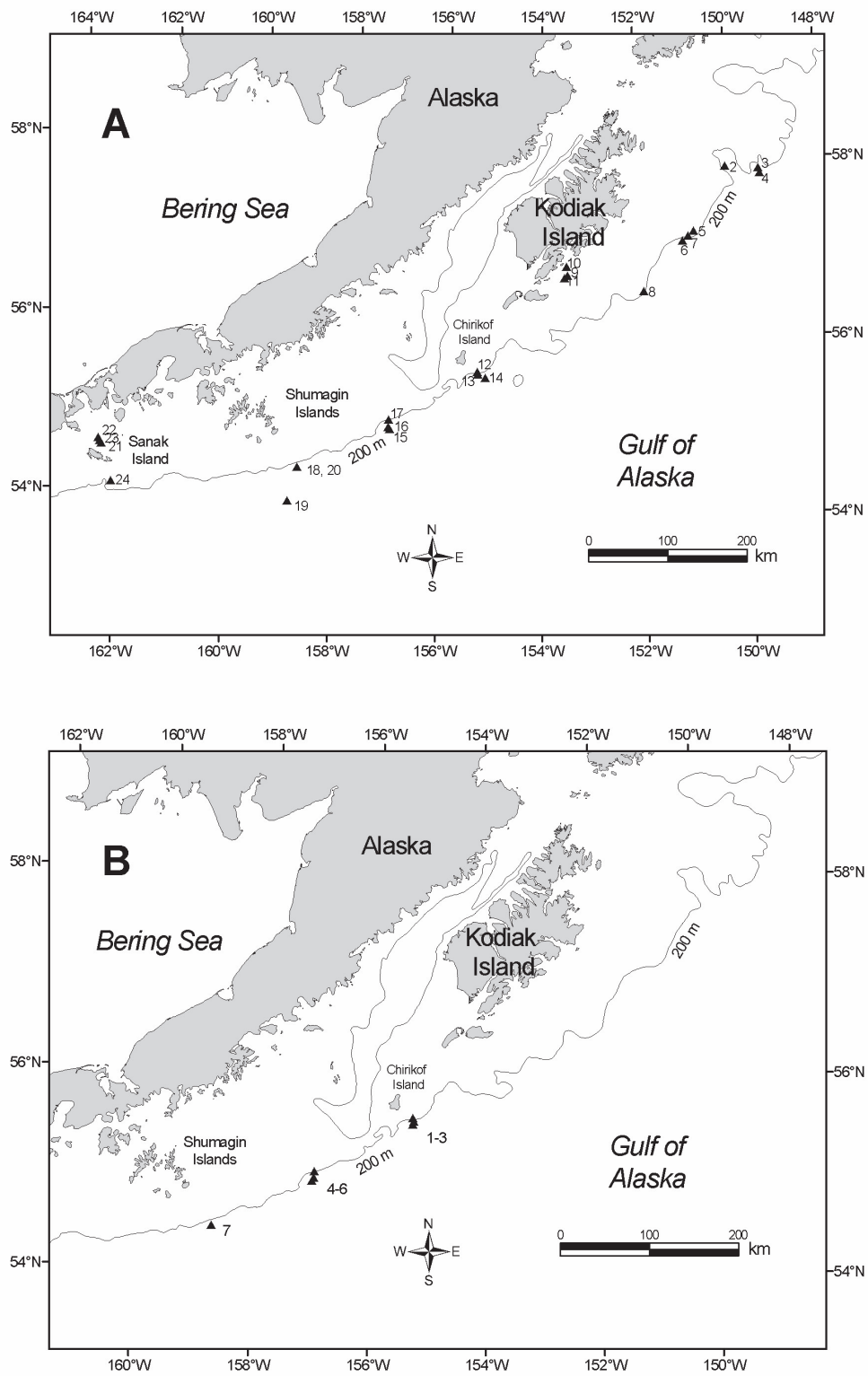
## Egg fertilization, incubation, and sampling

Eggs stripped from ripe females were fertilized with milt from multiple males using the procedures described by Blood et al. (1994), except males had so little milt ( $\leq 0.5$  ml per male) that it was first collected in plastic pipettes rather than being expressed directly into a bowl with the eggs. In some cases, females collected at one depth were held in live tanks until ripe males could be collected at other depths. Fertilized eggs were placed into one-gallon glass jars in a large 3.0°C refrigerator. In 2001, eggs remained in the 3.0°C refrigerator for the duration of the cruise. In 2002 and 2003, eggs were held in the 3.0°C refrigerator for 3–5 h, then examined

<sup>2</sup> Dunn, J. R., and W. C. Rugen. 1989. A catalog of Northwest and Alaska Fisheries Center ichthyoplankton cruises 1965–1988. Alaska Fish. Sci. Cent., Natl. Mar. Fish. Serv., NOAA, 7600 Sand Point Way NE, Seattle WA 98115-6439. AFSC Proc. Rep. 89-04, 87 p.

<sup>3</sup> Rugen, W. C. 2000. Alaska Fisheries Science Center Ichthyoplankton Cruise Database. [Online]. Available: <http://161.55.120.152/icc/openframe.cfm> (access date – August 2006).

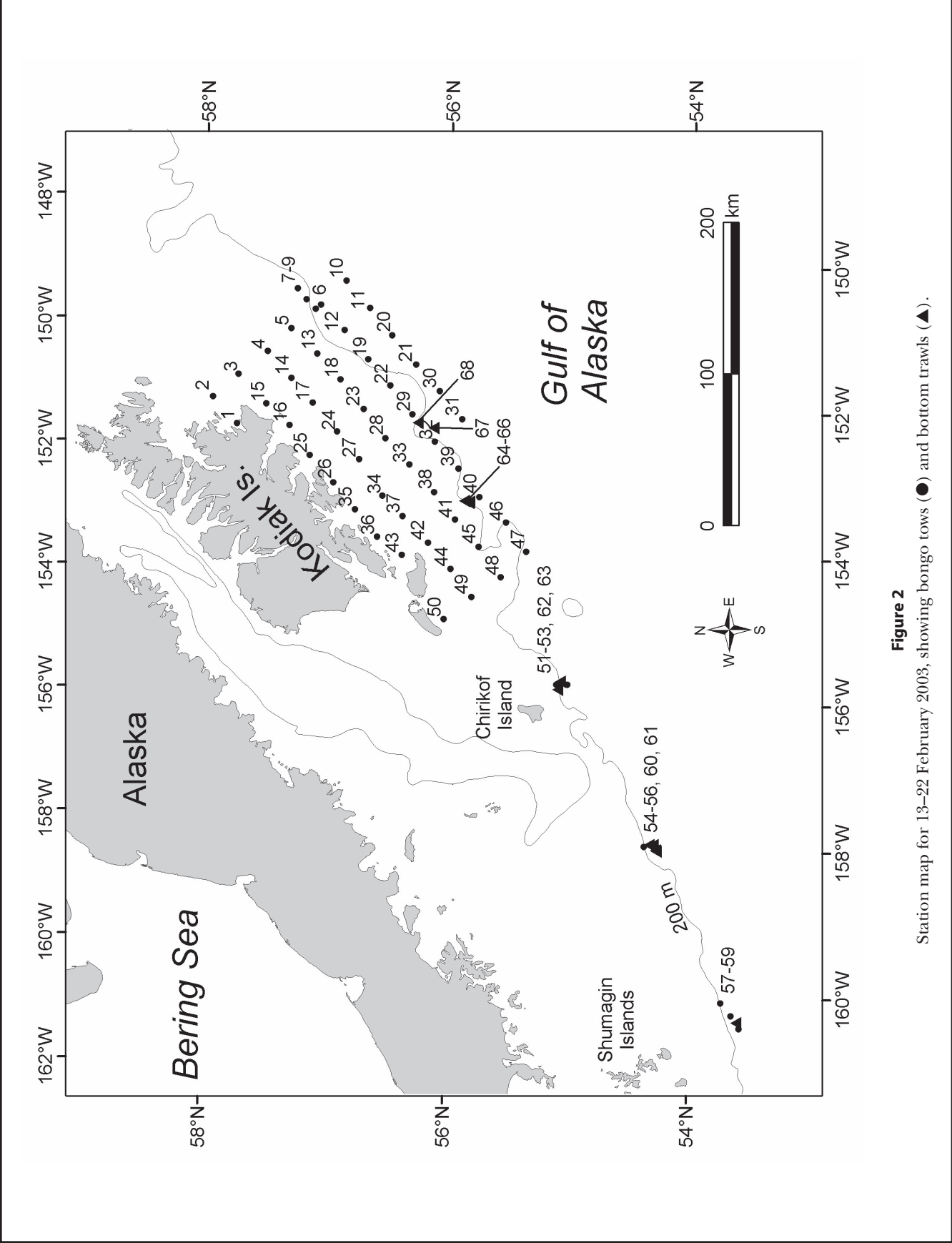




**Figure 1**

Station map for 2001 and 2002 cruises. A) 28 January–5 February 2001. Deep bongo tows and bottom trawls conducted at each station. B) 3–9 February 2002. Only bottom trawls conducted at each station.





**Figure 2** Station map for 13–22 February 2003, showing bongo tows (●) and bottom trawls (▲).

**Table 1**  
Station and biological data for arrowtooth flounder (*Atheresthes stomias*) spawning and egg incubation 2001–2003.

Year	2001	2002	2003
Date	2 February	7, 8, 9 February	18, 21 February
Time (local)	1400	1915, 2230, 0300	0800, 1500
Location	54°30.94'N, 158°46.83'W	54°30.88'N, 158°46.76'W; 54°28.99'N, 158°50.66'W; 54°29.04'N, 158°50.75'W	55°02.69'N, 157° 02.72'W; 56°15.23'N, 153° 17.41'W
Depth (m)	400	400, 400, 500	400, 300
Temperature at depth of spawning females (°C)	4.7	4.8, 4.8, 4.3	5.0, 5.4
<i>n</i> females	1	1, 2, 1	1, 5
Lengths (cm FL)	60	81; 55, 69; 72	58; 81, 71, 74, 52, 80
Weights (g)	N/A*	5566; 1734, 3044; 3950	N/A*
<i>n</i> males	3	8, 14, 31	16, 20
Length range (cm FL)	43–47	41–47, 36–50, 43–50	41–53, 41–57
Weight range (g)	N/A*	630–948, 616–1046, 636–1038	N/A*
Incubation location	Seattle, WA	Seattle, WA	Kodiak, AK
Egg incubation temperatures (°C)	3.0	3.0, 4.5, 6.0	3.0, 4.5, 6.0
Incubation sampling schedule	variable intervals every 12–24 h onboard ship; every 24 h thereafter	every 12 h; eggs did not survive incubation	sampled every 2 h until blastodisc formed, then every 12 h

\*Not available.

using a microscope (25×); floating eggs having a perivitelline space were assumed to be fertilized (Alderdice, 1988). Viable eggs were separated into three groups in multiple one-liter jars placed in small refrigerators set at 3.0°, 4.5°, and 6.0°C. Up to 10 eggs were sampled from each group at 2–24 h intervals for the duration of each cruise (Table 1). In 2001–2002, eggs were sampled every 12–24 h; in 2003, eggs were sampled every 2 h during the period of most rapid development until formation of the blastodisc. Eggs were sampled every 12 h thereafter. At the end of each survey, eggs were transferred into thermoses, placed in coolers, and transported to the rearing laboratory at the AFSC, Seattle, in 2001–2002, or to the AFSC Kodiak lab in 2003. In Seattle, eggs were placed in one-gallon glass jars in walk-in coolers (4.5°C and 6.0°C) or a refrigerator (3.0°C). Eggs reared in 2001 were incubated with no aeration until fungus developed after day 17; all jars were aerated and received daily 50% water changes thereafter. One jar was treated with Maracyn® and acriflavine to kill the fungus; eggs in this jar were not used for subsequent incubation and hatch data, but live diameters of eggs were recorded and newly hatched larvae were transferred to a larger tank to document first appearance of pigment. All jars were aerated in 2002 and received 50% water changes daily. In Kodiak, eggs were incubated in 10-inch diameter screened (1 mm) PVC tubes suspended in troughs supplied by a flow-through seawater system at approximately 5°C. Chillers were used in two troughs to reduce the water temperature to 3.0°C and 4.5°C and an aquarium

heater was used to increase the temperature in the third trough to 6.0°C. Eggs were sampled at 12–24 h intervals and held through hatching and the early yolk-sac stage. All eggs were staged according to developmental criteria described by Blood et al. (1994) with one exception: since the arrowtooth flounder embryo is not pigmented, those authors' stage 14 (first appearance of pigment) was not used, and subsequent stages, defined by morphological changes, were assigned a number equaling stage number –1 (e.g., stage 15 for walleye pollock eggs equals stage 14 for arrowtooth flounder eggs). Eggs were preserved in 5% buffered formalin. Some eggs were also preserved in Stockard's solution to clear the chorion and darken embryonic tissue.

Ichthyoplankton data were collected in 2001 and 2003 using a MARMAP bongo sampler (Posgay and Marak, 1980) with an inside diameter of 0.6 m and 0.505-mm mesh nets. Bongo nets were lowered at a rate of 40 m of wire/min and retrieved at a rate of 20 m/min, sampling obliquely from the surface to within 10 m of the bottom or to targeted depths where deeper, following standard MARMAP procedures (Smith and Richardson, 1977). Ichthyoplankton samples were preserved in a 5% buffered formalin-seawater solution and later sorted for ichthyoplankton, which were removed and identified to the lowest possible taxon at the Plankton Sorting and Identification Center in Szczecin, Poland (ZSIOP). Egg and larval identifications were verified at AFSC. A detailed account of sampling and identification protocols is available in Matarese et al. (2003). Position of embry-

onic and larval structures are presented as percentages of preserved standard length. All larval measurements reported are standard length.

### Analytical methods

For adult arrowtooth flounder, depth of capture was separated into three strata: 200 m (150–250 m), 400 m (350–450 m), and >450 m (460–650 m). The depth stratum for the deepest samples for adults, eggs, and larvae was changed from 600 m to >450 m after the first cruise to include data from all hauls; one of four trawls (502 m) and two of five bongo tows (525, 536 m) would have been excluded from analysis if the deepest stratum had remained as 600 m. Sex ratios were determined for each stratum using the mean proportion of the fish that were females in all hauls. An ANOVA tested for significant differences of the proportion of females among the depths. The proportions of females were transformed using the arcsine of the square root of the proportion to rescale the data to satisfy the assumptions of the ANOVA. Percent maturity was calculated for males and females examined for maturity states in 2001 and 2002.

For arrowtooth flounder eggs and larvae, depth of capture was separated into four strata defined by the maximum depth of the bongo array: <150 m, 200 m (150–250 m), 400 m (350–450 m), and >450 m (460–650 m). Abundances of arrowtooth flounder eggs and larvae collected in ichthyoplankton samples were calculated as number/10m<sup>2</sup> sea surface area (Kendall and Dunn, 1985). Up to 100 eggs from each station in 2001 were staged as described above to determine age and stage-specific vertical distribution; all eggs were staged in 2003. The diameters of 118 live reared eggs, 194 preserved reared eggs, and 510 randomly selected eggs collected in ichthyoplankton samples and preserved in 2001 were measured to the nearest 0.01 mm; 226 preserved reared eggs and 71 randomly selected eggs collected in ichthyoplankton samples in 2003 were also measured. Newly hatched larvae in the laboratory (129 of a total of 435) and up to 50 individuals from each ichthyoplankton sample were measured to the nearest 0.1 mm SL; newly hatched larvae were measured when alive, while field-collected larvae were measured after preservation in 5% formalin. Range, mean, mode, and standard deviation were calculated for egg diameter and larval length measurements. An ANOVA was used to test for significant differences in mean lengths of larvae collected from bongo tows that sampled to depths of 400 m and >450 m in 2001 (no larvae were collected in tows that sampled to 200 m) and 200 m, 400 m, and >450 m in 2003.

Egg incubation data were used to generate temperature-specific equations to estimate duration of develop-

ment and age of the eggs. Endpoints, midpoints, and duration of stage (in hours) were estimated for eggs incubated at each temperature, as described in Blood et al. (1994), except the midpoint of the last stage (19) was taken as the midpoint between the end of stage 18 and the sampling time during which the last egg hatched, rather than the time of 50% hatch as defined by Blood et al. (1994). A piecewise, least-squares, linear regression model (SAS, vers. 6.0, SAS Inst., Inc., Cary, NC) was applied to estimate age (hours) of eggs at a specific stage incubated at any temperature within the limits of our data.

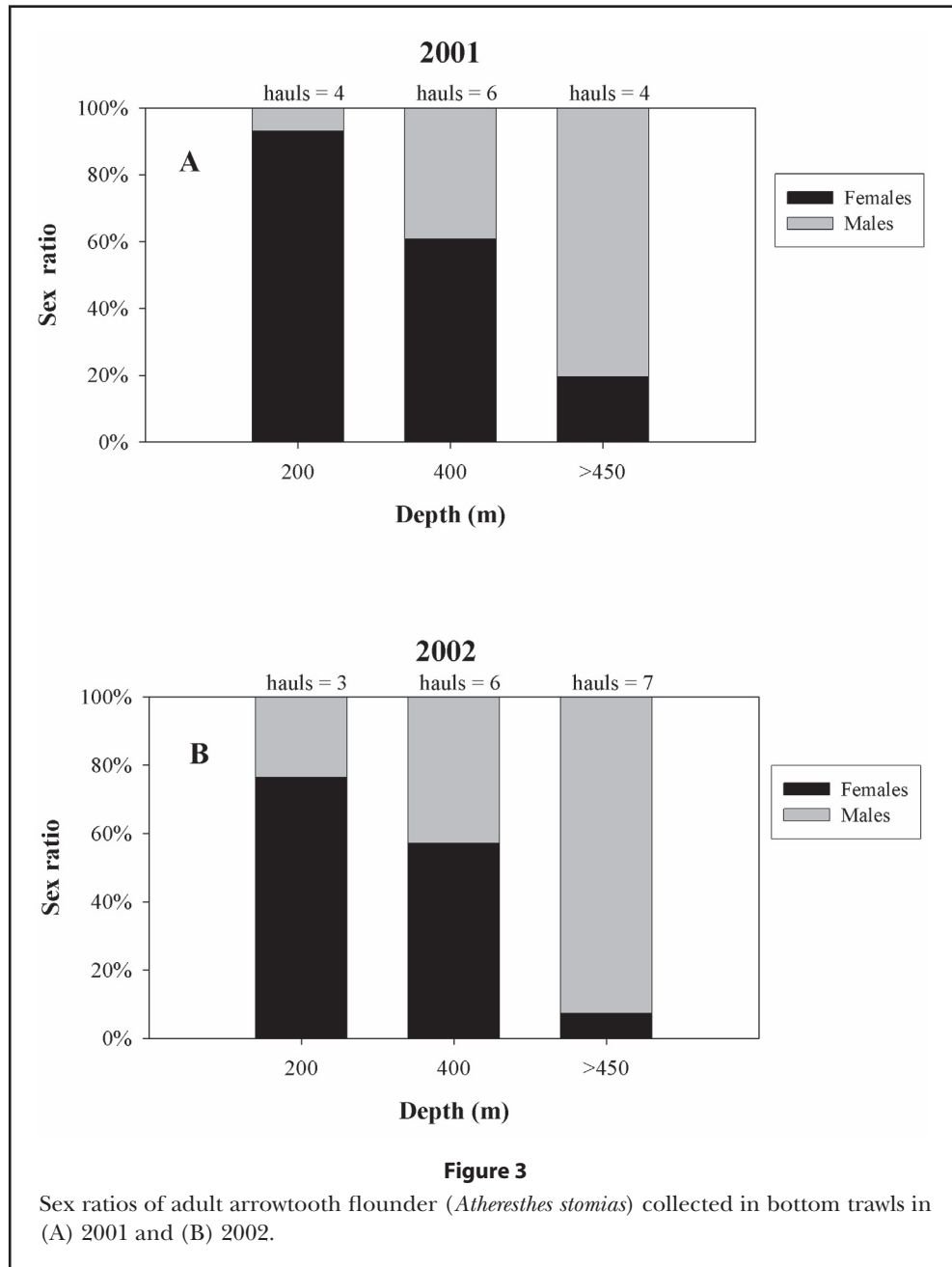
## Results

### Spawning and maturity of arrowtooth flounder

The sex ratio of arrowtooth flounder varied significantly by depth stratum (Fig. 3). Females dominated the catch at 200 m, the sex ratio was more evenly distributed at 400 m, and males dominated the catch at >450 m. The proportion of females in the catch at >450 m was significantly lower ( $P<0.05$ ) than the proportion of females at 200 m and 400 m, but the proportion was not significantly different between the 200 m and 400 m strata. Few females collected at 200 m and >450 m were mature (54 of 369) and only one female was ripe. At 400 m, 23–26% of the females were mature and 0–10% of the males were mature (Fig. 4); among these, 51% of females and less than 2% of males were ripe. Most ripe males were collected at depths >450 m: 50% of ripe males in 2001 and 100% of ripe males in 2002 were collected from this stratum. Females averaged 51.5 cm FL and males averaged 43.4 cm FL in 2001 and 47.8 cm FL and 39.6 cm FL, respectively, in 2002 (Fig. 5). Water temperature at the depths where spawning females were collected was 4.7°–4.8°C (400 m) and 4.2°C (500 m) (Table 1).

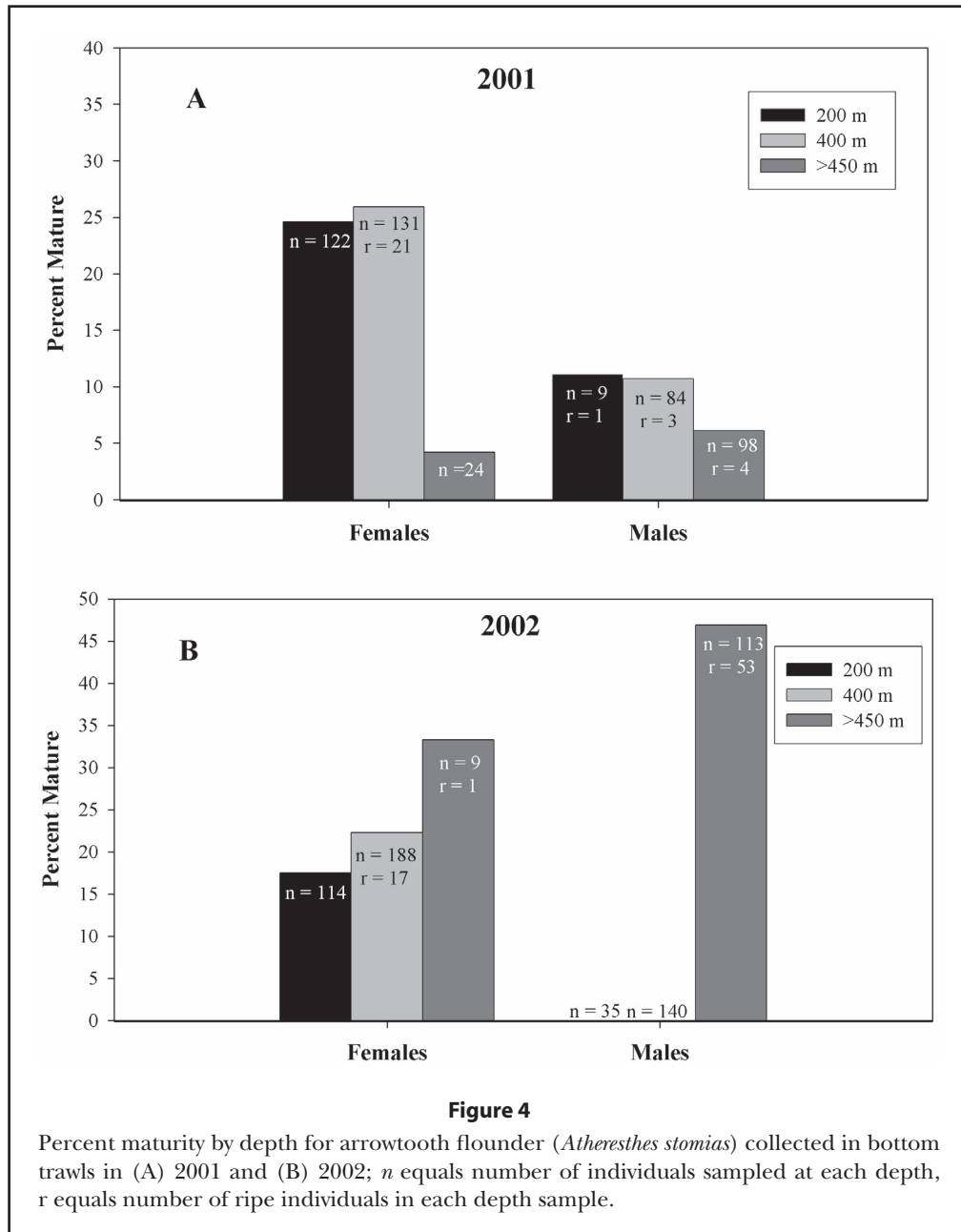
No maturity data were collected in 2003; however, many spent females were encountered in areas where in previous years we obtained ripe individuals. It was necessary to travel farther north than in 2001–2002 to find ripe females along the slope (Table 1, Fig. 2). The temperature at 400 m where the first spawning adults were collected in 2003 was warmer (5.0°C) compared to the prior two years. The second group of spawners (5 females, 20 males) was collected from 300 m (5.4°C); no spawners had previously been collected at that depth.

Macroscopic maturity states of selected adults in 2001 agreed with histological maturity states for all males and most females (Table 2). The distinction between mature and ripe males was difficult to assess at times; mature males had only a drop or two of milt that could be expressed and ripe males usually had about 0.5 ml of milt



(ca. 14 drops). It was difficult to express enough milt from males to assess their maturity and still have enough milt left to fertilize eggs. Histological examination of males classified as ripe confirmed that even though the quantity of milt produced was very small, there were more spermatozoa (Fig. 6A) than in those males classified as immature (Fig. 6B). The gonadal somatic index (GSI) for males was inconsistent; immature male GSI ( $n = 4$ ) ranged from 0.13 to 0.33 and mature male GSI ( $n = 3$ ) was 0.16–0.38. Histology slides prepared from a ripe female's ovaries show the hydrated, yolked eggs and

the postovulatory follicles that characterize this stage of maturity (Fig. 7A,B). The preliminary assessment of the 53-cm FL female (Table 2) as mature was incorrect; although that individual did have many small eggs in the lumen of the ovary, most of the larger eggs were atretic (Fig. 8) (as described by Zimmermann, 1997). The GSI calculated for females was distinct for each of the four maturity states. Immature female GSI ( $n = 3$ ) was 0.54–0.79 and spent female GSI ( $n = 5$ ) was 1.08–2.69; mature ( $n = 1$ ) and ripe ( $n = 1$ ) female GSI was 9.93 and 23.83, respectively.



### Description of eggs and morphological development

Arrowtooth flounder eggs are pelagic, with a smooth, clear chorion and homogeneous yolk. The perivitelline space is narrow and oil globules are absent. Live eggs fertilized at sea were 1.64–1.97 mm in diameter ( $n = 118$ , mean 1.83 mm, mode 1.84 mm, SD 0.06 mm); formalin-preserved eggs fertilized at sea had a wider size range, 1.58–1.98 mm ( $n = 419$ , mode 1.80 mm, SD 0.06 mm), but were slightly smaller on average (mean 1.82 mm). Eggs collected in ichthyoplankton tows had

a slightly greater mean diameter of 1.86 mm ( $n = 581$ , range 1.76–1.98 mm, mode 1.84 mm, SD 0.04 mm). The yolk and embryo are unpigmented throughout development (Fig. 9). Nineteen developmental stages were defined as follows:

**Stage 1** (precell – early stage) – Cytoplasm is concentrated at the animal pole, appearing as a raised cap of undivided cellular material. The edge of the cytoplasm is diffuse, with no clear margin.

**Stage 2** (2 cells – early stage) – Cytoplasm is divided into two equal-sized cells. The outer margins of the

cells are diffuse, whereas the margin between cells is well defined.

**Stage 3** (4 cells – early stage) – Cell cap consists of four cells of equal size.

**Stage 4** (8 cells – early stage) – The eight cells comprising the cell cap form a single-layer rectangle two cells wide by four cells long, with the four inner cells smaller than the four outer cells. The outer margins of the cells are now distinct.

**Stage 5** (16 cells – early stage) – Fourth and last division of cells in a single layer. Cells form a rough square four cells wide and four cells long.

**Stage 6** (32 cells – early stage) – Number of cells >16 and ≤32. The blastodisc is greater than one cell thick and has a round edge, appearing similar to a raspberry. During this stage, the cells multiply and become smaller, but the size of the blastodisc remains constant.

**Stage 7** (blastodermal cap – early stage) – Individual cells are not visible at 50× magnification. The blastodisc appears to sit on top of the yolk and the periblast begins to form.

**Stage 8** (early germ ring – early stage) – The center of the blastodisc becomes thinner and the cytoplasm appears granular, while the edge of the blastodisc remains thick. The center of the blastodisc (blastocoel) remains thin and granular and toward the end of this stage a portion of the germ ring that will form the embryonic shield bulges toward the center. In profile, the germ ring extends less than  $\frac{1}{4}$  of the way down the yolk.

**Stage 9** (germ ring extends  $\frac{1}{4}$  of the way down the yolk – early stage) – The embryonic shield is small and projects  $\frac{1}{4}$  of the way across the blastocoel.

**Stage 10** (germ ring extends to half-way down the yolk – early stage) – The embryonic shield is large and extends to the top of the yolk. The neural keel is prominent, but the margin of the embryonic shield is diffuse.

**Stage 11** (germ ring extends  $\frac{3}{4}$  of the way down the yolk – early stage) – The developing embryo is very slender and no differentiation of the body is apparent.

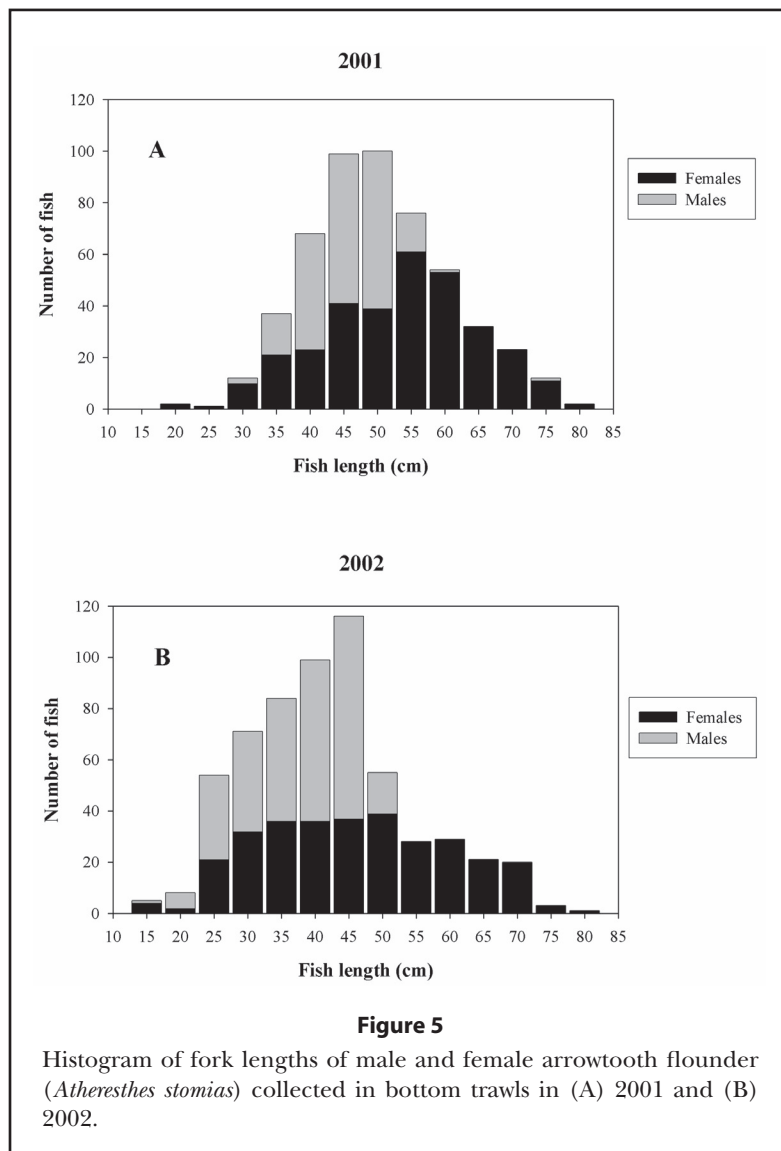
**Stage 12** (late germ ring – early stage) – The germ ring has almost completed overgrowth of the yolk, but the blastopore remains open. Optic vesicles are apparent and the notochord is visible ventrally. Myomeres (12–17) are visible and Kupffer's vesicle can be seen in the live egg.

**Stage 13** (early middle stage) – The blastopore is closed. The tail margin is indistinct and flat, but the medial portion of the tail begins to expand dorsoventrally. Kupffer's vesicle is visible in the Stockard's preserved egg and 19 myomeres can be counted.

**Stage 14** (late middle stage) – Optic vesicles are very apparent and the heart and hindbrain can be distinguished. The tail is plump and the tip appears to be lifting from the yolk surface, but the tail margin is still attached. About 27 myomeres are formed.

**Stage 15** (early late stage) – The tail tip is lifted away from the yolk surface.

**Stage 16** (tail extends to  $\frac{5}{8}$  of the way around the yolk – early late stage) – A greater portion of the tail is lifted from the yolk surface as it elongates and tapers toward the end. At the beginning of this stage the tail is opposite the head (embryo extends half-way around the yolk); at the end of the stage the tail extends to  $\frac{5}{8}$  of the





way around the yolk and the tip has begun to curve away from the longitudinal plane of the embryo. The finfold forms on the posterior half of the tail. Otic capsules are visible.

**Stage 17** (tail extends to  $\frac{3}{4}$  of the way around the yolk – middle late stage) – The tail continues to lengthen and taper while it bends further out of the longitudinal plane of the embryo. The anus is formed, positioned at 60% SL, and is the point at which the body bends. The dorsal finfold extends anteriorly to 50% SL.

**Stage 18** (tail extends to  $\frac{7}{8}$  of the way around top half of the yolk – middle late stage) – The embryo is now positioned around the top half of the yolk; the entire body of the embryo can be seen on one side of the yolk. The anus is now positioned at 50% SL and the finfold extends anteriorly to 30% SL.

**Stage 19** (tail completes full circle around top half of the yolk – late stage) – The tail of the embryo almost touches the head. The anus is at 40% SL and the dorsal finfold extends to just anterior to the anus.

## Egg incubation

Water temperature for eggs incubated in 2001 varied no more than 0.2°C above or below the target temperature of 3.0°C except on day 81 (Fig. 10A); average temperature was 3.1°C. Eggs were first sampled 22.5 h after fertilization, at which time they were classified as stage 4. Eggs began to hatch at stage 18 (512 h); midpoint of hatch was at 562 h. The hatching period lasted 143 h and the last sample was taken at 655 h. Larvae did not survive to yolk absorption and remained unpigmented.

Water temperature in the thermoses transported to Seattle in 2002 dropped to  $\leq 1^\circ\text{C}$  during shipping. Few of these eggs survived to hatch. Eggs incubated at 4.5°C survived only to stage 13; the 3.0°C and 6.0°C eggs appeared to have hatched prematurely (larvae were curled). Incu-

**Table 2**

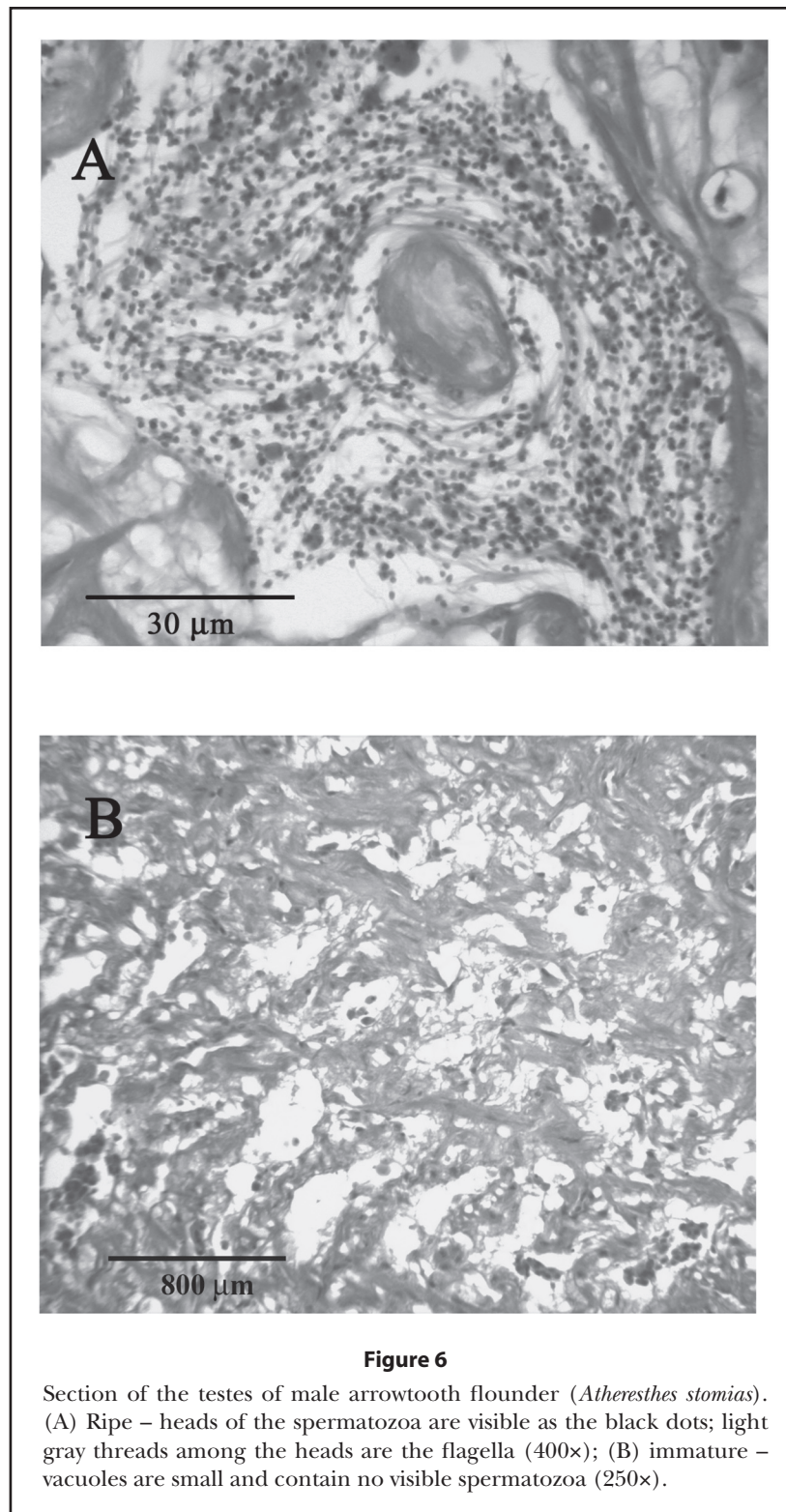
Collection data, gonadal somatic index (GSI<sup>1</sup>), macroscopic maturity states, and histological (microscopic) maturity states (based on Zimmermann, 1997, in part) for selected arrowtooth flounder (*Atheresthes stomias*) adults collected in 2001.

Date	Depth (m)	Sex	Fork length (cm)	Somatic weight (g)	Gonad weight (g)	GSI <sup>1</sup>	Macroscopic maturity	Histological maturity <sup>2</sup>	Comments
30-Jan	395	F	53	1399.6	36.71	2.69	Mature	Atretic – Spent	A few hydrated eggs remaining in ovary
30-Jan	395	F	54	1492.0	287.10	23.83	Ripe	Spawning – postovulatory follicles present	Many hydrated eggs present
30-Jan	395	F	55	1587.8	143.43	9.93	Mature	MN – Late developing	No hydrated eggs present
1-Feb	127	F	42	595.5	4.69	0.79	Immature	Immature	Immature
1-Feb	127	F	43	653.8	3.53	0.54	Immature	EP, LP – Immature	Immature
2-Feb	400	M	39	493.8	0.63	0.33	Immature	Immature	No spermatozoa visible
2-Feb	400	M	40	531.4	0.77	0.13	Immature	Immature	No spermatozoa visible
2-Feb	400	M	41	571.0	0.91	0.15	Immature	Immature	A few spermatozoa in early development
2-Feb	397	M	37	424.1	1.40	0.18	Mature	Mature	Only a few spermatozoa visible
2-Feb	397	M	40	531.4	0.97	0.16	Ripe	Mature	Only a few spermatozoa visible
2-Feb	397	M	43	656.1	1.07	0.16	Immature	Immature	No spermatozoa visible
2-Feb	397	M	49	962.6	3.69	0.38	Ripe	Mature	Many spermatozoa visible
2-Feb	397	M	51	1083.2	2.72	0.25	Mature	Mature	Only a few spermatozoa visible
5-Feb	390	F	59	2007.1	21.51	1.08	Spent	LP, CA – Spent	Atresia noted
5-Feb	390	F	60	2121.3	24.03	1.15	Spent	LP, CA – Spent	Atresia, a few hydrated eggs present
5-Feb	390	F	61	2239.4	15.32	0.69	Immature	LP, CA	Immature, not spent
5-Feb	390	F	69	3331.0	52.24	1.59	Spent	CA – Spent	Atresia, a few hydrated eggs present
5-Feb	390	F	70	3486.8	52.32	1.52	Spent	CA – Spent	Atresia noted

<sup>1</sup> Gonadal somatic index =  $100 \times \text{gonad weight} / (\text{somatic weight} - \text{gonad weight})$ .

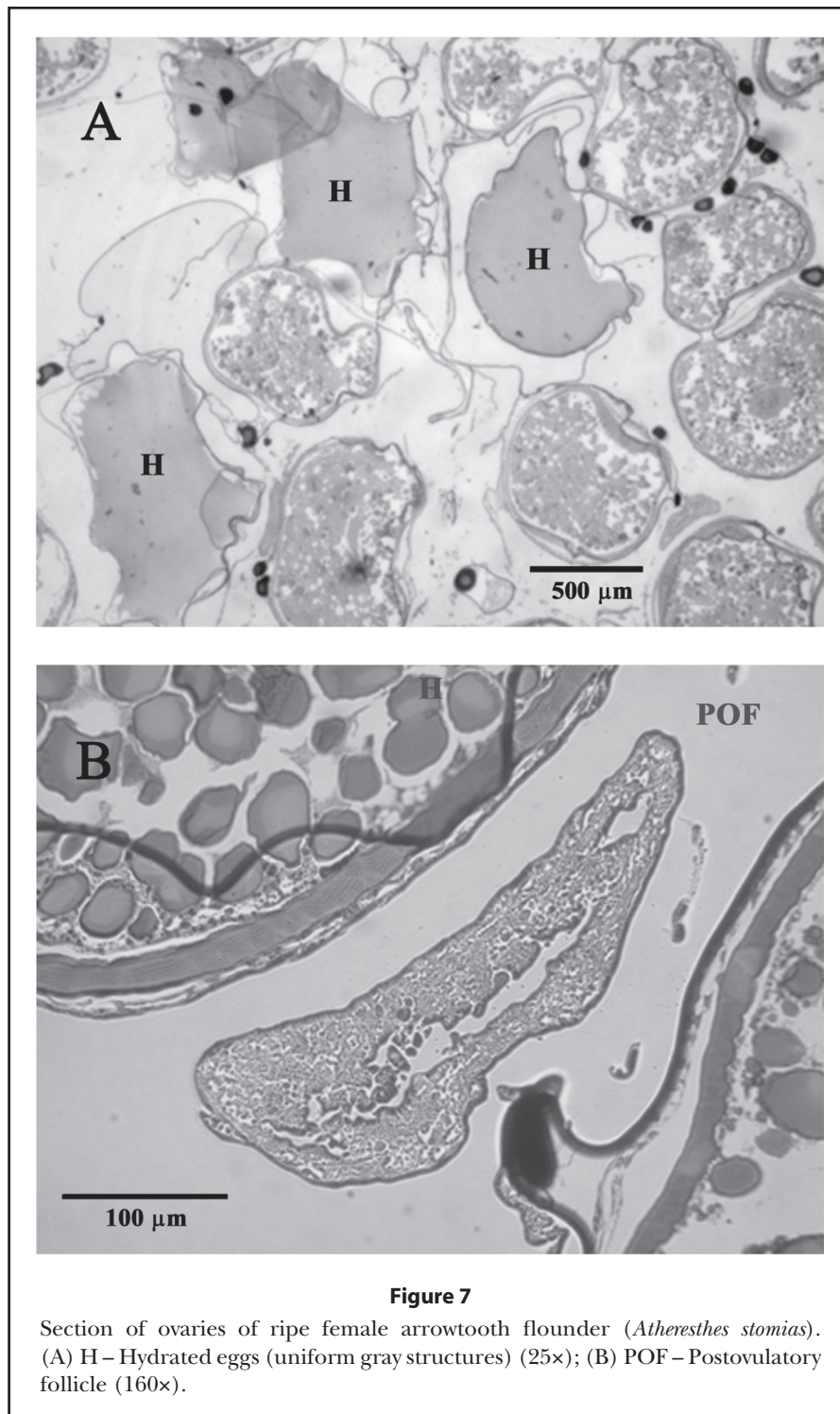
<sup>2</sup> Histological stages according to Zimmermann (1997): MN = migratory nucleus, EP = early perinucleus, LP = late perinucleus, CA = cortical alveoli.





bation data were not used because of the drop in water temperature during shipping. No larvae were measured and none survived long enough to develop pigment.

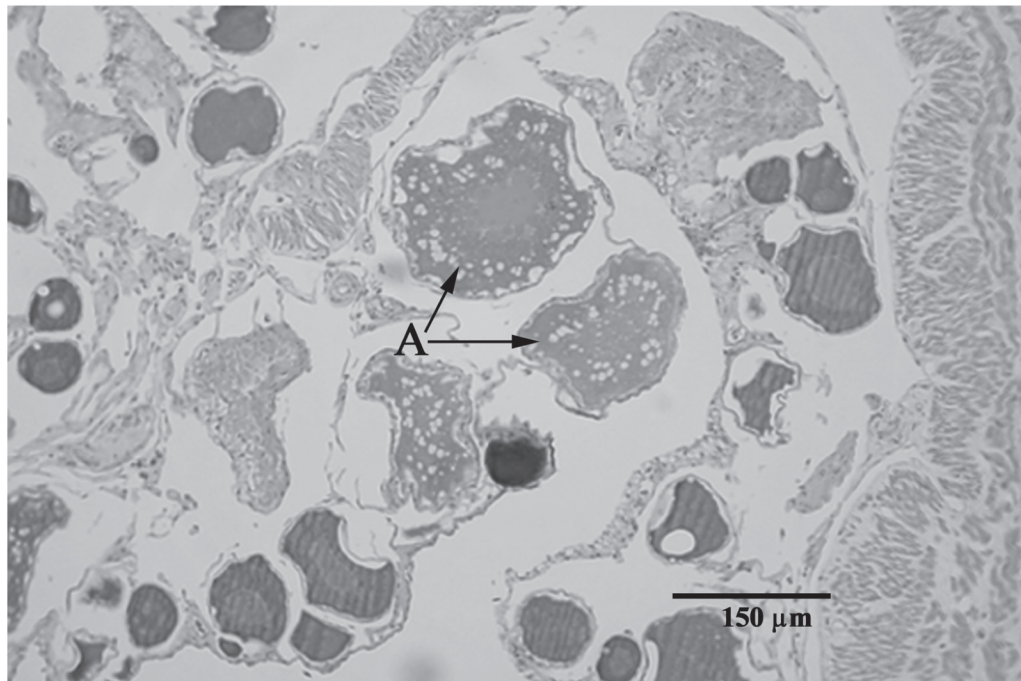
Water temperature for eggs incubated at 3.0°C in 2003 varied more than in 2001 (Fig. 10B) and averaged 3.2°C. Development to the end of stage 17 was docu-



mented; shortly thereafter, there were no eggs left to sample in the incubator.

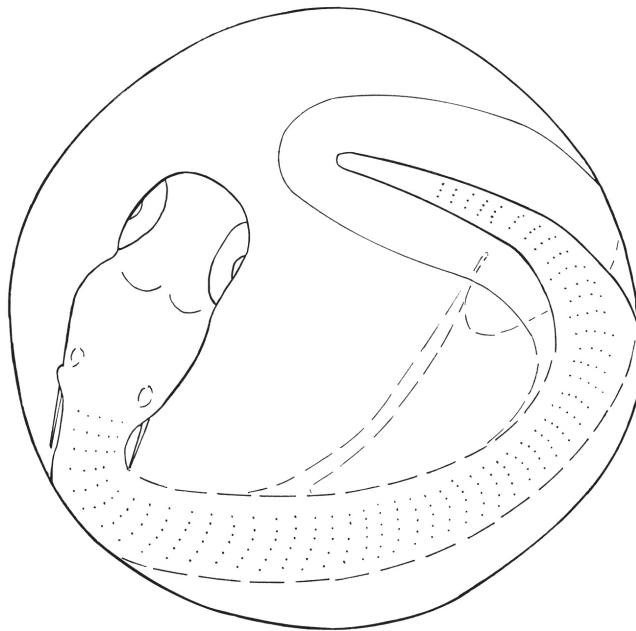
Few eggs incubated at 4.5°C survived past stage 16 and few hatched. Egg mortality was high and data were not considered reliable.

Eggs incubated at 6.0°C reached stage 19 in less than one-half of the time (276 h) required at 3.0°C (610 h; Fig. 10C); average temperature throughout incubation was 6.2°C. Hatching was first observed at 281 h; the last egg hatched at 353 h. No yolk-sac lar-



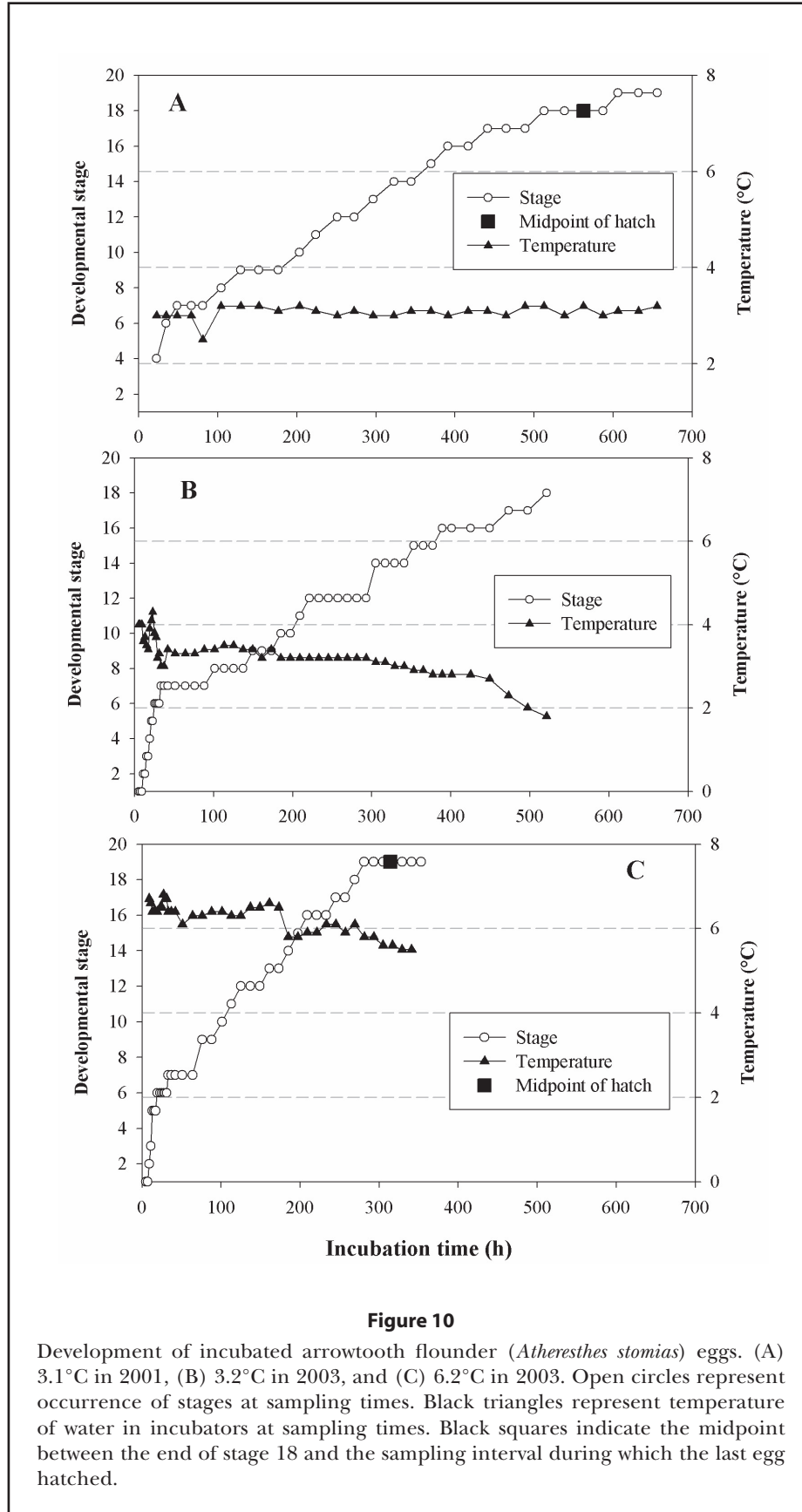
**Figure 8**

Section of ovaries of spent female arrowtooth flounder (*Atheresthes stomias*); A – Degenerating (atretic) ova (100×).



**Figure 9**

Arrowtooth flounder (*Atheresthes stomias*) egg; stage 18 (tail  $\frac{7}{8}$ s around top half of yolk).





**Table 3**

Endpoint, midpoint, and duration in hours (h) of stage of development of arrowtooth flounder (*Atheresthes stomias*) eggs incubated at 3.1°C (2001), 3.2°C (2003), and 6.2°C (2003).

Stage	3.1°C (2001)			3.2°C (2003)			6.2°C (2003)		
	Endpoint (h)	Midpoint (h)	Duration (h)	Endpoint (h)	Midpoint (h)	Duration (h)	Endpoint (h)	Midpoint (h)	Duration (h)
1				10.00	5.00	10.00	8.00	4.00	8.00
2				14.00	12.00	4.00	10.00	9.00	2.00
3				18.00	16.00	4.00	10.75	10.38	0.75
4	25.50			20.00	19.00	2.00	12.25	11.50	1.50
5	31.50	28.50	6.00	24.00	22.00	4.00	18.00	15.13	5.75
6	41.50	34.50	10.00	33.00	28.50	9.00	33.00	25.50	15.00
7	104.00	74.25	65.50	101.00	67.00	68.00	64.00	48.50	31.00
8	129.00	116.50	25.00	149.00	125.00	48.00	76.00	70.00	12.00
9	176.50	152.75	47.50	185.00	167.00	36.00	101.00	88.50	25.00
10	224.00	200.25	47.50	203.00	194.00	18.00	113.00	107.00	12.00
11	237.50	230.75	13.50	215.00	209.00	12.00	119.00	116.00	6.00
12	272.50	255.00	35.00	260.00	237.50	45.00	155.00	137.00	36.00
13	309.50	291.00	37.00	305.00	282.50	45.00	179.00	167.00	24.00
14	357.00	333.25	47.50	347.00	326.00	42.00	197.00	188.00	18.00
15	391.00	374.00	34.00	383.00	365.00	36.00	203.00	200.00	6.00
16	428.75	409.88	37.75	461.00	422.00	78.00	239.00	221.00	36.00
17	512.50	470.63	83.75	509.00	485.00	48.00	257.00	248.00	18.00
18	609.50	561.00	97.00				275.00	266.00	18.00
19	655.50	632.50	46.00				353.00	314.00	78.00

vae survived to yolk absorption and none developed pigment.

Endpoint, midpoint, and duration of each stage were estimated for the 2001 and 2003 3.0°C groups and the 2003 6.0°C group (Table 3). Data were incomplete for the 3.0°C groups. When the midpoints of stages at 3.0°C for each year were compared using an analysis of covariance (ANCOVA) using stage as the covariant, they were not found to be significantly different ( $P>0.05$ ). Therefore, midpoint data for 3.0°C development for 2001 and 2003 were pooled together. Stage duration, which equals the endpoint of a stage minus the endpoint of the previous stage (Blood et al., 1994), was very different between 2001 and 2003 for some stages (particularly stages 8, 10, 16, and 17); using midpoints moderated the degree of variability in development present among individual eggs. The 3.0°C and 6.0°C midpoint data were used to construct a piecewise nonlinear regression describing the relation between time to stage at the extremes of mean temperature (3.1°–6.2°C). The piecewise regression model has two separate components and is discontinuous between stages 6 and 7 (Fig. 11); the divergence of developmental rates after stage 6 required two equations to obtain the best fit over the entire incubation time. The two components are described by the following equations:

component 1: stages 1–6

$$\text{Age (h)} = 9.32 + 4.5(\text{stage}) - 2.08(\text{temp}),$$

adjusted  $R^2 = 0.923$ ,  
mean squared error = 8.269

component 2: stages 7–19

$$\begin{aligned} \text{Age (h)} = & -547.13 + 122.25(\text{stage}) \\ & + 49.47(\text{temp}) - 7.59(\text{stage})(\text{temp}) \\ & - 5.89(\text{stage}^2) + 0.18(\text{stage}^3), \end{aligned}$$

adjusted  $R^2 = 0.991$ ,  
mean squared error = 219.752.

Incorporating both temperature and stage as variables into the equations allows estimation of egg age at any temperature between 3.1°C and 6.2°C. Whereas separate equations for 3.1°C and 6.2°C would have given results closer to the recorded stage midpoints, the regression above allows for estimation of egg age at temperatures where spawning adults were found (4.3°–5.4°C). Incubation time at this in situ temperature range is estimated to be 372 h (5.4°C) to 477 h (4.3°C).

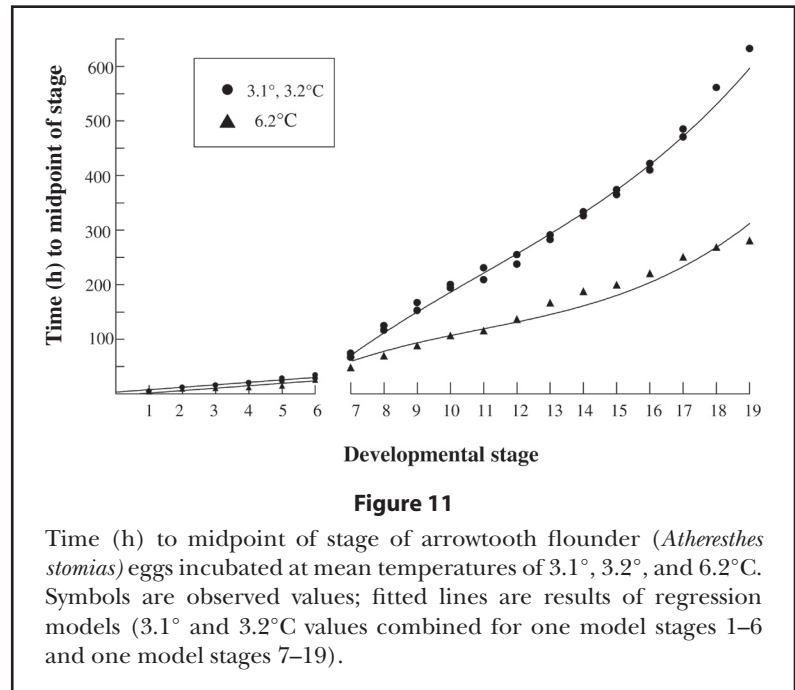
### Description of larvae

A total of 129 reared and 184 plankton-caught larvae was examined for morphological development and for

first appearance and changes in pigmentation. Newly hatched larvae are slender, lack pigment, and possess a large yolk mass (length  $\times$  depth = approx. 30%  $\times$  24% SL) (Fig. 12A). Size range at hatching is 3.9–4.8 mm SL (mode 4.3 mm, mean 4.4 mm, SD 0.2 mm) and preanal length is approximately 40% SL. At about 6.0 mm SL, the mouth begins to form and a horizontal streak of pigment is present on the eye, extending from each margin through the center (Fig. 12B). In addition, a small saddle of pigmentation, beginning as a single melanophore on the dorsal midline, is present at about 75% SL, between myomeres 36–39. In some specimens of this length, an additional small patch of pigment is present ventrally on the body over the posterior end of the yolk sac (Fig. 12C). Yolk absorption is complete between 6.5 and 7.0 mm SL and the mouth appears functional. Preanal length is reduced to approximately 25% SL.

Preflexion through postflexion arrowtooth flounder larvae 10.0–25.6 mm SL are illustrated and briefly described in Matarese et al. (1989), and are described in more detail here (Fig. 12D, 13A–B). In late preflexion larvae, the pigment patch over the end of the yolk sac has expanded dorsally over the hindgut, the posterior dorsal patch on the tail has expanded to between myomeres 36–45, and a second dorsal patch has formed at about 50% SL, between myomeres 21–27 (Fig. 12D). In addition, pigment appears on the crown and between the eyes, and 3–4 preopercular spines are visible. The dorsal patches fuse by late flexion and some lateral and ventral pigment develops posteriorly under the dorsal patch (Fig. 13A). Additional pigment on the crown and laterally on the gut appears along with nape pigment and melanophores on the mandible and jaw articulation. A second row of spines forms on the preoperculum. Dorsal body pigment in postflexion larvae is scattered (Fig. 13B). Pigment is concentrated on the caudal peduncle, crown, and posteriorly on the gut, and small melanophores are visible on the snout and isthmus.

Transforming specimens (Fig. 13C) are slender-bodied, with body depth approximately 27% SL. Fine pigment is scattered over most of the head, with larger melanophores present on the snout, upper and lower jaws, gular region, preoperculum, and operculum. The pectoral-fin base and proximal region of the pectoral-fin rays are moderately pigmented. The peritoneum is dark, and the lateral surface of the gut is covered with fine pigment and a few larger melanophores. Fine pigmentation covers the entire lateral line. Small,



**Figure 11**

Time (h) to midpoint of stage of arrowtooth flounder (*Atheresthes stomias*) eggs incubated at mean temperatures of 3.1°, 3.2°, and 6.2°C. Symbols are observed values; fitted lines are results of regression models (3.1° and 3.2°C values combined for one model stages 1–6 and one model stages 7–19).

faint melanophores cover about 75% of the body on the eyed side. Larger dark melanophores are present as blotches dorsolaterally on the body and dorsal-fin pterygiophores at approximately 20%, 35%, and 50% SL. Similar blotches are present ventrolaterally on the body and anal-fin pterygiophores at approximately 30%, 50%, and 80% SL. Two aggregations of pigment resemble bands at 70% and 90% SL. Melanophores are scattered between the bands and blotches near the dorsal and ventral body margins.

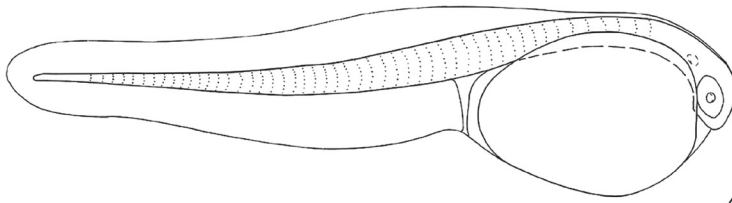
Light pigment outlines the hypural margin and is present on the entire fin in a subtle banding pattern. Light pigment is also present on the remnant finfold on the caudal peduncle.

Blind side pigment is greatly reduced. Anteriorly, pigment is present on the snout and gular region, with large internal spots on the peritoneum. The lateral line is pigmented starting at approximately 50% SL and there is a dorsolateral pigment blotch at about 50% SL. A row of melanophores is present along the base of the dorsal pterygiophores. Posteriorly, melanophores are present as remnants of bands at about 90% SL, and outlining the caudal peduncle and hypural margin.

### Distribution and abundance of eggs and early-stage larvae

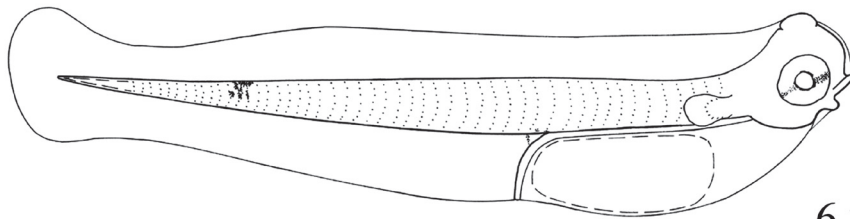
Arrowtooth flounder eggs were collected at 75% of deep bongo stations and also in eight shallower tows that were part of the 2003 survey grid (depth 160–205 m) (Fig. 14, Table 4). Except for one station, all eggs were collected

A



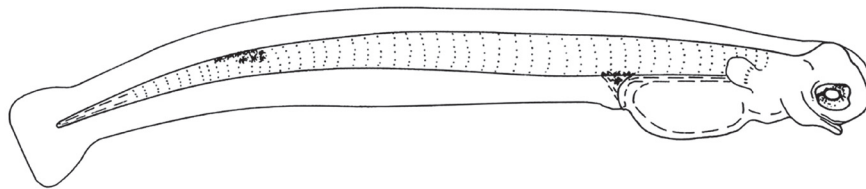
4.3 mm SL

B



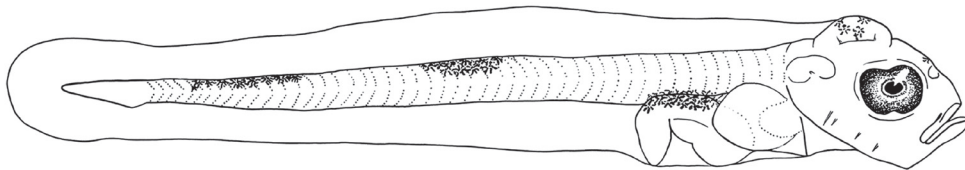
6.2 mm SL

C



6.1 mm SL

D



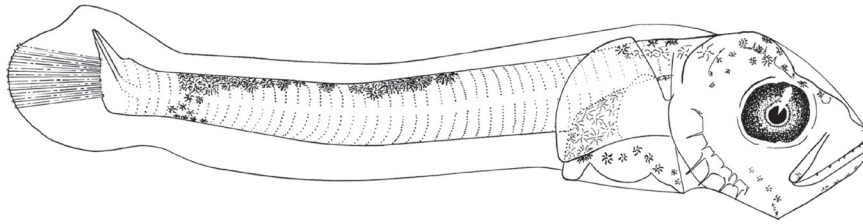
10.0 mm SL

**Figure 12**

Newly hatched and early larvae of arrowtooth flounder (*Atheresthes stomias*). (A) Newly hatched yolk-sac larva (reared); (B) yolk-sac larva (UW cat. no. 104797); (C) yolk-sac larva (UW cat. no. 104818); (D) preflexion larva (Matarese et al., 1989).

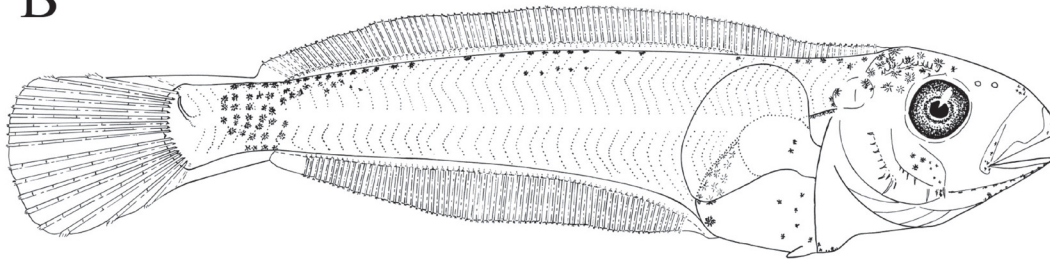


A



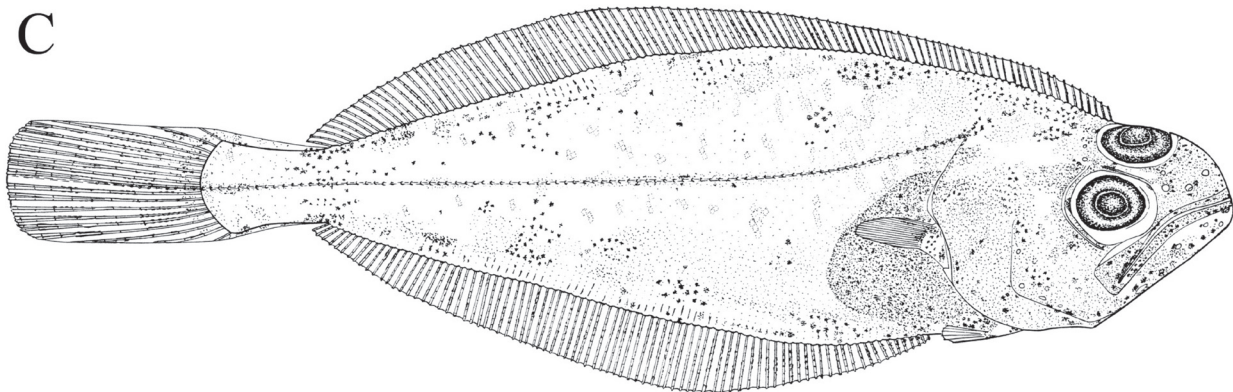
13.4 mm SL

B



25.6 mm SL

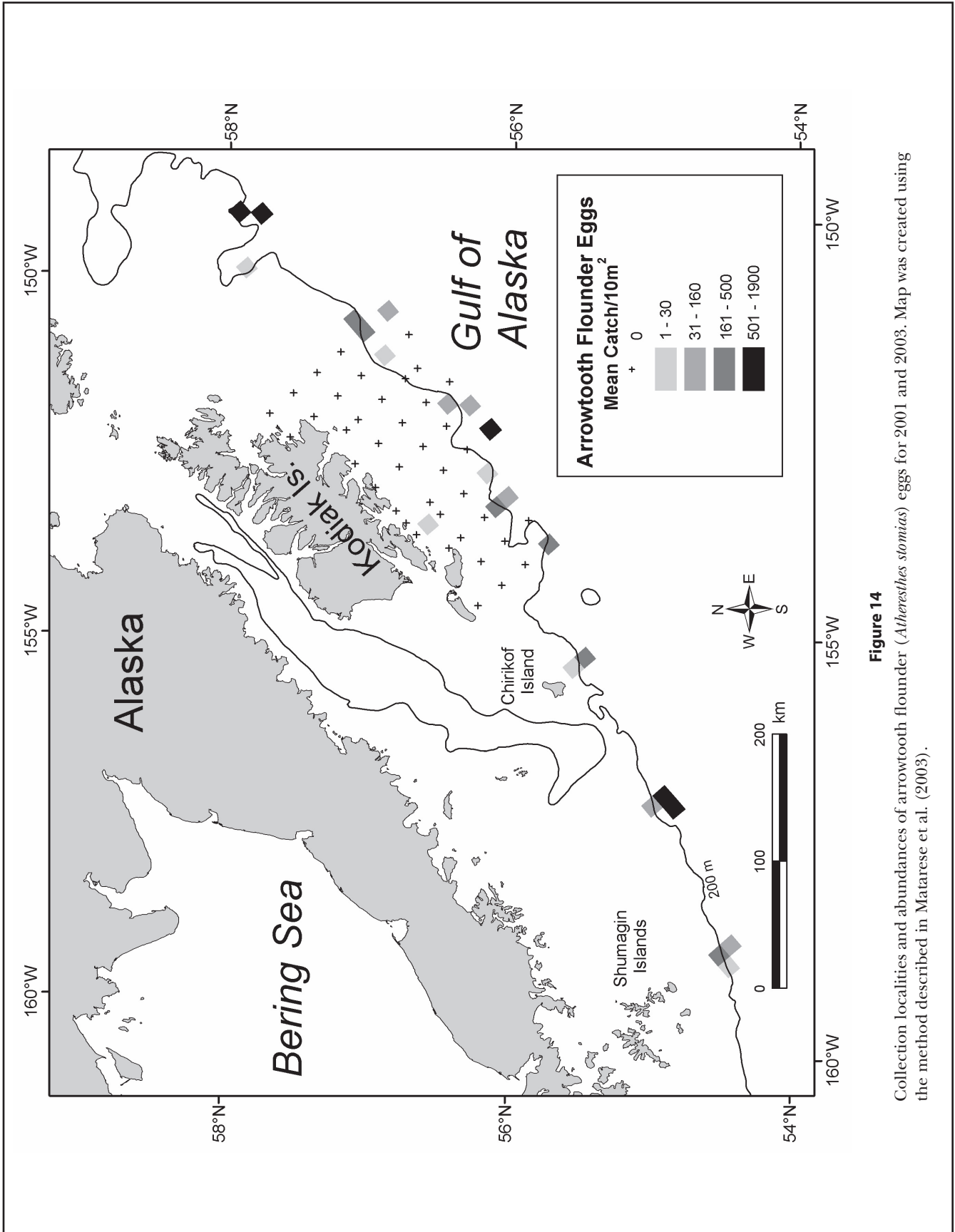
C



44.5 mm SL

**Figure 13**

Arrowtooth flounder (*Atheresthes stomias*). (A) Flexion larva (Matarese et al., 1989); (B) postflexion larva (Matarese et al., 1989); (C) transforming (UW cat. no. 104591).

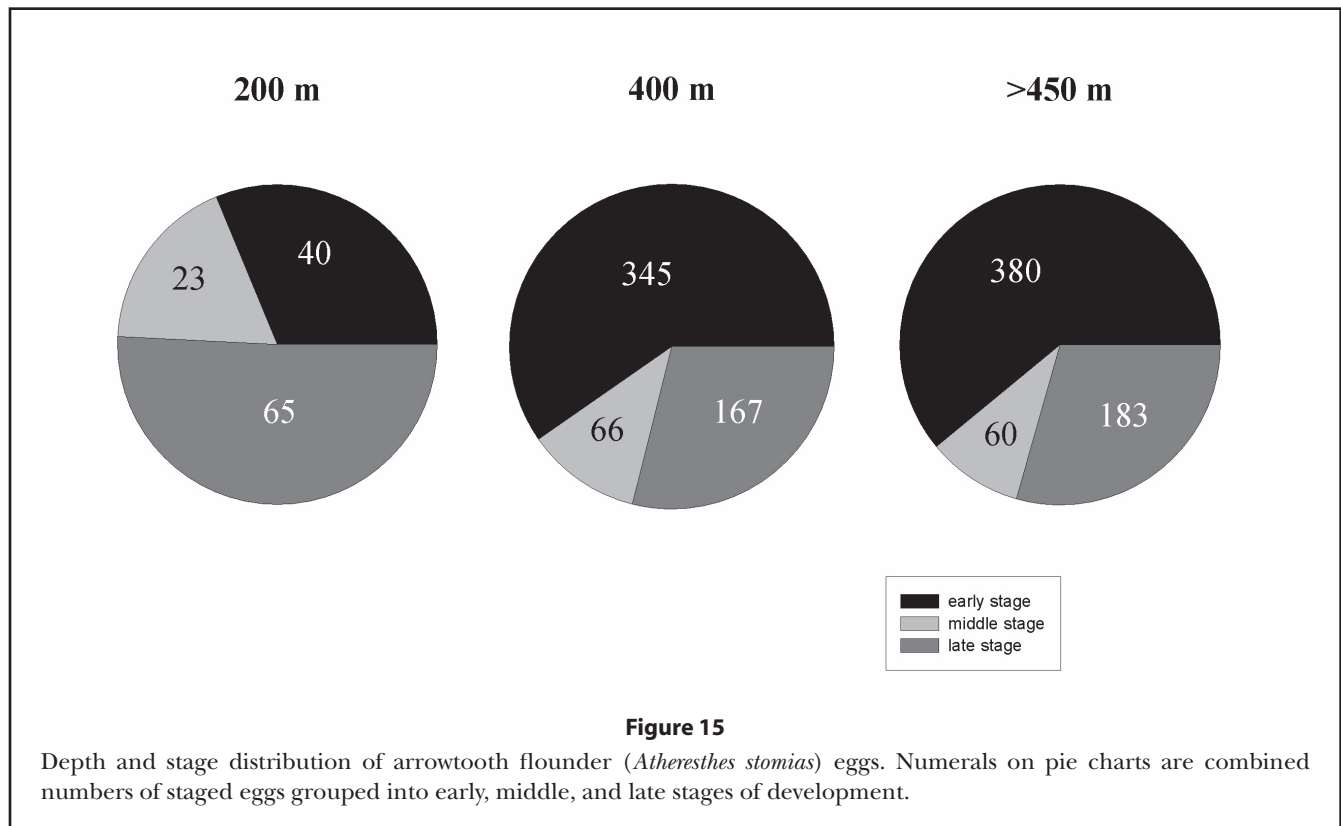


**Figure 14** Collection localities and abundances of arrowtooth flounder (*Atheresthes stomias*) eggs for 2001 and 2003. Map was created using the method described in Matarese et al. (2003).

**Table 4**

Station, haul, date, bottom depth, location, gear depth, and standardized catch of arrowtooth flounder (*Atheresthes stomias*) eggs and catch information plus mean length of larvae at each station for bongo tows in 2001 and 2003. Only stations with a positive catch for eggs and/or larvae are shown. Shaded rows denote stations that were part of the 2003 survey grid which limited gear depth to the lesser limit of either 10 m off bottom or 200 m.

Station	Haul	Date	Bottom depth (m)	Latitude (°N)	Longitude (°W)	Maximum gear depth (m)	Eggs catch/10m <sup>2</sup>	Larvae	
								Catch/10m <sup>2</sup>	Mean length (mm)
2		29-Jan-01	224	57.96	150.12	212	6.95	0	
3		29-Jan-01	419	57.92	149.42	415	1376.72	177.12	3.83
4		29-Jan-01	630	57.86	149.39	630	586.07	0	
5		30-Jan-01	179	57.24	150.85	172	310.23	0	
6		30-Jan-01	432	57.14	151.08	423	883.18	218.91	5.24
7		30-Jan-01	566	57.19	150.97	536	956.71	306.87	5.09
8		30-Jan-01	208	56.59	151.93	198	118.51	0	
11		31-Jan-01	137	56.74	153.55	127	13.60	0	
12		1-Feb-01	216	55.68	155.32	200	53.34	0	
13		1-Feb-01	415	55.65	155.30	401	1078.82	7.01	6.50
14		1-Feb-01	606	55.62	155.31	571	798.71	511.53	5.05
15		1-Feb-01	404	55.05	157.02	393	1875.26	28.63	6.08
16		1-Feb-01	578	55.03	157.05	525	1863.20	237.86	4.82
18		2-Feb-01	406	54.51	158.80	406	258.09	58.28	6.43
6	2	14-Feb-03	673	57.11	151.04	200	22.05	22.05	7.97
7	1	14-Feb-03	186	57.24	150.85	175	0	6.91	8.00
8	1	14-Feb-03	400	57.14	151.09	400	226.52	290.23	4.99
9	1	14-Feb-03	574	57.19	150.97	551	252.06	1138.32	6.09
10	1	14-Feb-03	968	56.94	150.79	200	95.02	14.62	7.50
11	1	14-Feb-03	911	56.80	151.11	202	0	26.59	8.10
12	1	14-Feb-03	884	56.97	151.35	203	7.12	28.48	8.43
13	1	14-Feb-03	100	57.14	151.59	90	0	6.48	7.50
19	2	14-Feb-03	298	56.83	151.68	201	0	6.89	8.00
20	2	15-Feb-03	1054	56.74	151.53	199	0	16.86	9.17
20	3	15-Feb-03	1046	56.74	151.53	606	0	25.71	8.90
21	3	15-Feb-03	1000	56.54	151.77	602	0	74.14	6.60
30	1	15-Feb-03	1952	56.39	152.07	200	26.14	13.07	8.60
30	2	15-Feb-03	1952	56.39	152.07	601	36.34	814.05	6.57
31	2	15-Feb-03	949	56.26	152.39	205	114.13	7.13	6.00
31	3	15-Feb-03	949	56.26	152.38	602	1139.34	762.55	5.87
32	2	16-Feb-03	132	56.43	152.63	122	0	11.71	7.40
39	1	16-Feb-03	168	56.29	152.93	160	6.36	6.36	7.80
40	1	16-Feb-03	1206	56.16	153.24	200	55.23	6.14	8.10
40	2	16-Feb-03	1134	56.16	153.25	600	179.25	590.02	5.92
40	3	16-Feb-03	462	56.21	153.32	446	221.17	475.10	6.35
45	2	17-Feb-03	244	56.17	153.82	200	0	6.68	13.50
47	2	17-Feb-03	789	55.88	153.86	200	7.14	7.14	8.00
47	3	17-Feb-03	767	55.87	153.87	601	394.01	1173.65	6.70
51	1	17-Feb-03	203	55.68	155.33	192	0	20.10	7.57
52	1	17-Feb-03	412	55.65	155.30	391	21.48	200.43	6.77
53	1	17-Feb-03	594	55.62	155.29	575	12.04	312.94	6.19
54	1	18-Feb-03	198	55.11	157.05	189	0	13.58	6.75
55	1	18-Feb-03	393	55.06	157.03	374	208.37	266.71	6.49
56	1	18-Feb-03	599	55.03	157.07	569	364.63	689.84	6.01
57	2	19-Feb-03	952	54.44	158.98	600	17.48	555.79	5.70
58	5	19-Feb-03	395	54.51	158.82	377	304.73	124.15	6.46
59	1	20-Feb-03	250	54.57	158.71	240	52.11	26.05	7.52



close to or along the continental shelf edge; eggs were collected from all except six stations over the continental slope (>160 m). Over 92% of all eggs were from tows sampling to depths  $\geq 400$  m and 58% of all staged eggs were in the early stage of development (Fig. 15). Less than 10% of the eggs were from tows sampling to depths  $\leq 200$  m, and about 50% of those eggs were in the late stage of development. Eggs were most abundant (1863–1875/10m<sup>2</sup>) at two locations approximately 125 km west of Chirikof Island (Stations 15–16, 2001); these stations had the greatest percentage of early-stage eggs. The greatest percentage of late-stage eggs was found east of Kodiak Island (Stations 5–7, 2001).

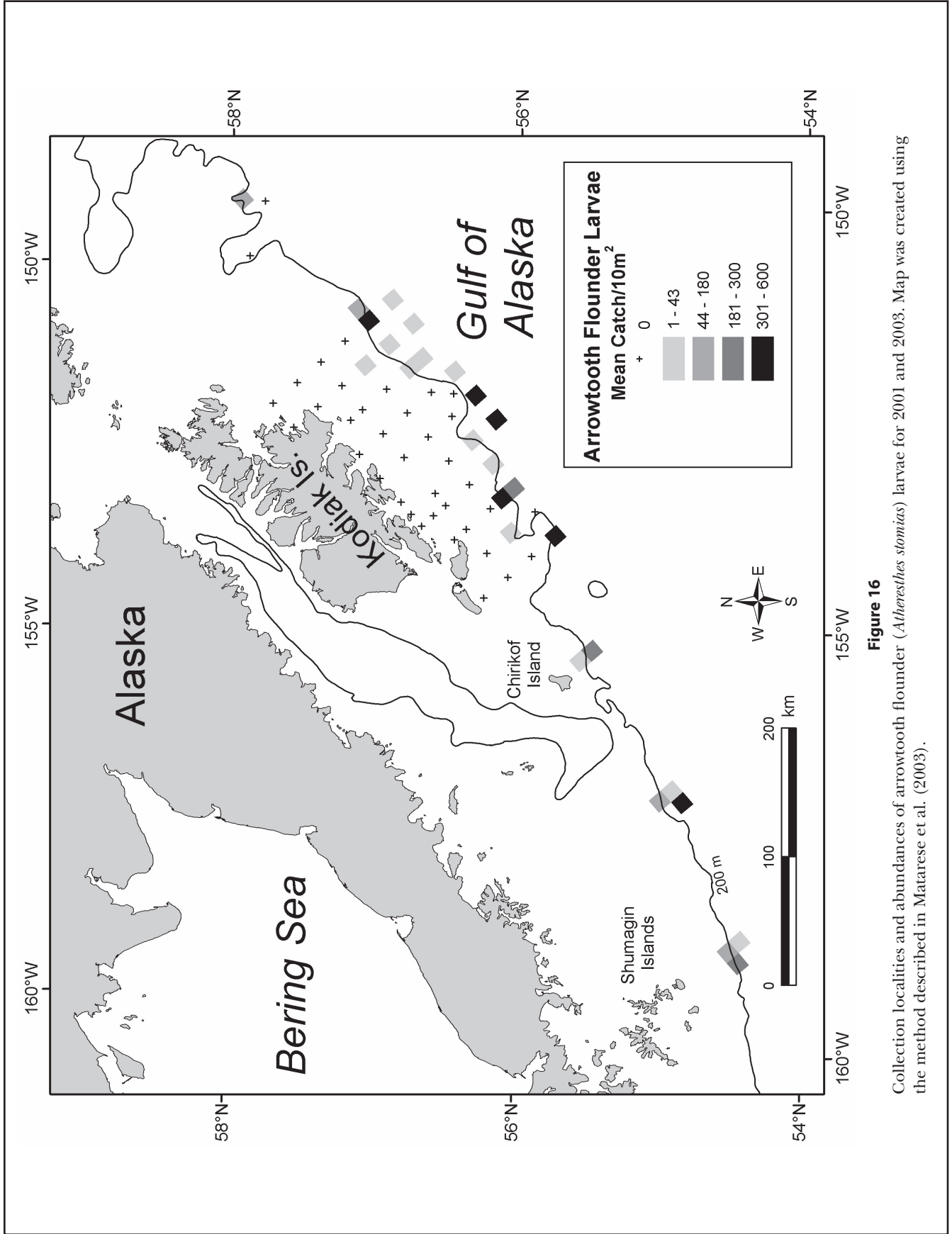
Depth of the tows having the greatest percentage of eggs was 400 m in 2001 and >450 m in 2003. Although more deep plankton tows were taken in 2003, more eggs were collected in 2001. In addition, there were more very early-stage eggs (stage 1–6) in 2001, indicating that spawning and fertilization had occurred just hours before the eggs were collected. For both years, early-stage eggs (stages 1–12) comprised the largest percentage of the catch in each of the three depth strata, except for the 200 m stratum in 2001, in which 60% of the eggs were late stage.

Arrowtooth flounder larvae were collected in 2001 and 2003 from 41 of 84 stations, all located close to or along the continental shelf edge (Fig.16). At station

clusters (2–3 closely-spaced stations sampling 2 or 3 depth strata) where larvae were collected, abundance increased with depth (Table 4). Highest abundances were at depths >450 m east and south of Kodiak Island (1138/10m<sup>2</sup> and 1173/10m<sup>2</sup>, Stations 9 and 47, 2003).

Most larvae collected in 2001 were yolk-sac stage. Lengths of larvae varied from 3.2 to 9.6 mm SL, but 90% were less than 7 mm SL (Fig. 17A). Although there was variation within depth groups, mean length of larvae collected in tows that sampled to depths of 400 m (5.0 mm SL) was not significantly different ( $P>0.05$ ) from the mean length of larvae collected in tows that sampled to depths >450 m (4.9 mm SL).

Larvae collected in 2003 were 4.0–13.5 mm SL; 59% of these larvae were less than 7 mm SL (Fig. 17B). Larvae collected in the 200 m stratum were the largest among the four depth strata groups: lengths were 5.4–13.5 mm SL, with a mean of 8.1 mm SL. These larvae were significantly larger than those collected in tows that sampled to 400 m and >450 m ( $P<0.05$ ). Mean length of larvae in the 400 m stratum (6.0 mm SL) was significantly less ( $P<0.05$ ) than the mean length in the >450 m stratum (6.3 mm SL) (Table 4). Many more larvae were collected in 2003 than in 2001; there were more sampling locations than in 2001, but mean abundance in tows that sampled to depths  $\geq 400$  m in 2003 was 250%



**Figure 16** Collection localities and abundances of arrowtooth flounder (*Atheresthes stomias*) larvae for 2001 and 2003. Map was created using the method described in Matarrese et al. (2003).

greater than mean abundance at those depths in 2001 (Table 4).

## Discussion

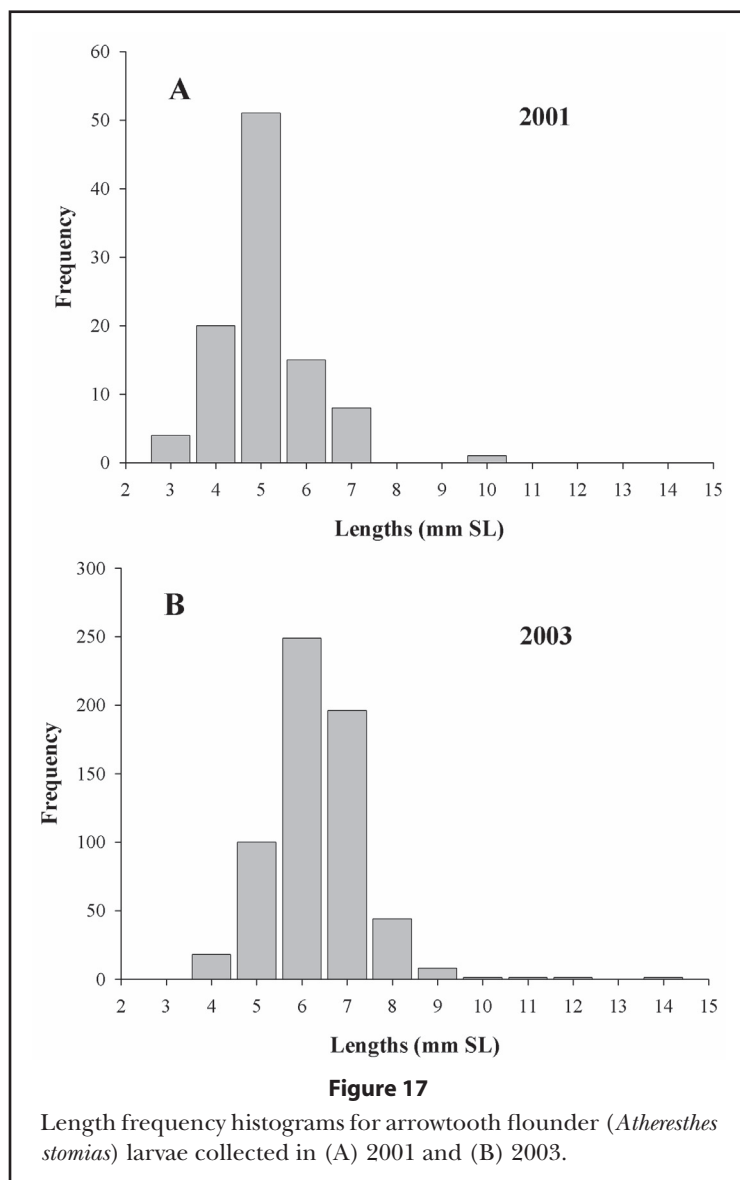
The spatial and temporal variability in spawning by GOA arrowtooth flounder and its early life history are poorly known. Hirschberger and Smith (1983) collected arrowtooth flounder in spawning condition at depths of 108–360 m along the continental shelf in March and May–August. Zimmermann (1997) collected arrowtooth flounder from depths of 66–165 m east of Kodiak Island in September, and concluded from histological examinations that spawning occurs after September. Farther south, Rickey (1995) found ripe female arrow-

tooth flounder off the Washington coast in December at a depth of 475 m.

Unlike previous studies, we used ichthyoplankton data to help pinpoint possible time, location, and depth of spawning. Prior to this study, the smallest arrowtooth flounder larvae we had collected were small preflexion larvae 6–7 mm SL. These larvae were first collected in late February, primarily over the outer continental shelf and upper slope, suggesting that early February (or perhaps late January) marked the beginning of spawning offshore and at depths >200 m. Only eggs of other species had been collected over the upper continental slope along with the small arrowtooth flounder larvae in February. Standard surveys conducted from 1971 to 1985 deployed ichthyoplankton gear to only 200 m and missed both eggs and early-stage larvae of arrowtooth flounder, and no surveys were scheduled during February over the outer continental shelf and upper slope after 1985.

During our study, spawning arrowtooth flounder were found along the continental slope southeast, south, and southwest of Kodiak Island at depths of 300–500 m in late January–February 2001–2003. The earliest spawning occurred at the more southerly sampling locations near the Shumagin Islands and later spawning was farther north, closer to Kodiak Island. Fewer ripe fish were found during the earliest cruise (28 January–5 February 2001) than in 2002 (trawling 6–9 February), and more spent females were found during the latest cruise (trawling 18–22 February 2003). For 2001–2003, spawning appears to begin before the end of January and accelerate through February.

In general, females were predominant in trawl catches at 200 m, males were predominant at >450 m, and the sex ratio was more nearly equal at 400 m. Sexually mature fish were usually found at  $\geq 400$  m. Although some ripe females were found at 300 m ( $n = 5$ ) and 500 m ( $n = 1$ ), most were found at 400 m ( $n = 38$ ). Some ripe males were found at 200 m ( $n = 1$ ), 400 m ( $n = 19$ ), and 600 m ( $n = 4$ ), but most were found at 500 m ( $n = 53$ ). Rickey (1995) suggested that the majority of spawning off Washington is at depths exceeding 366 m. Our data agree, suggesting that most spawning in the central GOA takes place at 400–500 m. The depth segregation of ripe females and males suggests movement by the males toward the females when ready to spawn, since most spent females and the majority of ripe females were found at 400 m.





Arrowtooth flounder eggs were smaller than we expected. In previous ichthyoplankton surveys, the smallest larvae collected were 6.0–7.0 mm SL. Larval length at hatching is usually 2.5–3.0 times the diameter of the egg (Ahlstrom and Moser, 1980), so it was expected that arrowtooth flounder eggs would be close to 3.0 mm diameter. Egg diameters are critical to identification, and we measured a large number of eggs to encompass the full size range. Reared, preserved arrowtooth flounder eggs range from 1.58 mm to 1.98 mm; field collected eggs are 1.76–1.97 mm. Prior to this study, arrowtooth flounder eggs may have been misidentified as sablefish (*Anoplopoma fimbria*), whose embryos are unpigmented, reported to range from 1.80 to 2.20 mm in diameter, and also are spawned over the outer continental slope at depths of 400 m in January and February (Mason et al., 1983; Kendall and Matarese, 1987). We measured reared sablefish eggs archived in the ichthyoplankton collection at AFSC to compare to the arrowtooth flounder egg diameters recorded from this study. From a sample of 316 sablefish eggs, 34% were  $\leq 1.97$  mm (range 1.85–2.09 mm, mean = 1.99, mode = 2.01). Since diameters overlap, arrowtooth flounder eggs may be difficult to distinguish from sablefish eggs until close to hatch when the anus is at 40% SL and myomeres (47–50) are formed (anus in sablefish is at 50–60% SL and myomeres = 61–66). However, most of the sablefish eggs in our collection are  $\geq 2.00$  mm and all of our reared arrowtooth flounder eggs are  $< 2.00$  mm. Along with the arrowtooth flounder eggs collected in the plankton in 2001 and 2003, many newly-hatched arrowtooth flounder larvae were present, but no sablefish larvae were found. Prior to this study, eggs 1.80–2.00 mm in size with unpigmented embryos that were collected at depths  $> 200$  m were all assumed to be sablefish because there was little information on the timing and depth of arrowtooth flounder spawning; arrowtooth flounder and sablefish eggs can now be differentiated, except for eggs that are 1.85–1.97 mm in diameter and younger than stage 19.

The length of time for egg development ranged from 632.5 h (26.4 days) at 3.1°C to 314 h (13.1 days) at 6.2°C (50% hatch). Over the range of temperatures where spawning adults were found (4.3°–5.4°C), incubation would take 372–477 h (15–20 days). The conditions under which the first group of eggs were reared (minimal water changes, no aeration to reduce movement) seemed the most optimal; eggs survived for over 630 h and larvae lived for an additional 144 h. No other group of eggs or larvae survived as long; the 6.2°C group in 2003 survived to hatch, but that was only for 314 h.

The regression model created from incubation data allows estimation of the age of arrowtooth flounder eggs at in situ temperatures. Assignment of discrete stages to continuous development is arbitrary; stages are ordinal data based on morphological criteria without consider-

ation for development time. However, ages predicted by the regression are estimates and are used as such. Bouwens et al. (1999) were not able to calculate spawning seasons from their hatch date estimates because they lacked data on egg incubation at various temperatures. Our regression model provides that data. During our first survey (2001), larvae were found within 24 h of the beginning of the cruise (station 3, 29 January). Time to the midpoint of stage 19 would take 429 h (approx. 18 days) at 4.8°C, the average temperature at depth where spawning adults were found. Therefore, spawning was occurring on 11 January; the beginning of spawning may have been mid-December because we collected near-flexion stage larvae on that day.

Arrowtooth flounder eggs are infrequently collected at depths  $< 400$  m; fewer than 8% of all eggs were collected at depths  $\leq 200$  m. Collections at shallower depths are likely the result of transport onto the shelf by a combination of strong onshore flow and tidal mixing (Bailey and Picquelle, 2002; Ladd et al., 2005). At standard ichthyoplankton stations (bongo gear lowered to 10 m off bottom or no deeper than 200 m), all eggs collected were stage 7 or later. The estimated 64 hours required to reach stage 7 at 4.8°C (average bottom temperature at depths where ripe adults were collected), as calculated by the regression equation, requires that eggs be advected only 0.2 cm/sec to ascend the vertical distance of 200–300 m to the depth our standard bongo tows sample. The weakest recorded current in Chiniak Trough (near stations 5–7 of the 2001 cruise) was 5 cm/sec in a northwest direction. This measurement was recorded in August–September, but the flow is not expected to be much different in winter<sup>4</sup>. Locations where late-stage eggs were collected in 2001 are all near troughs and canyons identified as areas where downwelling relaxation and cross-shelf flow typically occur in winter; tidal mixing is also important in these areas where canyon bathymetry diverts flow onto the shelf and aids in cross-isobath transport. The combination of strong, fluctuating tidal currents and steep, convoluted bathymetry results in enhanced vertical mixing (Ladd et al., 2005) and is likely the mechanism by which arrowtooth flounder eggs and larvae are advected to shallower depths and onto the continental shelf. Whereas all sampling for this study was carried out using bongo gear, any information on the vertical distribution of arrowtooth flounder eggs and larvae is circumstantial, since no vertical resolution is possible with bongo tows. Although coupling of shallow and deep tows at the same stations provides a strong indication of basic vertical distribution, only

<sup>4</sup> Ladd, Carol. Personal commun. 2005. Office of Oceanic and Atmospheric Research, Pacific Marine Environmental Laboratory (PMEL), 7600 Sand Point Way NE, Seattle, WA 98115-6439.



depth-stratified sampling will provide conclusive evidence and finer details.

Arrowtooth flounder larvae are easily distinguished from newly-hatched planktonic larvae of sablefish and Pacific halibut by preanal length (arrowtooth flounder = about 40% SL, sablefish = 50–60% SL, Pacific halibut = about 30% SL), number of myomeres (arrowtooth flounder = 47–50, sablefish = 61–66, Pacific halibut = 49–51), and size (arrowtooth flounder size at hatch = 3.9–4.8 mm SL, sablefish = 5–6 mm SL, Pacific halibut = 7.8–8.5 mm SL). From the first appearance of pigment (about 6.2 mm SL), arrowtooth flounder larvae are easily identified from other pleuronectids, most of which have complete bands on the body and/or postanal ventral melanophores (Matarese et al., 1989). In addition, arrowtooth flounder are the only pleuronectid larvae in the GOA that possess preopercular spines, which are present by 10.0 mm SL.

Size at hatch is smaller than the 8–9 mm SL estimated by Bouwens et al. (1999), who used a length frequency profile of planktonic arrowtooth flounder larvae and a linear regression model using total length, otolith diameter, and otolith focal diameter to determine size at hatch. In our study, reared arrowtooth flounder hatched at lengths of 3.9–4.8 mm SL; larvae in this size range were also present in our plankton samples.

Most eggs and small larvae (ca. 4 mm SL) were collected in tows to depths  $\geq 400$  m. Less than 5% of eggs in 2001, and 10% of eggs and 5% of larvae collected in 2003, were found in tows to depths  $\leq 200$  m. The large number of eggs and yolk-sac larvae in tows to depths  $\geq 400$  m, coupled with lower numbers or absence of eggs and early-stage larvae in tows to depths  $< 400$  m, suggests spawning and hatching occur at  $\geq 400$  m depth. The smallest larva collected from depths  $\leq 200$  m was 5.4 mm SL (mid- to late yolk-sac stage). All of our bongo tows were oblique and did not sample discrete depths, so we do not know the size of the largest larvae at  $\geq 400$  m. Annual mean length of larvae at  $\geq 400$  m was 5.0–6.1 mm SL; it is likely that larvae begin to ascend the water column prior to complete yolk sac absorption, which occurs after they reach 6.2 mm SL (Fig. 12B–C). Although a grid of stations directly east and south of Kodiak Island was surveyed in February 2003, only two larvae were found at stations inshore of the outer continental shelf (Fig. 16). For 2003, our data suggest that any onshore transport of larvae (Bailey and Picquelle, 2002) must have occurred later in the year.

Prior to this study, Doyle et al. (2002) grouped arrowtooth flounder and Pacific halibut larvae together as part of a distinct early-spring shelf edge and slope assemblage. Pacific halibut larvae were also found in our deep-water samples. This flatfish shares a similar early-life-history pattern with arrowtooth flounder of eggs being spawned, incubated, and hatched at great

depths in winter along the shelf edge and slope in the GOA (Thompson and Van Cleve, 1936).

While our study clearly describes the early life history of arrowtooth flounder in the GOA, taxonomic issues make similar studies in the Bering Sea more problematic. In the Bering Sea, the Kamchatka flounder has a distribution that partially overlaps that of arrowtooth flounder. Kamchatka flounder ranges farther west to the Sea of Okhotsk and northern Sea of Japan and south only into the western GOA. In regions where the species overlap, Kamchatka flounder tend to occupy greater depths than arrowtooth flounder (Mecklenburg et al., 2002). Eggs and larvae of Kamchatka flounder are unknown and therefore cannot be distinguished from those of arrowtooth flounder. As a result, in the Bering Sea where both species spawn (Dolganov, 2000), eggs and larvae can only be identified at the generic level (*Atheresthes* spp.). Since Kamchatka flounder are uncommon in the GOA and are not known to spawn there, all *Atheresthes* collected in the GOA are assumed to be arrowtooth flounder. Future studies comparing *Atheresthes* larvae from non-overlapping regions of their geographic ranges will be helpful for determining taxonomic characters useful for distinguishing the two species. Molecular studies comparing DNA samples from larvae with samples from adults of known identity may also help resolve this problem.

Although the most basic questions concerning GOA arrowtooth flounder spawning, egg incubation and development, and egg and larval distribution and abundance have been addressed, this study has given rise to more questions about their spawning and early life history. Does spawning begin in December, as suggested by the duration of egg development and the occurrence of preflexion larvae in the plankton at the end of January? How has arrowtooth flounder managed to become so numerous, given that depth distributions of ripe males and females infrequently overlap and males have very little milt? Now that the eggs and early larvae can be identified, directed studies can obtain more precise data on their vertical distribution and abundance. This additional information, in addition to recruitment studies on late larvae and juveniles, may help to explain why arrowtooth flounder dominates the GOA groundfish assemblage.

## Acknowledgments

The authors thank the officers and crew of the NOAA Ship *Miller Freeman* for their efforts at sea. We also thank the following individuals for providing at-sea assistance collecting, spawning, and rearing arrowtooth flounder eggs and larvae: Rachael Cartwright, Jim Stark, and Duane Stevenson; William Floering (PMEL); Fumihito Muto (formerly Hokkaido University); and Erin

MacDonald (formerly UW). Elizabeth Chilton helped us greatly at sea, spent many hours setting up the Kodiak Lab incubation tubes, sampled eggs and larvae in the lab, and took helpful photographs of incubating eggs. Lisa Appesland, Frank Morado, Dan Nichol, Jim Stark, and Mark Zimmermann assisted in the histological preparations and interpretation of maturity data. Beverly Vinter (retired) illustrated the egg and larvae. Kathy Mier provided statistical analyses. Jeff Napp and Gary Stauffer accommodated requests for ship time and supported all facets of this research. Kevin Bailey, Janet Duffy-Anderson, and Jeff Napp reviewed early drafts and made helpful suggestions. Gary Duker provided invaluable editing during the in-house review. Three anonymous reviewers provided numerous comments and suggestions which greatly improved the manuscript. This research is contribution FOCI-0618 to NOAA's Fisheries-Oceanography Coordinated Investigations.

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