Otolith Formation and Body Shrinkage due to Fixation in Larval Cod *(Gadus morhua)*

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OTOLITH FORMATION AND BODY SHRINKAGE DUE TO FIXATION IN LARVAL COD (*Gadus morhua*)

by

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ABSTRACT

Radtke, R. L., and K. G. Waiwood. 1980. Otolith formation and body shrinkage due to fixation in larval cod (*Gadus morhua*). Can. Tech. Rep. Fish. Aquat. Sci. 929: *iii* + 10 p.

Daily increments were found to develop in the otoliths of larval cod with the first increment formed the day after hatching. Scanning electron microscope data pointed to the formation of a "metamorphic" check that occurred at the time of yolk-sac absorption. With this information it might be possible to accurately age and determine the growth of this important commercial fish species. Fixation of reared cod larvae in ethanol resulted in body shrinkage which was dependent upon the age of the larvae and independent of the time of death. These data give a strong alternative to formalin fixation.

Key words: Ageing, cod, otolith, fixation, shrinkage, scanning electron microscope

RÉSUMÉ

Radtke, R. L, and K. G. Waiwood. 1980. Otolith formation and body shrinkage due to fixation in larval cod (*Gadus morhua*). Can. Tech. Rep. Fish. Aquat. Sci. 929: *iii* + 10 p.

On a relevé dans les otolithes des larves de morue des accroissements journaliers dont le premier se produit le lendemain de l'éclosion. Des données obtenues au microscope électronique à balayage ont indiqué l'existence d'un arrêt de la métamorphose qui se produirait au moment de l'absorption du sac vitellin. Grâce à cette information, il serait possible de déterminer précisément l'âge et le taux de croissance de cette espèce commercialement importante. La fixation par l'éthanol de larves de morue élevées provoque un rétrécissement du corps qui dépend de l'âge des larves mais qui est indépendant du moment de la mort. Ces données permettent de remplacer avantageusement la fixation par le formol.

INTRODUCTION

The importance of larval mortality as a determinant of year-class strength is generally accepted for numerous fish species (Hjort 1914; Tanaka 1972). Larval fish survival has been shown to be related to growth (Ivlev 1961), and recent studies have focused on this finding (Hunter 1976). Unfortunately, measurements of growth rate of larval fish in field studies lack the precision associated with estimates for juveniles and adults where techniques for determining size at age are fairly well established.

Historically, age determination of adult fish has involved interpretation of concentric ring formation in hard tissues. Although scales and bones have been used, otoliths are preferred for many species including cod (Dannevig 1933; Six and Horton 1977). Otoliths are formed as calcium carbonate in the form of aragonite (Irie 1955; Degens et al. 1969) in the labyrinth of teleosts. Three otoliths are found on each side of the brain cavity in the semicircular canals and are named the sagitta, lapillus, and the asteriscus (Lowenstein 1971). The sagitta is the largest otolith and is the one most often utilized in yearly and daily ageing studies. Only recently have otoliths been utilized for age determination in larval fish. Previously, age estimates were based primarily on length frequencies which are dependent upon the initial larval size and the conditions of fixation (Blaxter 1971; Schnack and Rosenthal 1978).

Daily growth increments in otoliths were first observed in adult fish (Pannella 1971, 1974). Subsequent works by Brothers et al. (1976), Ralston (1976), Struhsaker and Uchiyama (1976), Taubert and Coble (1977), Barkman (1978), and Radtke (1978) have shown that some species of fish do have daily growth rings and that larvae can be aged by this method. Furthermore, Struhsaker and Uchiyama (1976) found that back calculation of body length from otolith radii could be done accurately for larvae showing daily increments.

However, Brothers et al. (1976) showed that daily increments form at different times in different fish species. Some species can hatch with rings already formed while others might not form increments until yolk-sac absorption. Thus, it is necessary to study the formation of increments in each species before exact age determinations can be made.

The following study was undertaken to establish the pattern of otolith formation in larval cod (*Gadus morhua*). Of particular interest was the ontogenetic timing of circuli formation and verification of the daily increment pattern. The effect of age, death, and fixation on shrinkage of larval cod was also determined.

METHODS AND MATERIALS

BODY SHRINKAGE MEASUREMENTS

Adult cod (35-50 cm) were collected by otter trawl from Passamaquoddy Bay in April-June, 1978 and transported to the Biological Station in St. Andrews. They were held for 9-10 mo at 8°C in 2,500-L, circular tanks. Cod came into natural spawning condition in March 1979. Mature fish were removed from the tanks, anaesthetized (MS 222), rinsed of anaesthetic and stripped by applying pressure to the abdomen. The eggs and milt were collected into chilled glass beakers (without water) and the fertilized eggs were placed directly into incubators. These consisted of either a 13-L plexiglas trough (15 x 10 x 90 cm) supplied with running water or a 1,000-mL Imhoff cone in which water was exchanged daily. The embryos were held at 4°C ± 0.5 and maintained under natural photoperiod with the use of incandescent lights and timers. Salinity varied from 18-25 ppt, reflecting the variability induced by tides and spring runoff. The larvae hatched after 19 d of incubation. After hatching, larvae were fed daily a supply of zooplankton which was collected by towing a 120-µm mesh meter net.

Every day after hatching, 10 larvae were measured (TL) to the nearest .5 mm and either placed directly into 60% ethanol or allowed to sit for 15 min (after death), after which they were again measured and placed into 60% ethanol. After 60 d in 60% ethanol, all cod larvae were transferred into 95% ethanol. After 14 d in 95% ethanol, they were removed and again measured.

AGEING MEASUREMENTS

The pH of the 60% ethanol used in the above study was found to be 6.1. This was low enough to cause dissolution of the otoliths. It is important to note this problem so that fellow workers do not meet with the same failure. The otoliths in fish this size are only slightly calcified and very little change in acidity will dissolve them. For safe fixation larvae to be used for otolith studies should be fixed in 100% to 95% alcohol and possibly a buffer added. Hence, all cod larvae stored in 60% ethanol could not be used for ageing and a further rearing study was conducted at the Biological Station in St. John's, Newfoundland, during June 13-16, 1979.

Fertilized cod eggs were obtained from ripe cod caught in cod traps off St. John's, Newfoundland, and were placed in 10-gal aquaria which were suspended in a 4° ± 1°C waterbath. The photoperiod was kept at 12/12 using fluorescent lights and a timer. The salinity of the rearing water was 32 ‰. Larval fish were collected each day for 4 d and were placed directly into 95% ethanol.

EXTRACTION, PREPARATION, AND INSPECTION OF OTOLITHS

After length measurements the ethanol was allowed to evaporate from each individual larva being dissected. The larva was then immersed in glycerol which cleared the specimen and made the otoliths visible. All three otoliths (sagitta, lapillus and asteriscus) on the back side of the brain were removed from every fish where possible, and the sagitta, the largest of the otoliths, was used for increment determinations. Under powered 80X magnification, the otoliths were then teased from the cranial area of the larva using fine insect needles which were mounted on wood rods. The otoliths were washed with 95% ethanol, dried and mounted on glass slides with Flo-Texx (Lerner Laboratory, Stanford, Conn.). The mounted otoliths were then viewed under the compound microscope at 1125X with increment counts and otolith diameters being noted.

SCANNING ELECTRON MICROSCOPE

Additional samples of 30-d-old larval cod were obtained from the Statens Biologiske Stasjon Flodevigen, in Arendal, Norway. Otoliths from these larvae were used for scanning electron microscope studies (SEM). The otoliths were glued to viewing stubs with 5-min epoxy and were then ground with one-micron diamond polishing compound (Buehler Ltd., Evanston, Ill., U.S.A.). They were then etched with 7% EDTA (pH 7.4) for 1-5 min after which they were gold-coated in an Edwards vacuum evaporator with continual rotation on a planetary specimen holder. The coated otoliths were viewed in a Cambridge Steroscan Mark 2A SEM.

RESULTS

BODY SHRINKAGE OF COD LARVAE

The cod larvae reared at 4°C took 4 d to reach yolk-sac absorption by which time approximately 50% had died. The rest fed on zooplankters and survived for a further 6 d when the last were collected and preserved.

The larvae shrank from 9-15% within 15 min after death and from 11-20% totally when placed in ethanol. Although there was no significant difference in the percent shrinkage over the first 3 d after hatching, cod larvae from days 4-6 shrank significantly less than those from days 1-3 (Table 1). After 74 d of fixation, there was no significant difference in shrinkage between the larvae fixed alive and those fixed 15 min after death (Table 1).

Ethyl alcohol seemed to produce a constant amount of shrinkage regardless of the time of fixation. In no case did fixation retard shrinkage.

INCREMENT FORMATION IN LARVAL COD OTOLITHS

Otoliths were present when the larvae hatched although they were extremely small (diameter averages .0252 ± .0012 mm). Increment formation started the day after hatching. (The nucleus of the otolith is not considered to be an increment.) The concentric increments consisted of alternate light and dark zones when seen under transmitted light (Fig. 1). The number of increments continued to correspond with the age of the larva (Table 2).

The small otoliths of the early developing larvae were disc shaped and showed daily increments concentric to the nucleus. Otolith diameters increased with age of the fish. As the otoliths grew they became elongated with a definite rostrum (Fig. 2). Daily increments can be seen in the sagitta and lapillus of older fish.

The mean number of increments for each daily group of cod larvae was equal to the number of days from hatching and within each group the standard deviation of increment number and otolith diameter was small.

The increments in otoliths formed before yolk-sac absorption and they appeared to be lighter than those formed after yolk-sac absorption. Examination of the otoliths from 30-d-old cod showed a definite dark band or check at a time that corresponded to yolk sac absorption (Fig. 3). This check is evident in the lapillus in the light micrograph and can be seen in the sagitta from the scanning electron micrograph.

DISCUSSION

BODY SHRINKAGE IN COD LARVAE

Most ichthyoplankton is preserved in formalin, which can result in varying degrees of body shrinkage. Blaxter (1971) and Schnack and Rosenthal (1978) found that shrinkage in formalin can be as high as 22%, contingent upon the initial larval size. The same pattern was observed in the present study (Appendices 1-6) where smaller larvae shrank relatively more than larger larvae when fixed in ethanol. This may be because the younger fish contain relatively more water which was replaced by the alcohol or that a more rigid bone structure is present in the older fish.

These data are applicable to ichthyoplankton studies. The normal tow time for ichthyoplankton samples is 15 min, and if the larval fish are preserved in alcohol, it is possible to back calculate the live length for a given length. Ethanol causes all larvae to shrink to a relative constant size regardless of when they were killed. These results indicate that a larva killed at the beginning of a tow would still only shrink as much as a larva killed towards the end of the tow. Alcohol preservation is poor for identification studies of larval fish and formalin fixation results in the decalcification of otoliths, but in those cases where larval growth, ageing, and identification are needed two samples could be taken, one preserved in formalin and the other in 95% ethanol.

Although more research is needed on shrinkage, especially pertaining to older juveniles, the present data suggest an alternative to formalin fixation.

INCREMENT FORMATION IN LARVAL COD OTOLITHS

The otoliths were present in cod larvae at the time of hatching, which shows that the otoliths form early in the developmental stages. This would agree with Radtke (1978) who found that the otoliths were the first calcified tissues to form in embryo *Fundulus heteroclitus*. Scott's (1973) study of otolith development in the larvae of the northern sand lance, *Ammodytes dubius*, proposed that the otoliths first formed in the post-larvae at a mean fish length of about 2.4 cm. However, this information is a result of back calculations, not direct observations. Otoliths in larvae appear to form early in the developmental stages and may only be found by micro-dissection.

No increments were formed before hatching. Increments started to form 1 d after hatching and continued to form on a daily basis. It appears that increment formation is different for each species. Radtke (1978) and Brothers et al. (1976) found that two to four increments can form in those fish species which have large eggs and long incubation times. However, Brothers et al. (1976) also found that the northern anchovy *Engraulis mordax*, had no increment formation until 6 d after hatching or at the time of yolk-sac absorption. It seems that the larvae, which have long incubation periods, form increments before hatching, while the larvae that have short incubation periods do not start increment

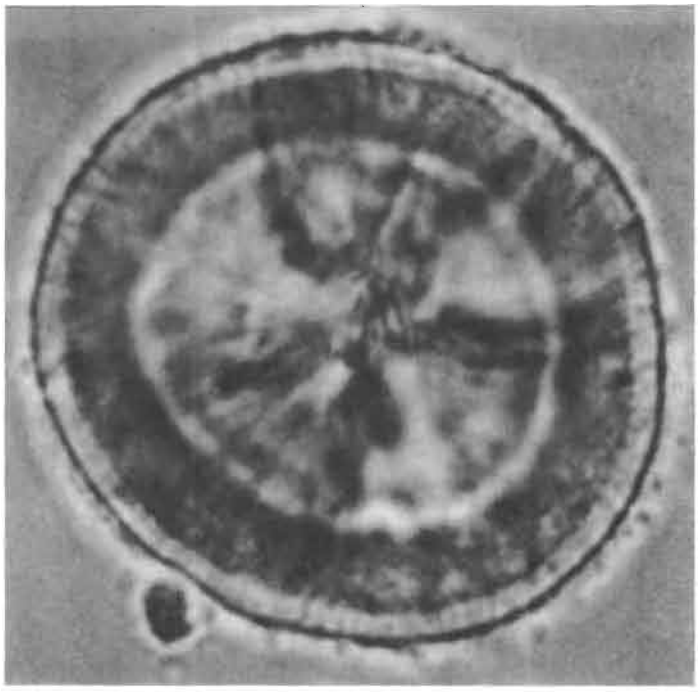

Fig. 1. Otolith from a 3-d-old cod larva (*Gadus morhua*) that shows two increments. The nucleus of the otolith is not counted as an increment since it formed over the period of embryological development - 3625X.

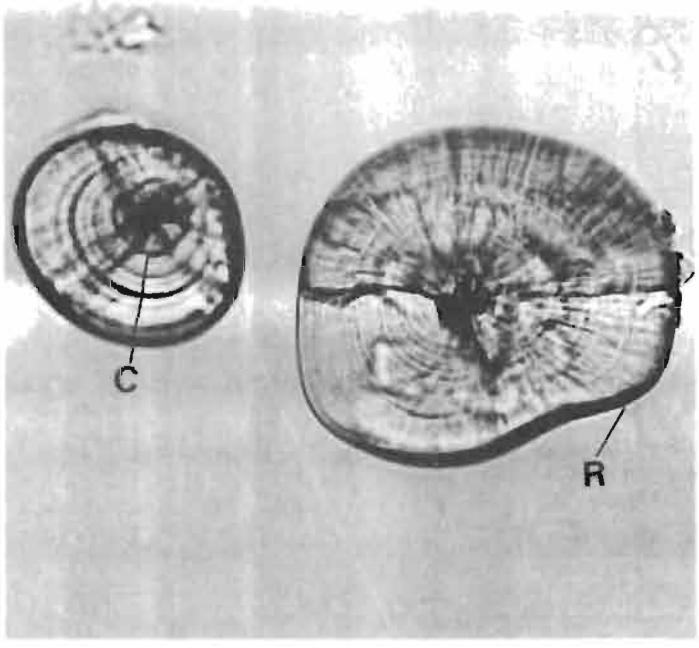

Fig. 2. The sagitta (larger otolith and lapillus) of a 30-d-old cod (*Gadus morhua*) larva which shows many daily growth increments - 375X. R - rostrum, C - check.

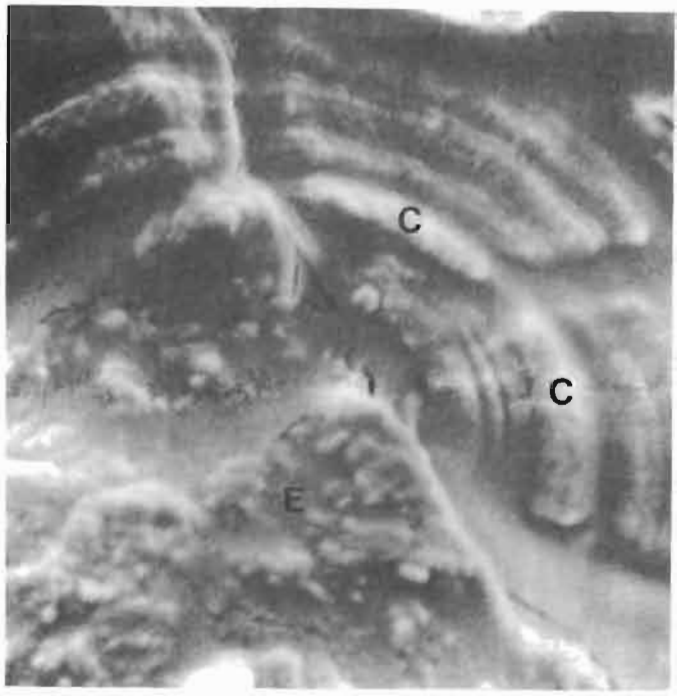

Fig. 3. A scanning electron micrograph of a sagitta from a 30-d-old cod (*Gadus morhua*) larva with a check that corresponds to the time of yolk-sac absorption. Faint increments can be observed inside the check. C - check, E - epoxy resin - 1500X.

formation until after hatching or yolk-sac absorption.

Cod larvae fit the developmental pattern exhibited by the northern anchovy and other pelagic spawning fish; that is, the eggs are small and development continues after hatching. Cod cannot feed upon hatching as the digestive system is still developing. However, cod larvae showed increment formation that started upon hatching. The older cod showed a definite mark or check that corresponded to the time of yolk-sac absorption. This check may be similar to the metamorphic checks seen in capelin (*Mallotus villosus*) otoliths by Bailey et al. (1977). Thus the otoliths appear to be a record of the physiological changes that take place during a larval fish's life. It might be possible to determine the time taken to reach yolk-sac absorption by the examination of the increments formed before the yolk-sac absorption check occurs.

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REFERENCES

Bailey, R. F. J., K. W. Able, and W. C. Leggett. 1977. Evidence for the presence of metamorphic check in capelin (*Mallotus villosus*) otoliths and implications for age determination. J. Fish. Res. Board Can. 34: 2008-2014.

Barkman, R. C. 1978. The use of otolith growth rings to age young Atlantic silversides, *Menidia menidia*. Trans. Am. Fish. Soc. 107: 790-792.

Blaxter, J. H. S. 1971. Feeding and condition of Clyde herring larvae. Rapp. P.-v. Rêun. Cons. int. Explor. Mer. 160: 128-136.

Brothers, E. B., C. P. Mathews, and R. Lasker. 1976. Daily growth increments in otoliths from larval and adult fishes. Fish. Bull. 74: 1-8.

Dannevig, A. 1933. On the age and growth of the cod (*Gadus callarias* L.) from the Norwegian Skagerrack Coast. Fiskeridirektoratets skrifter, Vol. IV, No. 1, Bergen.

Degens, E. T., W. G. Deuser, and R. L. Haldrich. 1969. Molecular structure and composition of fish otoliths. Mar. Biol. 2: 105-113.

Hjort, J. 1914. Fluctuations in the great fisheries of Northern Europe viewed in the light of biological research. Rapp. P.-v. Rêun. Cons. int. Explor. Mer 1: 5-38.

Hunter, J. R. [ed.] 1976. Report of a Colloquium on larval fish mortality studies and their relation to fishery research, Jan. 1975. NMFS Circ. 395: 1-5.

Irie, T. 1955. The crystal texture of the otolith of a marine teleost *Pseudosciaena*. J. Fac. Fish. Anim. Hus. Hiroshima Univ. 1: 1-13.

Ivlev, U. S. 1961. Experimental ecology of the feeding of fishes (Transl. D. Scott). Yale University Press, New Haven.

Lowenstein, O. 1971. The labyrinth, p. 207-240. *In* W. S. Hoar and D. J. Randall [ed.]. Fish physiology, Vol. 5. Academic Press, New York.

Pannella, G. 1971. Fish otoliths: Daily growth layers and periodical patterns. Science 173: 1124-1127.

1974. Otolith growth patterns: an aid to age determination in temperate and tropical fishes, p. 28-39. *In* T. B. Bagenal [ed.]. Ageing of fish. Unwin Brothers, Ltd. Surrey.

Radtke, R. L. 1978. The formation and growth of otoliths in the estuarine fish, *Fundulus heteroclitus*. Ph.D. Thesis, University of South Carolina, Columbia, South Carolina.

Ralston, S. 1976. Age determination of a tropical reef butterflyfish utilizing daily rings of otoliths. Fish. Bull. 74: 990-994.

Schnack, D., and H. Rosenthal. 1978. Shrinkage of Pacific herring larvae due to formalin fixation and preservation. Ber. Dtsch. Meeresforsch. 26: 222-226.

Scott, J. S. 1973. Otolith structure and growth in northern sand lance, *Ammodytes dubius*, from the Scotian Shelf. Intern. Comm. Northw. Atl. Fish. Res. Bull. 10: 107-116.

Six, L. D., and H. F. Horton. 1977. Analysis of age determination methods for yellowtail rockfish, canary rockfish, and black rockfish off Oregon. Fish. Bull. 75: 405-414.

Struhsaker, P., and J. H. Uchiyama. 1976. Age and growth of the nehu, *Stolephorus purpureus*, from the Hawaiian Islands as indicated by daily growth increments of sagittae. Fish. Bull. 74: 9-17.

Tanaka, M. 1972. Studies on the structure and function of the digestive system in teleost larvae. V. Epithelial changes in the posterior-gut and protein ingestion. Jap. J. Ich. 19: 172-180.

Taubert, B. D., and D. W. Coble. 1977. Daily rings in otoliths of three species of *Lepomis* and *Tilapia mossambica*. J. Fish. Res. Board Can. 34: 332-340.

Table 1. Percent shrinkage in larval cod (*Gadus morhua*). Method A - 74 d after direct fixation in ethanol; Method B - 15 min after death and 74 d after fixation in ethanol. Each value represents the mean and 95% confidence interval of five animals.

Day after hatching	Method	% shrinkage (±95% CI) 15 min after death	% total shrinkage (±95% CI) after 74 days of fixation
1	A	13.50 ± 2.59	18.28 ± 3.00
	B		20.78 ± 4.27
2	A	15.19 ± 3.31	19.58 ± 5.18
	B		21.20 ± 2.42
3	A	13.36 ± 2.62	18.72 ± 1.18
	B		20.80 ± 4.30
4	A	13.39 ± 3.34	13.13 ± 4.88
	B		15.27 ± 4.96
5	A	11.66 ± 4.78	9.78 ± 3.24
	B		15.23 ± 5.90
6	A	9.29 ± 1.59	12.20 ± 5.29
	B		14.41 ± 2.73

Table 2. Cod larvae (*Gadus morhua*) reared at 4°C with 10 specimens taken each day. Otolith diameters and increment formation.

Age (days after hatching)	Otolith diameter (mm) ± S.D.		Increment number ± S.D.	
1	.0252	.0012	.6	.52
2	.0287	.0012	1.6	.70
3	.0298	.0012	2.5	.53
4	.0327	.0011	3.6	.52

APPENDIX 1

Percent shrinkage in cod (*Gadus morhua*) larvae at day 1 after hatching.

Live length (mm)	Length (mm) 15 min after death	% shrinkage	Length (mm) after storage in 95% ethanol	% total shrinkage
4.55			3.65	19.78
4.12			3.33	19.17
4.65			3.96	14.84
4.52			3.76	16.80
4.33			3.43	20.79
4.27	3.71	13.11	3.39	20.61
4.38	3.91	10.73	3.71	15.30
4.35	3.64	16.32	3.28	24.60
4.61	4.01	13.02	3.67	21.04
4.56	3.89	14.69	3.54	22.37
Mean S.D.				
4.43 ± .17	3.83 ± .15	13.57 ± 2.09	3.57 ± .22	19.53 ± 3.10

APPENDIX 2

Percent shrinkage in cod (*Gadus morhua*) larvae at 2 d after hatching.

Live length (mm)	Length (mm) 15 min after death	% shrinkage	Length (mm) after storage in 95% ethanol	% total shrinkage
5.26			4.57	13.12
5.17			4.17	19.03
5.31			4.17	21.47
5.39			4.07	24.49
5.10			4.09	19.80
5.42	4.73	12.73	4.40	18.12
5.45	4.50	18.83	4.34	21.37
5.27	4.42	16.13	4.04	23.34
5.35	4.50	15.89	4.16	22.24
5.25	4.61	12.36	4.15	20.95
Mean S.D.				
5.30 ± .12	4.55 ± .12	15.19 ± 2.67	4.22 ± .17	20.39 ± 3.19

APPENDIX 3

Percent shrinkage in cod (*Gadus morhua*) larvae at 3 d after hatching.

Live length (mm)	Length (mm) 15 min after death	% shrinkage	Length (mm) after storage in 95% ethanol	% total shrinkage
5.33			4.33	18.76
5.31			4.32	18.64
5.28			4.24	19.70
5.33			4.30	19.32
5.29			4.38	17.20
5.36	4.80	10.45	4.36	18.66
5.43	4.74	12.71	4.56	16.02
5.21	4.46	14.40	3.97	23.80
5.26	4.41	16.16	3.99	24.14
5.13	4.48	13.07	4.01	21.83
Mean S.D.				
5.29 ± .08	4.58 ± .18	13.36 ± 2.11	4.25 ± .20	19.81 ± 2.67

APPENDIX 4

Percent shrinkage in cod (*Gadus morhua*) larvae at 4 d after hatching.

Live length (mm)	Length (mm) 15 min after death	% shrinkage	Length (mm) after storage in 95% ethanol	% total shrinkage
5.45			4.63	15.05
5.36			4.63	8.77
5.29			4.53	14.07
5.43			4.91	9.58
5.17			4.23	18.18
5.12	4.33	15.43	4.14	19.14
5.15	4.62	10.29	4.57	11.26
5.37	4.74	11.73	4.51	16.01
5.47	4.95	9.52	4.87	10.97
5.48	4.65	14.96	4.44	18.98
Mean S.D.				
5.32 ± .14	4.66 ± .22	12.39 ± 2.69	4.57 ± .27	14.20 ± 3.90

APPENDIX 5

Percent shrinkage in cod (*Gadus morhua*) larvae at 5 d after hatching.

Live length (mm)	Length (mm) 15 min after death	% shrinkage	Length (mm) after storage in 95% ethanol	% total shrinkage
5.50			5.01	8.99
5.51			5.11	7.26
5.63			5.20	7.64
5.42			4.78	11.81
5.39			4.68	13.18
5.37	4.60	14.34	4.51	16.01
5.38	4.52	15.99	4.16	22.82
5.39	4.80	10.95	4.63	14.10
5.62	5.21	7.30	5.04	10.10
5.33	4.81	9.76	4.64	13.13
Mean S.D.				
5.45 ± .11	4.79 ± .27	11.66 ± 3.50	4.78 ± .32	12.50 ± 4.62

APPENDIX 6

Percent shrinkage in cod (*Gadus morhua*) larvae at 6 d after hatching.

Live length (mm)	Length (mm) 15 min after death	% shrinkage	Length (mm) after storage in 95% ethanol	% total shrinkage
5.48			5.00	8.26
5.30			4.27	19.45
5.31			4.76	10.36
5.46			4.85	11.17
5.44			4.80	11.76
5.34	4.76	10.86	4.25	14.98
5.43	4.91	9.58	4.83	11.05
5.31	4.84	8.85	4.75	10.55
5.42	4.89	9.78	4.80	11.44
5.55	5.14	7.39	5.05	9.01
Mean S.D.				
5.40 ± .09	4.91 ± .14	9.29 ± 1.28	4.74 ± .27	11.80 ± 3.23

APPENDIX 7

Otolith growth of cod (*Gadus morhua*) reared at 4°C 1 d after hatching. Data are ± S.D.

	Otolith diameter (mm)	Increment number
1	.024	0
2	.023	0
3	.026	1
4	.024	1
5	.025	1
6	.025	0
7	.026	1
8	.027	1
9	.026	1
10	.026	0
	.0252 ± .0012	.6 ± .52

APPENDIX 8

Otolith growth of cod (*Gadus morhua*) reared at 4°C 2 d after hatching. Data are ± S.D.

	Otolith diameter (mm)	Increment number
1	.028	1
2	.026	0
3	.029	2
4	.029	2
5	.030	2
6	.030	2
7	.029	2
8	.028	1
9	.029	2
10	.029	2
	.0287 ± .0012	1.6 ± .70

APPENDIX 9

Otolith growth of cod (*Gadus morhua*) reared at 4°C 3 d after hatching. Data are ± S.D.

	Otolith diameter (mm)	Increment number
1	.031	3
2	.029	2
3	.029	2
4	.030	3
5	.031	3
6	.029	2
7	.028	2
8	.032	3
9	.030	3
10	.029	2
	.0298 ± .0012	2.5 ± .53

APPENDIX 10

Otolith growth of cod (*Gadus morhua*) reared at 4°C 4 d after hatching. Data are ± S.D.

	Otolith diameter (mm)	Increment number
1	.031	3
2	.033	4
3	.034	4
4	.033	4
5	.033	4
6	.033	4
7	.032	3
8	.033	3
9	.034	4
10	.031	3
	.0327 ± .0011	3.6 ± .52