CHAPTER 2
Collection and Preservation of Material for Otolith Analysis

John L. Butler

National Marine Fisheries Service,
Southwest Fisheries Science Center, P.O. Box 271,
La Jolla, CA 92038, USA

Correct citation: Butler, J. L. 1992. Collection and preservation of material for otolith analysis, p. 13–17. *In* D. K. Stevenson and S. E. Campana [ed.] Otolith microstructure examination and analysis. Can. Spec. Publ. Fish. Aquat. Sci. 117.

Introduction

The methods used for collecting, handling and preserving fish for otolith analysis vary, depending on the nature of the material that is required and the objectives of the study. Larval and juvenile fish are collected using different methods than those used to collect adult fish; handling and preservation techniques are similar. Problems associated with gear selectivity and shrinkage are common to all life stages. Improper preservation techniques can cause otolith damage.

Sampling and Gear Selectivity

The methods used to collect adult fish are as diverse as the methods of fishing. These methods include spearing, traps and weirs, hook and line, trolling, night lighting, bottom trawls, pelagic purse seines, gill nets, lift nets and pumping as well as nonconventional methods such as poisons and electrofishing. These methods may be employed by the investigator or the investigator may simply sample the catch from fishermen who use these techniques. All of these methods are to some degree selective in terms of the species and size of fishes collected. Spear fishing and baited traps tend to select for large individuals. Hook and line and set lines select large individuals (Klein 1986), but selectivity is also a function of hook size (Ralston 1982, 1990) and bait size (Løkkeborg 1990). The selectivity of nets is well known and is often used as a management strategy to regulate the harvest of fish. The size of the mesh opening largely determines the size of fish collected. Gill nets, for example, are more size selective than other nets such as tangle nets. The mesh size of bottom trawls determines the minimum size of fish collected while avoidance determines the maximum size (Hemmings 1973). Other techniques such as poisons (Weinstein and Davis 1980) and electrofishing are not selective if all of the stunned fish are collected.

Sampling gear used to collect larval and juvenile fishes are also size selective. The minimum size of larvae collected in plankton nets is determined by mesh size and extrusion (Lenarz 1972; Colton et al. 1980; Lo et al. 1989). The maximum size is determined by net avoidance (Lenarz 1973; De Ciechomski and Sanchez 1983; Somerton and Kobayashi 1989) and time of day (Bridger 1956). Large-volume plankton pumps and plankton nets sample small fish larvae equally efficiently (Taggart and Leggett 1984).

The catchability of larval fishes affects apparent growth and mortality rates estimated from individuals collected in plankton nets (Morse 1989). Estimated growth rates of larvae collected in plankton nets are strongly influenced by the size and age of the largest individuals. Because avoidance is a function of size and condition, there is a strong possibility that captured individuals will be the slowest growing members of their cohort. More research needs to be done comparing the estimated growth rates of large larvae collected in plankton nets and similar sized larvae collected by small mesh midwater trawls.

The degree of size selection is important in age and growth studies because growth varies among individuals of a cohort. Collecting samples using highly selective sampling gear will reduce the observed variability in age and growth. This is particularly troublesome if the objective of the study is to analyze the effect of environmental variability on growth or to back-calculate dates of hatching. As an example, consider a study designed to monitor changes in the hatch date distribution of a cohort as it progresses from the pelagic egg stage to that of a bottom-dwelling juvenile. Representative sampling of the eggs and early larvae is relatively straight forward with a gear such as a bongo net. However, once the fish reach the late larval stage, a severe sampling problem exists; how can all members of a cohort be representatively sampled when: (1) the slow-growing fish are still small pelagic larvae and can be captured only with small-mesh gear, (2) the intermediate-growth fish are too large for bongo nets, but just right for pelagic trawls such as Tucker trawls, and (3) the very fastest-growing fish have metamorphosed to a bottom-dwelling form and can be captured only with a bottom trawl? While different gear types can be used to representa-

tively sample each size range, how can these samples subsequently be recombined to form one sample from which the hatch date distribution for the entire cohort can be determined? Unless the catchabilities of each gear type are identical (or known) and the numbers at each size and in each habitat can be calculated, data derived from the various samples cannot just be combined. Yet, if hatch dates from only one gear type are examined, the hatch date distribution will be skewed either to slow-growing (early hatch date) or fast-growing (late hatch date) fish. There is no easy answer to this problem. However, the problem must be recognized before interpretation of the data is begun, and preferably at the sampling design stage.

Preservation and Shrinkage

Sample preservation techniques differ among life stages. Adult and juvenile fishes may be frozen for later analysis, but if freezer space is limited otoliths may be removed at sea. Larvae collected with plankton nets are usually preserved in the field. Fish which are not processed immediately or which are preserved or frozen can be expected to shrink before measurement and otolith removal.

Plankton samples are usually preserved at sea and larval fish removed at a later time. Plankton samples may be preserved with 5% formalin buffered by marble chips, sodium borate or sodium carbonate or preserved with 95% ethanol. Although otolith dissolution is a problem with formalin preserved samples (see below), Ré (1983) reports obtaining intact otoliths from plankton samples preserved with formalin. Brothers et al. (1976), Methot and Kramer (1979), and others recommend alcohol (ethanol) preservation. The ethanol used to initially preserve samples should be changed within 24 hours because water in the tissues of gelatinous plankton or fish flesh quickly dilutes the preservative. Ethanol solutions less than 80% are too acidic for safe larval otolith preservation. In addition, ethanol produced from petrochemicals may be contaminated with sulfuric acid, and denatured ethanol has been altered to make it unpotable. For these reasons the pH of the preservative should be checked and, if necessary, the ethanol should be buffered with 20 nM tris(hydroxymethyl)aminoethane (such as Sigma 7-9). Regardless of what type of preservative is used, the pH of samples kept for long periods of time should be monitored. It is also advisable to avoid placing too many specimens in the same sample container since they may not be properly preserved (this is especially true of ethanol). Another problem to avoid is the evaporation of preservative from sample containers which are not completely sealed.

Otolith dissolution is a problem with samples preserved in either alcohol or formalin. Nothing is more frustrating than to spend a month at sea making collections and to find later that all of the otoliths are dissolved, etched, pitted or discoloured. Damaged otoliths may provide some information after etching, but seldom a complete record. Although buffering helps, preserved samples may still become acidic with time. Therefore it is strongly recommended that larval fish destined for otolith analysis be separated from the rest of the sample as soon as possible after collection. Since small otoliths have a higher surface area: volume ratio than large otoliths, larval otoliths will dissolve more rapidly than juvenile otoliths. Removing the otoliths from the larvae as soon as possible and storing them, either dry or mounted on microscope slides, eliminates the possibility of lost time and effort due to otolith damage.

Preservation, whether with chemicals or freezing, and handling affect fish size. Upon death, osmoregulatory functions cease; fish in seawater begin to lose water to the medium, while fish in freshwater begin to absorb water. Consequently, marine species shrink after death; preservation and freezing cause additional shrinkage. The degree of shrinkage varies with species, type and strength of preservative, the time between death and preservation, and the size of the fish (Table 1). Fish larvae with unossified skeletons shrink most. Handling and the time from death to preservation affect shrinkage; the degree of shrinkage itself is a function of fish size (Theilacker 1980). Because autolysis and shrinkage begin as soon as larval fish die, it is recommended that the duration of plankton-net tows used to collect larval fish for otolith analysis be limited. It is also important to preserve the sample as soon as possible after it is collected. Use of standardized collections procedures (e.g., duration of tow, time between end of tow and preservation) is also recommended.

Leak (1986) used the relationship of otolith size to live fish length measured in the laboratory to correct for shrinkage of field collected larvae. This approach has promise but must be used with caution because Reznick et al. (1989) found larger otoliths relative to standard length in slower growing guppies in the laboratory. Some have suggested that stunted fish have larger heads (and therefore probably larger otoliths) relative to body length than faster growing fish. Larval herring from the same eggs reared in a mesocosm, but experiencing different growth rates (Wespestad and Moksness 1989), had otoliths of different relative sizes (Erland Moksness, pers. comm.). Butler (1989) found different allometric relationships of otolith size and fish length from juvenile anchovies

TABLE 1. Shrinkage correction factors for different species of fish.

Species	Preservative	Treatment	Percent shrinkage
Anchoa mitchilli[1]	95% ethanol	Bongo net	22–30
Catostomus commersoni[2]	Davidson's B		3.2
Clupea harengus[3]	2% formalin	15 ppt SW	11–12
	2% formalin	34 ppt SW	14–16
	4% formalin	15 ppt SW	11–14
	4% formalin	34 ppt SW	9–13
	10% formalin	15 ppt SW	5–7
Clupea pallasi[4]	4% formalin	—	8
	4% formalin	Net	13–43
Dicentrarchus labrax[5]	4% formalin		5.3
	70% ethanol		5.6
	4% formalin	Net 5 min	12.4
	70% ethanol	Net 5 min	18.9
	4% formalin	Net 10 min	15.8
	70% ethanol	Net 10 min	23.1
	4% formalin	Net 15 min	19.8
	70% ethanol	Net 15 min	24.2
	4% formalin	Net 20 min	22.4
	70% ethanol	Net 20 min	26.0
Engraulis mordax[6]	5% formalin		8
	80% ethanol		0
	Bouin's		8
	—	Net	8–19
Esox americanus[2]	Davidson's B		0
E. lucius[2]	Davidson's B		3.5
E. lucius[7]	Freezing		5.4
Etheostoma nigrum[2]	Davidson's B		0
Gadus morhua[8]	95% ethanol	—	0
	—	Death	30–40
Limanda ferruginea[9]	Ice	Death	1.5
Merluccius bilinearis[10]	4% SW formalin		3.4–4.3
	95% ethanol		4.8–7.0
	Freezing		1.4
M. productus[11]	3% formalin		4.5–4.6
	80% ethanol		4.4
Onchorhynchus nerka[12]	10% formalin		4.6–6.8
O. gorbuscha[12]	3.8% formalin		2.3–4.1
O. keta[12]	3.8% formalin		1.8–4.8
O. nerka[12]	3.8% formalin		2.0–4.3
Paralichthys lethostigma[14]	4% FW formalin	1 h–6 yr	0–6.6
	4% SW formalin	1 h–6 yr	5.2–9.4
Parophrys vetulus[15]	80% ethanol		3.2
	10% formalin		5.1
Perca fluviatilis[7]	Freezing		1.7

Continued

TABLE 1. Shrinkage correction factors for different species of fish. *(Cont'd)*

Species	Preservative	Treatment	Percent shrinkage
Pimephales notatus[2]	Davidson's B		4.3
Pleuronectes platessa[16]	4% formalin		2.4–2.7
Psuedopleuronectes americanus[17]	4% formalin		3.7
Rhinichthys atratulus[2]	Davidson's B		3.1
Trachurus symmetricus[6]	Bouin's		8
Thunnus albacares[18]	Freezing brine		0.9–2.6
Siganus caniculatus[19]	4% SW formalin		0
S. guttatus[19]	4% SW formalin		0
S. vermiculatus[19]	4% SW formalin		0
Sphyraena argentea[6]	Bouin's		8

[1]Leak (1986); [2]Leslie and Moore (1986); [3]Blaxter (1971); [4]Hay (1981); [5]Jennings (1991); [6]Theilacker (1980); [7]Treasurer (1990); [8]Radtke (1989); [9]Lux (1960); [10]Fowler and Smith (1983); [11]Bailey (1982); [12]Burgner (1962); [13]Parker (1963); [14]Tucker and Chester (1984); [15]Laroche et al. (1982); [16]Lockwood (1973); [17]Pearcy (1962); [18]Anonymous (1974); [19]Rosenthal and Westernhagen (1976).

collected during EL Niño years and normal years. If otolith size is used to calibrate shrinkage of larval fish, it is recommended that the allometric relationship be determined from live larvae.

Shrinkage is rarely documented but will affect growth rates calculated from otolith information. Before combining data from fish preserved by different methods, corrections must be made for shrinkage (Watanabe et al. 1988). Owen et al. (1989) found that systematic variation in handling time affected growth rates calculated from daily increments in the otoliths of larval northern anchovy collected at two different localities. These results indicate that as much care must be devoted to measuring or estimating live fish size as is devoted to accurately determining the number and, in the case of back-calculated growth, the width of daily otolith increments.

Otolith Storage

Otolith storage procedures vary with life stage and the method of analysis. Adult otoliths are often stored dry in numbered trays, in labeled vials, envelopes or capsules. Adult and juvenile otoliths may also be stored in liquid filled vials. The liquid may be water, ethanol, or glycerin and water. Thymol is added to water or glycerin solutions to prevent the growth of mold (Chilton and Beamish 1982).

Otoliths for daily increment analysis may be stored dry or mounted on labeled microscope slides for later analysis. Mounting media should be clear and have optical properties near that of glass. Mounting media that harden completely allow polishing of the otoliths. Small otoliths may be stored dry in covered trays used to store foraminifera and coccoliths. Some investigators store otoliths in oil on microscope slides (Brothers 1987), but the slides must be stored in a horizontal position.

References

ANONYMOUS. 1974. p. 38–39. *In* Inter-Am. Trop. Tuna Comm. Annual Rep. for 1973.

BAILEY, K.M. 1982. The early life history of the Pacific hake, *Merluccius productus*. Fish. Bull. U.S. 80: 589–598.

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

BRIDGER, J.P. 1956. On day and night variation in catches of fish larvae. J. Cons. int. Explor. Mer 22: 42–57.

BROTHERS, E.B. 1987. Methodological approaches to the examination of otoliths in aging studies, p. 319–330. *In* R.C. Summerfelt and G. E. Hall [ed.]. Age and growth of fish. Iowa State University Press. Ames, IA.

BROTHERS, E.B., C.P. MATHEWS, AND R. LASKER. 1976. Daily growth increments in otoliths from larval and adult fishes. Fish. Bull. U.S. 74: 1–8.

BURGNER, R.L. 1962. Studies of red salmon smolts from the Wood River Lakes, Alaska. *In* T.S.Y. Koo [ed.] Studies of Alaska red salmon. Univ. Wash. Pub. Fisheries N.S.I. (6): 247–314.

BUTLER, J.L. 1989. Growth during the larval and juvenile stages of the northern anchovy, *Engraulis mordax*, in the California Current during 1980–1984. Fish. Bull. U.S. 87: 645–652.

CHILTON, D.E., AND R.J. BEAMISH. 1982. Age determination methods for fishes studied by the groundfish program at the Pacific Biological Station. Can. Spec. Publ. Fish. Aquat. Sci. 60: 102 p.

COLTON, J.B. JR., J.R. GREEN, R.R. BYRON, AND J.L. FRISELLA. 1980. Bongo net retention rates as effected by towing speed and mesh size. Can. J. Fish. Aquat. Sci. 37: 606–623.

DE CIECHOMSKI, J.D., AND R.P. SANCHEZ. 1983. Relationship between ichthyoplankton abundance and associated zooplankton biomass in the shelf waters off Argentina. Biol. Ocean. 3: 77–101.

FOWLER, G.M., AND S.J. SMITH. 1983. Length changes in silver hake (*Merluccius bilinearis*) larvae: Effects of formalin, ethanol and freezing. Can. J. Fish. Aquat. Sci. 40: 866–870.

HAY, D.E. 1981. Effects of capture and fixation on gut contents and body size of Pacific herring larvae. Rapp. P.-v. Réun, Cons. int. Explor. Mer 178: 395–400.

HEMMINGS, C.C. 1973. Direct observation of the behaviour of fish in relation to fishing gear. Helgol. wiss. Meeresunters. 24: 348–360.

JENNINGS, S. 1991. The effects of capture, net retention and preservation upon lengths of larval and juvenile bass, *Dicentrarchus labrax* (L.). J. Fish Biol. 38: 349–357.

KLEIN, S.J. 1986. Selectivity of trawl, trap, longline and set-net gears for sablefish, *Anoplopoma fimbria*. Natl. Mar. Fish. Serv. Northw. and Alaska Fish. Ctr. Processed Rep. 86-06. 84 p.

LAROCHE, J.L., S.L. RICHARDSON, AND A.A. ROSENBERG. 1982. Age and growth of a pleuronectid, *Parophrys vetulus*, during the pelagic larval period in Oregon coastal waters. Fish. Bull. U.S. 80: 93–104.

LEAK, J.C. 1986. The relationship of standard length and otolith diameter in larval bay anchovy, *Anchoa mitchilli*, (Val.): a shrinkage estimator. J. Exp. Mar. Biol. 95: 167–172.

LENARZ, W.H. 1972. Mesh retention of larvae of *Sardinops caerulea* and *Engraulis mordax* by plankton nets. Fish. Bull. U.S. 70: 839–848.

1973. Dependence of catch rates on size of fish larvae. Rapp. P.-v. Réun. Cons. int. Explor. Mer 164: 270–275.

LESLIE, J.K., AND J.E. MOORE. 1986. Changes in lengths of fixed and preserved young freshwater fish. Can. J. Fish. Aquat. Sci. 43: 1079–1081.

LO, N.C.H., J.R. HUNTER, AND R.P. HEWITT. 1989. Precision and bias of estimates of larval mortality. Fish. Bull. U.S. 87: 399–416.

LOCKWOOD, S.J. 1973. Weight and length changes of O-group plaice (*Pleuronectes platessa* L.) after preservation in 4% neutral formalin. J. Cons. int. Explor. Mer 35: 100–101.

LØKKEBORG, S. 1990. Reduced catch of under-sized cod (*Gadus morhua*) in longlining by using artificial bait. Can. J. Fish. Aquat. Sci. 47: 1112–1115.

LUX, F.E. 1960. Length shrinkage of yellowtail flounder between live and landed condition. Trans. Am. Fish. Soc. 89: 373–374.

METHOT, R.D. JR., AND D. KRAMER. 1979. Growth of northern anchovy, *Engraulis mordax*, larvae in the sea. Fish. Bull. U.S. 77: 413–423.

MORSE, W.W. 1989. Catchability, growth, and mortality of larval fishes. Fish. Bull. U.S. 87: 417–446.

OWEN, R.W., N.C.H. LO, J.L. BUTLER, G.H. THEILACKER, A. ALVARIÑO, J.R. HUNTER, AND Y. WATANABE. 1990. Spawning and survival patterns of larval northern anchovy, *Engraulis mordax*, in contrasting environments — a site-intensive study. Fish. Bull. U.S. 87: 673–688.

PARKER, R.R. 1963. Effects of formalin on length and weight of fishes. J. Fish. Res. Board Canada 20: 1441–1455.

PEARCY, W.G. 1962. Ecology of an estuarine population of winter flounder, *Pseudopleuronectes americanus* (Walbaum). Part III. Distribution, abundance, growth, and production of juveniles: survival of larvae and juveniles. Bull. Bingham Oceanogr. Coll. 18(1): 39–64.

RADTKE, R.L. 1989. Larval fish age, growth, and body shrinkage: information available from otoliths. Can. J. Fish. Aquat. Sci. 46: 1884–1894.

RALSTON, S. 1982. Influence of hook size in the Hawaiian deep-sea handline fishery. Can. J. Fish. Aquat. Sci. 39: 1297–1302.

1990. Size selection of snappers (Lutjanidae) by hook and line gear. Can. J. Fish. Aquat. Sci. 47: 696–700.

RÉ, P. 1983. Daily growth increments in the sagitta of pilchard larvae *Sardina pilchardus* (Walbaum, 1792), (Pisces: Clupeidae). Cybium 7: 9–15.

REZNICK, D., E. LINDBECK, AND H. BRYGA. 1989. Slower growth results in larger otoliths: an experimental test with guppies (*Poecilia reticulata*). Can. J. Fish. Aquat. Sci. 46: 108–112.

ROSENTHAL, H., AND H. VON WESTERNHAGEN. 1976. Effects of formalin fixation on length, weight and condition factors of three siganid species. Arch. Fisch. Wiss. 27: 37–43.

SOMERTON, D.A., AND D.R. KOBAYASHI. 1989. A method of correcting catches of fish larvae for the size selection of plankton nets. Fish. Bull. U.S. 87: 447–455.

TAGGART, C.T., AND W.C. LEGGETT. 1984. Efficiency of large-volume plankton pumps, and evaluation of a design suitable for deployment from small boats. Can. J. Fish. Aquat. Sci. 41: 1428–1435.

THEILACKER, G.H. 1980. Changes in body measurement of larval northern anchovy, *Engraulis mordax*, and other fishes due to handling and preservation. Fish. Bull. U.S. 78: 685–692.

TREASURER, J.W. 1990. Length and weight changes in perch, *Perca fluviatilis* L., and pike, *Esox lucius* L., following freezing. J. Fish Biol. 37: 499–500.

TUCKER, J.W. JR., AND A.J. CHESTER. 1984. Effects of salinity, formalin concentration and buffer on quality of preservation of southern flounder (*Paralichthys lethostigma*) larvae. Copeia 1984: 981–988.

WATANABE, Y., J.L. BUTLER, AND T. MORI. 1988. Growth of Pacific saury, *Cololabis saira*, in the northeastern and northwestern Pacific Ocean. Fish. Bull. U.S. 86: 489–498.

WEINSTEIN, M.P., AND R.W. DAVIS. 1980. Collection efficiency of seine and rotenone samples from tidal creeks, Cape Fear River, North Carolina. Estuaries 3: 98–105.

WESPESTAD, V.G., AND E. MOKSNESS. 1989. Observations on growth and survival during the early life history of Pacific herring *Clupea pallasi* from Bristol Bay, Alaska, in a marine mesocosm. Fish. Bull. U.S. 88: 191–200.