

AGEING LABORATORY/FISHERIES MANAGEMENT DIVISION

PROTOCOL

PREPARATION OF OPERCULA FOR AGE ESTIMATION

September 29, 2023

Contents

Introduction	1
Structure of Opercula	1
Clean Opercula	1
Store Clean Opercula	2
Equipment and Supplies	3

Introduction

In the VMRC Ageing Lab we age Tautog *Tautoga onitis* using their opercula. Therefore, in this protocol we will use Tautog as an example to demonstrate how to prepare their opercula for age estimation. The protocol will first briefly introduce the structure of Tautog opercula, and then describe the detailed process of cleaning and storing them.

Structure of Opercula

A Tautog operculum is a small, bony flap that protects the fish's gills. It is assembled by four bones: the preopercle, opercle, interopercle, and the subopercle. Since the opercle is the largest of the opercular bones and is used for ageing (hereafter referred to as "operculum").

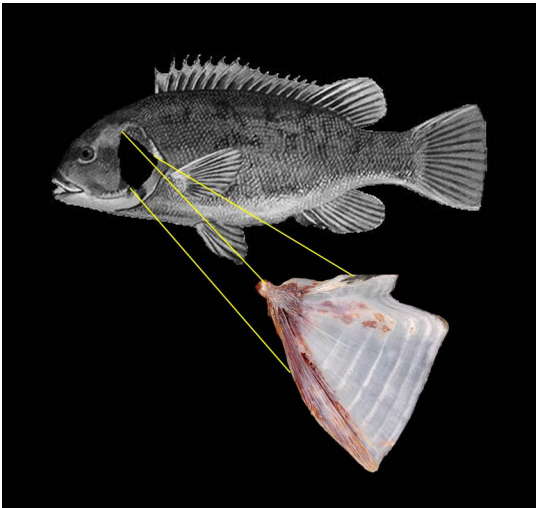


Figure 1: Tautog operculum position

The operculum is a triangle-shaped bone that is posterior to the eye and mouth and positioned on each of the fish's lateral sides (Figure 1), joined to the fish via a ball-and-socket hinge. This hinge enables the operculum to easily open and close. Opercular growth begins here and each successive year, new growth is added.

[Click here](#) to find the detailed descriptions

on how to remove Tautog operculum at the website of Center for Quantitative Fisheries Ecology (CQFE)/Old Dominion University (ODU).

Clean Opercula



Figure 2: The set-up for cleaning opercula.

Opercula that need to be processed are kept in the freezer. Remove them from the freezer to allow them to thaw. With the VWR[®] hot plate on the wet-lab dissection table, fill a 1000-ml beaker with tap water, set it on the hot plate and heat it to a temperature that is just below boiling (Figure 2). Remove the first operculum from its envelope while the water is warming. The operculum will most likely have frozen skin and cartilage matter still attached to it (Figure 3).

If the envelope is still intact and the data is readable, it can be used again but needs labeled with a new label containing all the biological information and a fish ID. **If not (most likely), use a new envelop and discard the old one.** Begin "boiling" the operculum; it should take about 5-6 minutes to loosen the attached material. Using the 8-inch forceps, remove the operculum from the beaker; take off

the skin and cartilage using the 4-inch, broad-tipped forceps (Figure 4).

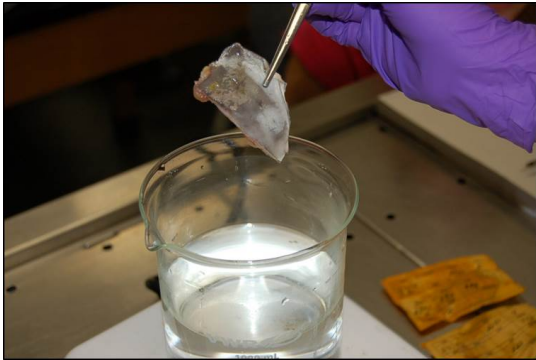


Figure 3: Boiling frozen operculum



Figure 4: Removing skin and connective tissue from operculum.

If some of the matter will not come off, a longer time of boiling may be required. After finishing cleaning, dry the clean operculum and store in a new envelop labeled with the same information as on the old envelop plus a newly assigned fish ID. The envelopes are in order by fish ID and bound with rubber bands.

Store Clean Opercula

Put the envelopes with the clean opercula in a cardboard storage box. Usually use one rubber band for 5 ~ 10 envelopes and one box for 50 ~ 75 envelopes depending on the size of each operculum. Figure 5 shows a clean operculum with 7 annuli numbered. Historic markers are recorded in this growth like reproduction checks and annuli. Annuli represent

the speeding up and slowing down of growth in response to changing seasons; a translucent band corresponds to faster growth, i.e., warmer months, and an opaque band corresponds to slow growth, i.e., winter months. An opaque band is counted as one annulus, and this fish was 7 years old.



Figure 5: Clean operculum with 7 annuli numbered

Equipment and Supplies

Item	Specification
Stirring Hot Plate	VWR® 7"x7"
Dissecting Forceps	8-inch and 4-inch, broad-tipped forceps
Glass Beaker	1,000-ml
Storage Box (Cardboard)	5"x4"x10.5"
Ultra Fine Point Permanent Marker	Sharpie®
Coin Envelopes	4.25"x2.5"
Paper Towels	