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TECHNIQUES OF OTOLITH TREATMENT FOR AGE AND GROWTH  
DETERMINATION OF THREE WHITE-BLOODED FISH SPECIES

TECHNIKA PRZYGOTOWANIA OTOLITÓW RYB BIAŁOKRWISTYCH  
W CELU OKREŚLENIA ICH WIEKU I TEMPZA WZROSTU

Institute of Fisheries Oceanography and Protection of Sea  
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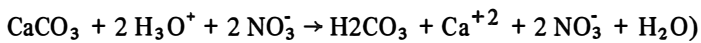
To determine age and growth rate of the white-blooded fish from their otoliths, some special techniques of otolith preparation is called for. Decalcination in 5% HNO<sub>3</sub> yields positive results for *Chaenocephalus aceratus* only. Burning of otoliths is suitable for *Champocephalus gunnari* and *Chaenocephalus aceratus*, the best results being produced for *Pseudochaenichthys georgianus*.

Otoliths of the white-blooded fish species: *Champocephalus gunnari*, *Chaenocephalus aceratus* and *Pseudochaenichthys georgianus*, similarly to otoliths of all the *Chaenichthyidae* make it very difficult to process large amounts of material collected for mass age reading. Čugunova (1939, 1959), Ricker (1958), Lee (1920), Vovk (1955) and many others have read the fish age from scales; according to Pliszka (1964), however, it is often impossible to back-read the age of those species with very small scales or lacking them, in which case the only way is to use bones and/or otoliths. Nowhere in the literature, however, can one find a description of a technique for age reading from otoliths treated in a traditional way (soaking in water or glycerin and illumination in chloral hydrate and cedar oil) proved futile: longitudinal and transverse gringing was extremely difficult to do and the fractures were irregular, or else the entire otolith became cracked and disintegrated. The difficulties resulted from small size and considerable thickness of the otoliths. All this does not permit direct readings to be obtained; moreover, the fact that the otoliths are milky-white renders and illumination ineffective.

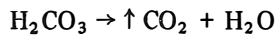
Attempts to develop a reasonably efficient way to improve the legibility of the otoliths were based on observations reported by Christensen (1964) who burned otoliths of sole and other species and analysed the fractures. For the practical reasons the techniques described by Smith (1968) and Albrechtsen (1968) could not have been used. Otoliths of the three species listed at the beginning were prepared for age determination by decalcination and burning.

#### Decalcination

5% HNO<sub>3</sub> was used as a decalcinating agent; various microscopic techniques employ 5–7% solutions of this acid for decalcination of hard tissues, bones and teeth, prior to mounting. The otoliths were decalcinated by HNO<sub>3</sub> – soaking in a glass dish. Gas bubbles were observed to form during the reaction:



Carbonic acid disintegrated into water and carbon dioxide released as gas bubbles:



The decalcination time depended on the otolith type (fish species) and size (species and age).

As the *Pseudochaenichthys georgianus* otoliths are rather lumpy in appearance, decalcination occurs on the edges only and does not penetrate inside. Even when the reaction proceeded for more than an hour, no ring pattern came out, the otolith surface dissolving and the inside remaining opaque. Although those rings nearest to the edges

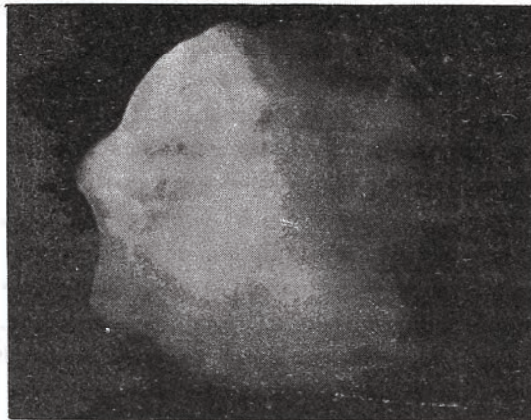


Fig. 1. A *Pseudochaenichthys georgianus* otolith after decalcination

Table 1

Otolith dimensions and their ratio in the fish species studied

Species	Measurement				A : B : C
	Fish body length (cm)	Otolith length (mm)	Otolith width (mm)	Otolith thickness (mm)	
		A	B	C	
<i>Pseudichaenichthys georgianus</i>	48.0	5.55	4.07	2.58	1 : 0.73 : 0.46
	45.0	5.54	4.00	2.72	1 : 0.72 : 0.49
	46.0	5.34	4.14	2.63	1 : 0.77 : 0.49
<i>Champscephalus gunnari</i>	25.0	2.43	2.30	1.04	1 : 0.95 : 0.43
	29.0	2.58	2.48	1.12	1 : 0.96 : 0.43
	28.0	2.47	2.32	1.02	1 : 0.94 : 0.41
<i>Chaenocephalus aceratus</i>	45.0	4.69	3.55	1.62	1 : 0.77 : 0.35
	52.0	5.65	4.27	2.03	1 : 0.70 : 0.35
	51.0	5.13	3.60	1.80	1 : 0.70 : 0.35

were distinctly visible, the effect was regarded unsatisfactory (Fig. 1) therefore the results of decalcination were for this species negative.

The small size and considerable thickness (Table 1) of the *Champscephalus gunnari* otoliths and their characteristic conical shape allowed no positive effects be obtained either. A prolonged soaking resulted in significant losses, whilst the thickest centre remained opaque. As only the marginal rings were visible, this technique was found inadequate for *Champscephalus gunnari*.

The best results were obtained when the technique was applied to the *Chaenocephalus aceratus* otoliths. The otoliths were thin enough to yield rings all the way through to the centre. The duration of soaking depended on the otolith size and was determined experimentally: 5 minutes for more than 15 – cm long fishes and about 15 minutes for large individual longer than 45 cm. Otoliths of older fishes (more than 60 cm long) required even an hour of soaking, which was not always enough for thick otoliths.

It is recommended to control the reaction of decalcination by taking an otolith out of the solution every now and then and rinsing with water, whereby the formation of gas bubbles is prevented by removing the acid, thus facilitating the inspection.

The decalcinated otoliths placed in a dish with water were examined under a binocular microscope (x25 ocular magnification) in transmitted or incident light. In both types of illumination the ring pattern of the *Chaenocephalus aceratus* otoliths was well-visible transmitted light producing a better contrast (Fig. 2 and 3).

#### Burning

Burning proved satisfactory in each case; it is thus a technique suitable for otoliths of each of the species mentioned. An electric hot plate (800 W, 220 V) has been allowed to

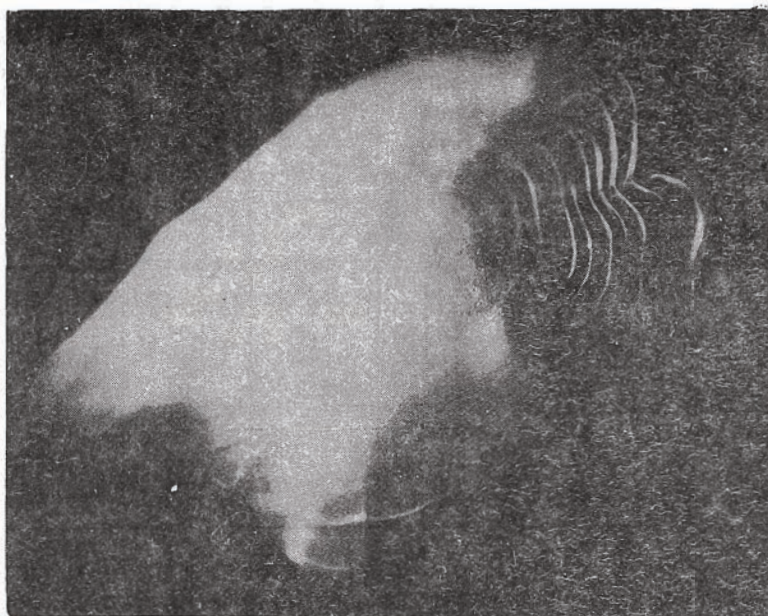


Fig. 2. A *Chaenocephalus aceratus* otolith after decalcination photographed in incident light

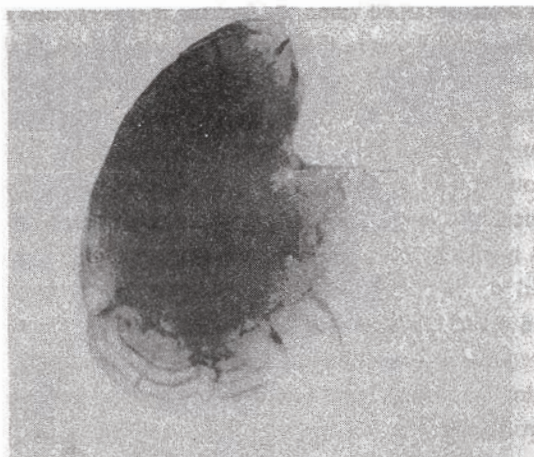


Fig. 3. A *Chaenocephalus aceratus* otolith after decalcination photographed in transmitted light

warm up for about 30 minutes before the otoliths were burned. In order to avoid harmful effects of burning (cracking, burning out), the otolith to be burned was placed on a thin piece of steel put onto the hot plate. A proper adjustment of this steel plate resulted in the formation of an air cushion. The time of burning was determined experimentally to last from several seconds to a few minutes depending on the otolith thickness. After burning, the otolith should show a black and glossy surface.

Burned otoliths were broken. The uniform thickness made it possible for otoliths of *Champscephalus gunnari* and *Chaenocephalus aceratus* to obtain reasonably good fractures pressing with one's fingers only. A more secure procedure involved placing an otolith on a thick layer of febric and fracturing by pressing with a fingernail edge. A fracture should run longitudinally and at the same time through the centre (nucleus).

In the case of the *Pseudochaenichthys georgianus* otoliths, the breaking is easily done with a needle. The otolith is placed on a folded fabric and pressure applied to the needle, its tip in the cavern between the convex and flattened sides (Fig. 7). The fabric prevents an uncontrolled cracking and splitting up of the otolith. Broken halves of the otolith were mounted in a rubber stopper and placed in water. Further examination and age reading were performed under a binocular microscope in incident light. The technique proved satisfactory for the three species studied (Figs 4,5,6). A proper burning time is, however essential; it is better to under – burn than to over – burn an otolith.

Particularly accurate were the fractures in the *Pseudochaenichthys georgianus* otoliths. An oval, somewhat flattened 0.1 mm thick central nucleus, never cracking on otolith breaking, was being frequently found in the centre.

The otolith radius measured on the fracture, typical of a species was used in the age reading according to Vovk (1955). A curvilinear relationship between fish body length and otolith radius was found, from which annual increments could be read based on the measurements made on otoliths prepared by means of one of the two techniques described. Owing to the good results obtained for all the three species, the second techniques is particularly recommended.



Fig. 4. Fracture of burned otolith of *Chaenocephalus aceratus*

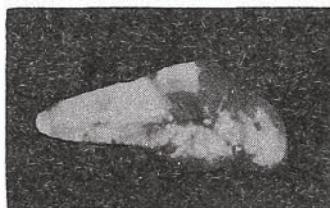


Fig. 5. Fracture of burned otolith of *Champscephalus gunnari*

## SUMMARY

1. The white-blooded fish otoliths can be prepared for age and growth rate reading by means of decalcination or burning.

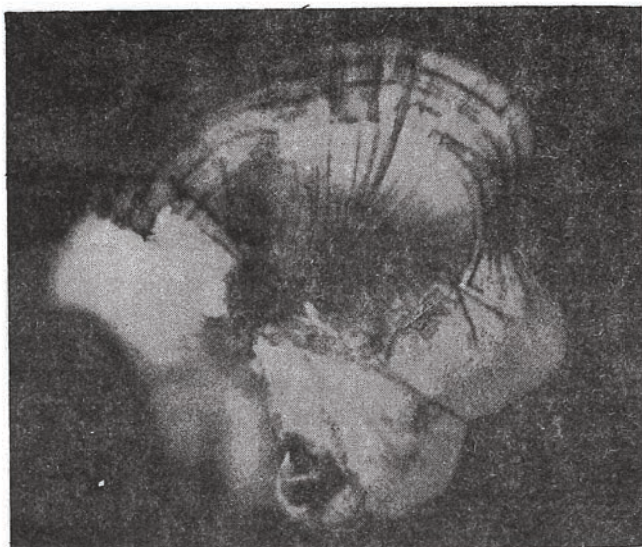


Fig. 6. Fracture of burned otolith of *Pseudochaenichthys georgianus*



Fig. 7. Cross-section of *Pseudochaenichthys georgianus* otolith

2. Decalcination is effected with 5% HNO<sub>3</sub>; positive results, however, were obtained for *Chaenocephalus aceratus* only. The decalcination time was about 5 minutes, 15 minutes and even 1 h for 15 cm, 45–60 cm and more than 60 cm long fishes, respectively.
3. Burning on an electric hot plate for a few seconds to a few minutes allows a regular, clearly-visible ring pattern to be obtained on a fracture. Satisfactory results can be obtained for *Chaenocephalus aceratus* and *Champscephalus gunnari*, while particularly good effects were obtained for *Pseudochaenichthys georgianus*.
4. Measurements taken on otoliths or their fractures are a starting point for developing a growth rate models of the species studied.

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#### TECHNIKA PRZYGOTOWANIA OTOLITÓW TRZECH GATUNKÓW RYB BIAŁOKRWISTYCH DO OKREŚLANIA WIEKU I TEMPY WZROSTU

##### Streszczenie

Pobrane do badań wieku i tempa wzrostu otolity ryb białokrwistych można przygotować do odczytu metodą odwapniania lub spiekania (prażenia). Do odwapniania należy stosować 5% roztwór HNO<sub>3</sub>, ale pozytywne efekty otrzymać można jedynie dla *Chaenocephalus aceratus*. Czas odwapniania otolitów ryb 15 cm długości wynosił 5 min., ryb 45–60 cm około 15 minut a dla ryb większych niż 60 cm nawet jedną godzinę.

Prażenie przez kilka sekund do kilku minut na rozgrzanej płytce elektrycznej pozwala uzyskać na przełomie regularną, czytelną strukturę. Zadowalające efekty uzyskać można w ten sposób dla

*Chaenocephalus aceratus* i *Champscephalus gunnari* a szczególnie dobre dla *Pseudochaenichthys georgianus*. Pomiarы na otolitach lub ich przełomach są punktem wyjścia do ustalenia modeli tempa wzrostu tych gatunków.

Хойнацки Ю., Пиотр Пальчевски

ТЕХНИКА ПРИГОТОВЛЕНИЯ ОТОЛИТОВ ТРЁХ ВИДОВ АНТАРКТИЧЕСКИХ  
БЕЛОКРОВНЫХ РЫБ ДЛЯ ОПРЕДЕЛЕНИЯ ИХ ВОЗРАСТА И ТЕМПА РОСТА

Р е з ю м е

Изъятые отолиты антарктических белокровных рыб для определения возраста и темпа роста можно подготовить к отсчету методом обезкальцивания или прожаривания. Для обезкальцивания необходимо применять 5% раствор  $\text{HNO}_3$ . Положительные результаты возможно однако получить лишь для *Chaenocephalus aceratus*. Время обезкальцивания отолитов рыб длиной 15 см составляет 5 мин. рыб длиной 45–60 см около 15 мин., а для рыб больше чем 60 см даже один час.

Прожаривание в течение нескольких секунд до нескольких минут на розогретой электрической плитке позволяет получить в переломе правильную отчетливую структуру. Удовлетворительные эффекты можно получить таким образом для *Chaenocephalus aceratus* и *Champscephalus gunnari* а особенно для *Pseudichaenichthys georgianus*. Измерения на отолитах или их переломах являются отправной точкой для получения модели темпа роста этих видов рыб.

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