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*Maria NAGIĘĆ, Celestyn NAGIĘĆ,*
*Konrad DĄBROWSKI, Eugenia MURAWSKA*

# MARKING OF JUVENILE WHITEFISH *COREGONUS LAVARETUS* (L.) WITH TETRACYCLINE ANTIBIOTICS

# ZNAKOWANIE NARYBKU SIEI *COREGONUS LAVARETUS* (L.) ANTYBIOTYKAMI Z GRUPY TETRACYKLIN

**Institute of Ichthobiology and Fisheries**
**Olsztyn**

Tetracycline (TC), when consumed along with zooplankton by the young-of-the-year whitefish, is deposited in the skeleton as calcium-tetracycline compounds, which – in the UV light – form yellow-gold fluorophores. The fluorescent mark is particularly distinct in vertebrae and otoliths, remaining in those skeletal elements for 24 months and presumably longer. An attempt to utilise artificial feed, based on frcezedried krill substituting live zooplankton used so far, as a TC carrier was made.

## INTRODUCTION

The tetracycline antibiotics (TC), both administered orally and intraperitoneally injected to the coregonids, form calcium – tetracycline chelates in developing bones, the compounds being visible in the UV light as yellow-gold fluorophores. Such marks were still clearly visible after 24 months (Nagięć and Nagięć, 1982).

The oral administering of tetracycline seems to be a very promising technique of small fish marking. Over the last ten years, the predominant procedure of lake stocking in the Mazurian Lake District has been the release of juveniles weighing slightly more than 500 g, which has resulted from the advancement of whitefish and ablen cage rearing

techniques in lakes. A mass marking of the fishes would allow to estimate the contribution of stocking to commercial catches.

The whitefish, the object of the present study, are difficult to feed pelleted food, while to administer the medicine with live zooplankton rules our any possibility of estimating the antibiotic dosage. Besides, feeding with TC-marked zooplankton is a very time-consuming technique, impossible to apply on a commercial scale, hence the present attempt to substitute live zooplankton with an appropriate artificial feed.

The antibiotic deposition in whitefish bones was followed.

## MATERIALS AND METHODS

The young-of-the-year whitefish were caught with a small trawl on July 7, 1981 in a pond at the Szwaderki Fish Farm in the Mazurian Lake District. The individual weight of the fish placed in 30-l troughs ranged from 580 to 2,530 mg, amounting most often to 1000 mg. Each trough was stocked with 30 individuals. The fishes were fed an artificial diet consisting of freeze-dried krill (70%), yeasts (24%), fish oil (3%), and vitamin mix (3%). The basic feed was supplemented with 4 doses of tetracycline. A daily portion was about 6% of the body weight. The pellet diameter ranged within 540–1100 $\mu$m, with a mean of 780 $\mu$m. The following TC doses were used:

A = control (no TC);
B = 20 mg TC/g feed = 1.2 mg TC/fish ) day = 1200 ppm;
C = 70 mg TC/g feed = 4.2 mg TC(fish) day = 4200 ppm;
D = 150 mg TC/g feed = 9.0 mg TC(fish) day = 9000 ppm;
E = 250 mg TC/g feed = 15.0 mg TC(fish) day = 15 000 ppm.

The fish were fed three times a day: at 8.00, 13.00, and 19.00 hours. On July 9 the control feed was administered; on July 10 the feeding experiment following the above pattern was started. The experiment was run for 9 days, i.e., until July 19, 1981.

The whole experiment, its design consisting of 5 replicates for each type of diet, was run in an old, wooden hatchery, its water supply coming from the surface of the Lake Maróz. Every day a few individuals were killed to check the food take-up.

Bones, without any preliminary grinding, were examined under the microscope in the ultra-violet light (Nagięć and Nagięć, 1982) 3 and 9 days after the experimental diet had beed started.

## RESULTS

The fish were eagerly feeding on the artificial feed in spite of the fact that they had been transferred from an open, fertile pond rich in zooplankton to an artificial habitat of the hatchery (30-l troughs, electric light, noise produced by the flowing water). A slightly reduced feeding intensity was recorded on the fifth day among those individuals offered

Readability of antibiotics built up in bones

| lot and dose | Examination after 3 days | Examination after 9 days |
|---|---|---|
| A – control | no tetracycline fluoresence | |
| B<br>1,200 ppm | Subtle fluorescence on the edge of the otolith (Fig. 1) and gill rakers. Distinct fluorescence in caudal vertebrae (Fig. 2) | Distinct fluorescence in the following skeletal elements: otolith (Fig. 3), trunkvertebrae (Fig. 4), gill rakers, ribs and scales. |
| C<br>4,200 ppm | Distinct fluorescence in caudal vertebrae, Fairly good in otoliths, gill rakers, hypurals and dental plates, | Distinct fluorescence in the following skeletal elements: otoliths, verterbrae, gill rakers, ribs, branchial archs and scales. |
| D<br>9,000 ppm | Distinct fluorescence in the following skeletal elements: otoliths, vertebrae (especially caudal) gill rakers, branchial archs, opercular bones, hyporals, lepidotrichia, scales. | |
| E<br>15,000 ppm | Distinct fluorescence in the all above mentioned elements.<br>Otoliths (Fig. 5), vertebrae (Fig. 6) | Otoliths (Fig. 7), vertebrae (Fig. 8) |

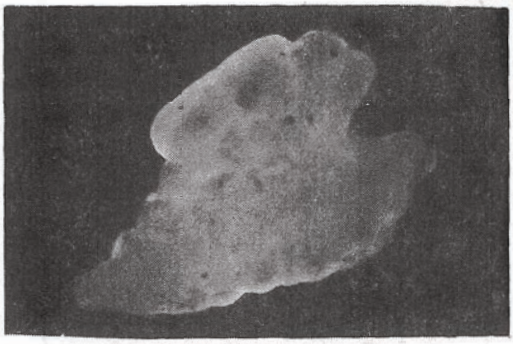

Fig. 1. Subtle fluorescence on the otolith margin adge of an otolith. Whitefish, 5.8 cm long, 2.53 g. weight. After 3 days of experimental feeding with diet 8, 1200 ppm of TC daily. Magn. 50x

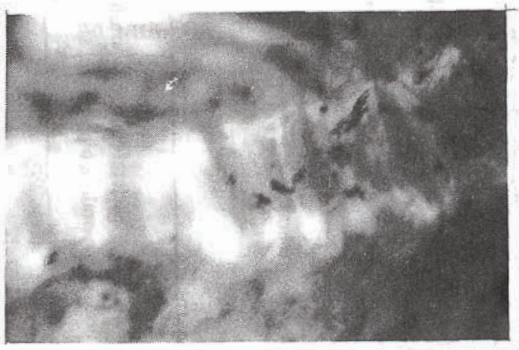

Fig. 2. The same specimen as in Fig. 1. Distinct fluorescence in the last caudal vertebrae. Side view. Magn. 50 x

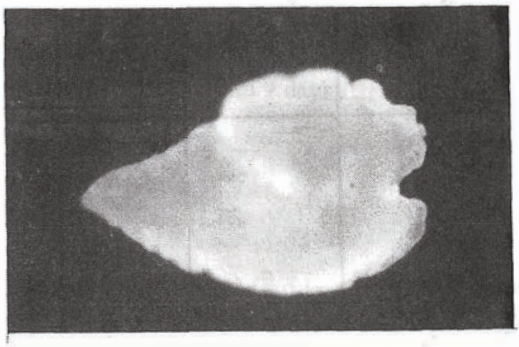

Fig. 3. Distinct fluorescence on the whole surface of the otolith. Whitefish 5.7 cm long, 2.2 g. weight After 9 days experimental feeding with diet 8,1200 ppm of TC daily. Magn.50 x

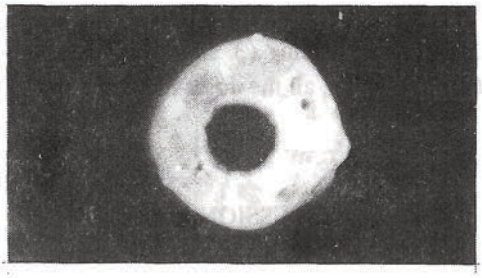

Fig. 4. The same specimen as in Fig. 3. Trunk vertebra. Upper view. Magn 50 x

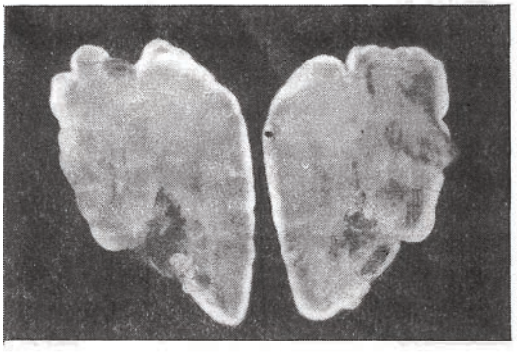

Fig. 5. Intensive fluorescence on the edge of an otolith. Whitefish 4.2 cm long, 0.9 g. weight. After 3 days of experimental feeding (15000 ppm of TC daily). Magn 50x

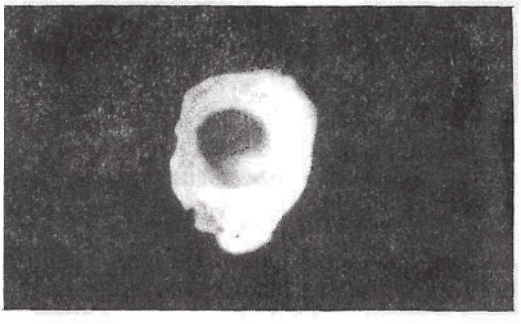

Fig. 6. The same specimen as in Fig. 5. Trunk vertebra. Upper view. Magn 50x.

the highest antibiotic doses (150 and 150 mg TC/g feed). The feeding intensity was higher in white troughs than in the dark ones.

Mortality was low and evenly distributed among the replicates although the water temperature reached 25°C. Only 18 out of the 750 experimental individuals died.

The readability of the deposited antibiotic was checked twice: after 3 and 9 days of feeding the pelleted food, 2–3 individuals from each replicate being examined. The results are summarised in Table 1 and presented in Figs. 1–8. After 3 days of using the pellets, a well-marked difference in otolith fluorescence between the diet B- (Fig. 1) and

diet E (Fig. 5) fed fish was recorded. Significant differences in the bone images were also produced by different duration of feeding (3 and 9 days), as illustrated by otoliths (Figs. 5 and 7). After 9 days of feeding, all the doses including the lowest one produced a distinct fluorescence.

## DISCUSSION

Oral administering of bone-marking substances seems to be a very attractive procedure for the small fish mass marking in population studies. An attempt to label the *Stisostedion vitreum* juveniles by bathing them in various concentrations of oxytetracycline for different periods of time brought meagre results (Scidmore and Olson, 1969). Injections cannot be applied to small individuals. An attempt to spray the rose bitterling with a fluorescent substance produced a serious mortality, the marks themselves quickly disappearing (Solomon et al., 1982).

Some authors (Behrens Yamada et al., 1982) tried strontium instead of antibiotics.

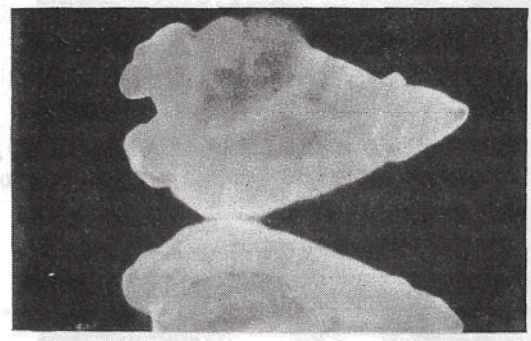

Fig. 7. Distinct fluorescence on the whole surface of the otoliths. Whitefish 4.8 cm long, 1.1 g.weight. After 9 days experimental feeding (15000 ppm of TC daily). Magn. 50x

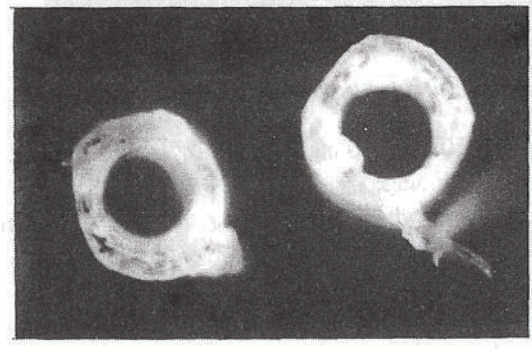

Fig. 8. The same specimen as in Fig. 7. Trunk vertebra. Upper view. Magn. 50 x.

Dosage of antibiotics administrated orally in different types of experiments

| Species and size | Aim of the experiment | Antibiotic used | Kind of diet | Dose per day (mg/kg body weight) | No of days fed | Total dose (mg/kg body weight) | Mark quality | Remarks | Author |
|---|---|---|---|---|---|---|---|---|---|
| *Oncorhynchus nerka*, 5 – 10 g | mass-marking | CTC | dry pellets | 250 – 1000 | 2 – 5 | 1000 – 2000 | good | vertebrae centre analysed; marks easily visible 42 mo later | Weber and Ridgway 1967 |
| *Salmo salar* | | | | | | | | | |
| 20 g | mass-marking | OTC | agar cubes | 250 | 5 | 1250 | good | vertebrae centres analysed; | Odense and Logan 1974 |
| 20 g | mass-marking | OTC | agar cubes | 250 | 10 | 2500 | good | marks easily visible 11 mo later | |
| near400 g | mass-marking | OTC | agar cubes | 660 | 9 | 5940 | good | marks easily visible 4 mo later | |
| *Salmo gairdneri* 0,18 g | mass-marking | TC | dry pellets | 100 – 700 | 4 – 8 | 400 – 5600 | good in all doses | anterior ribs examined; easily visible 12 mo later | Trojan 1973 |
| | | | beef liver | 100 – 700 | 4 – 8 | 400 – 5600 | | | |
| *Coregonus lavaretus* 0,8 g | mass-marking | CTC | crustacean plankton | 500 – 700 | 4 – 8 | 2800 – 4000 | good | vertebrae centres and tail peduncle analysed; | Nagięć M. and Nagięć C. 1983 |
| 1,5 g | mass-marking | TC | „ | 700 | 13 | 9100 | good | marks easily visible 38 mo later | |
| 12,0 g | mass-marking | TC | „ | 500 | 10 | 5000 | good | marks easily visible 24 mo later | |
| | | | | | | | | marks visible 13 mo later | |
| *Coregonus lavaretus* 1 g | mass-marking | TC | dry krill pellets | 1200 – 15000 | 9 | 10800 – 135000 | good | mark visibility not checked | present work |
| *Cyprinus carpio* 35 – 77 g | side effects of antibiotics | OTC | Trouvit pellets | 50 | 48 | 2500 | | demonstrable levels of OTC in serum, cellular immunity not affected, humoral immune response depressed. | Rijkers et al. 1980 |

The first is permanently deposited not only in vertebrae and otoliths, but also in scales, which would make it possible to identify a fish without killing it. It remains to find out if tetracycline remains long enough in fin rays, more and more often substituting scales in age determination.

The following problems have to be solved in order to efficiently mass mark the fish with internal markers: a choice of a marker (antibiotic); a diet composition; the magnitude of a dose; possible side effects produced by the marker; persistence of the label.

Any antibiotic-containing food has to be willingly and fast taken up by the fish as neither the remains nor the extent of leaching (TC is water-soluble) can be measured. Thus the oral doses are always higher than the injected ones, although the actual dose reaching the body in the latter case can be higher.

Table 2 shows how difficult to solve is the problem of finding a proper dose. Daily doses ranged from 100 to 15,000 mg TC/kg body weight, the duration of feeding ranging from 2 to 13 days. Particularly difficult is to set a dose for small fish (about 1 g individual

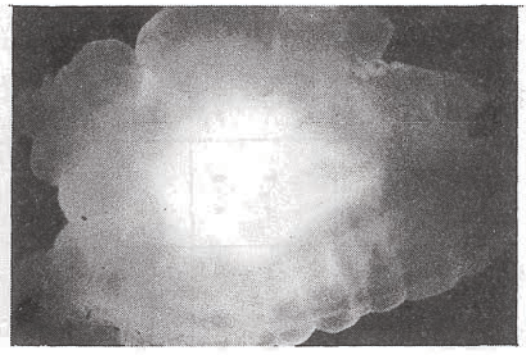

Fig. 9. Distinct fluorescence in the centre of the otolith 24 months after marking. Note increase in the otolith size. Whitefish 23.2 cm long, 93 g. weight. Magn. 32x

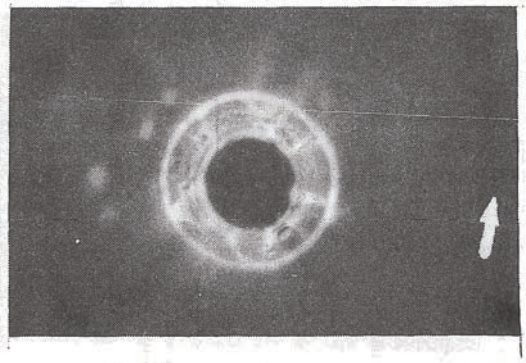

Fig. 10. Trunk vertebra from the same fish as in Fig. 9. Note increase in the vertebra diameter, the vertebra edge marked with an arrow. Magn. 50x

weight). The gut content analyses performed on the experimental fish showed the krill-based feed to be consumed; a question of How much? remains open.

In the experiment described, the only index of adverse tetracycline side effects was the mortality, low and similar for various doses. Conversely, Meunier and Boivin (1978) recorded increasing mortality of juvenile carp and trout of about 50 g body weight, brought about by intraperitoneally injected tetracycline and reduced weight increments of the survivors. The deposition of calcium-tetracycline compounds in bones would suggest the antibiotic to be inactive metabolically. However, long-term negative side effects cannot be ruled out. Weber and Ridgway (1967) found no negative effects of a 250 mg/kg/d dose administered with pelleted feeds for 5 consecutive days to the Pacific salmon, and Rijkers et al. (1981) observed an intensified growth of carp offered oxytetracycline with food. Antibiotics are fairly commonly used for therapeutic purposes in fish cultures, but such procedures have to be strictly controlled as TC-resistant pathogenic bacteria can occur in fishes and oxytetracycline-treated carp show a decreased blood serum immunoglobulin level (Rijkers et al., 1981). Oxytetracycline in vitro retards cell proliferation, which allows to presume that in vivo it reduces the fish immunity (Grondel and Boesten, 1982). Therefore, the TC doses as low as possible are imperative for the marking purposes.

In the previous experiment involving TC offered with live zooplankton to fish of 1.7 g individual weight, the fluorophores were well-visible after 24 months (Fig. 9 and 10). It proved impossible to keep the fish alive after the termination of krill-based feeding. The distinctiveness of fluorescent marks in otoliths and vertebrae allows to assume their persistence for several years.

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*M. Nagięć, C. Nagięć, K. Dąbrowski, E. Murawska*

## ZNAKOWANIE NARYBKU SIEI (*COREGONUS LAVARETUS* L.) ANTYBIOTYKAMI Z GRUPY TETRACYKLIN

### STRESZCZENIE

Nasze wcześniejsze doświadczenia wykazały, że tetracyklina (TC), zjedzona przez narybek siei jako domieszka do żywego zooplanktonu, wbudowuje się w szkielet w formie związków wapniowo-tetracyklinowych, które w świetle ultrafioletowym fluoryzują złociście-żółto. Fluoryzujący znaczek jest szczególnie wyraźnie widoczny w kręgach i otolitach i w tych elementach utrzymuje się do 24 miesięcy, a prawdopodobnie i dłużej. Celem niniejszego doświadczenia jest ocena możliwości użycia jako nośnika substancji znakującej, sztucznej paszy w zastępstwie dotąd stosowanego żywego planktonu. Skład zastosowanej w doświadczeniu paszy był następujący: liofilizowany kryl, drożdże, olej rybny, mieszanka witaminowa i cztery dawki TC (20, 70, 150 i 250 mg TC/g w paszy). Pasza ta podawana w formie granulatów o średnicy ok. 800 μm była chętnie pobierana przez 1-gramowy narybek siei. Wielkość dziennej dawki wynosiła 6% ciężaru ciała. W czasie karmienia co kilka dni przeprowadzano obserwacje kości w świetle ultrafioletowym. Już po trzech dniach wszystkie ryby z wyjątkiem tych, które dostawały najniższą dawkę TC były dobrze poznakowane. Po pięciu dniach obserwowano mniej intensywne pobieranie paszy o najwyższych dawkach TC (150 i 250 mg TC/g paszy). Dziewięciodniowe karmienie doprowadziło do bardzo dobrego poznakowania wszystkich ryb. Śmiertelność była nieznaczna.

M. NAGIĘĆ, C. NAGIĘĆ, K. DĄBROWSKI, E. MURAWSKA

## МАРКИРОВКА МОЛОДИ ПРОХОДНОГО СИГА (COREGONUS LAVARETUS L.) АНТИБИОТИКАМИ ГРУППЫ ТЕТРАЦИКЛИНА

### Р Е З Ю М Е

На основании опытов, поставленных нами раньше установлено, что при поедании молодью проходного сига тетрациклина в виде добавки к живому зоопланктону происходит встраивание в скелет кальциево-тетрациклиновых соединений. Эти соединения при УФ-излучении отличаются золотисто-жёлтой флюоресценцией. Флюоресцирующие метки особенно чётко заметные по позвонкам и отолитам, где они сохраняются на протяжении 24 месяцев и вероятно дольше. Целью настоящего опыта являлась оценка возможности применить в виде

носителя метки искусственный корм в место применявшегося до сих пор живого планктона. В исследуемом корме содержались; лиофилизированный криль, дрожжи, рыбье масло, смеси витаминов и четыре уровня тетрациклина (ТЦ) (20, 70, 150 и 250 мг ТЦ/г корма). Получение гранулированного (диаметром около 800 м) корма молодью сига, весом 1 г, являлось хорошим. Величина дневного рациона составляла 6% от веса рыбы. В период кормления, спустя каждые несколько дней, велись наблюдения костей под УФ-излучением. Через 3 дня у всех особей за исключением получавших самую небольшую дозу ТЦ, установлено наличие чёткой метки. Через 5 дней наблюдалось уменьшение интенсивности в получении корма содержащего самые большие дозы ТЦ (150 и 250мг ТЦ/г корма). После девятидневного кормления были получены очень хорошие метки у всех особей. Смертность рыбы являлась незначительной.

Author's address:
Institute of Ichthyobiology and Fisheries
Academy of Agriculture and Technology
10–957 Olsztyn, Poland

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