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Fish embryology

A DEVICE FOR TRANSPORTING FISH GAMETES AND FERTILISED EGGS IN A MAGNETIC FIELD

URZĄDZENIE DO TRANSPORTU GAMET I ZAPŁODNIONYCH JAJ RYB W POLU MAGNETYCZNYM

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A device for transporting and storage of fish gametes, developing eggs, and newly hatched fry in a constant, adjustable (3–9 mT) magnetic field was constructed. The device has a relatively large, thermally insulated, biological material storage compartment, exposed to highly uniform (± 2.5%) magnetic field.

INTRODUCTION

Transportation of fish eggs from spawning ground to the hatchery is one of the major operations in the fisheries practice. The widely used conventional method involves obtaining sperm and roe from spawners after capture, artificial fertilisation of eggs, and transporting them in water-filled containers to hatcheries, frequently situated far away from the spawning ground. Another method, albeit more seldom used, involves transporting, in separate containers and without water, the eggs and sperm obtained in the spawning ground, fertilisation occurring after delivery to the hatchery.

It has been shown that the other method may be more appropriate for some salmonid species. It allows to reduce handling time in the spawning ground; the gametes under anabiosis are not affected by shocks induced by transportation and do not respire; moreover, transportation of water is avoided, hence more space is saved and more efficient the whole procedure becomes (Cykowska et al. 1973; Winnicki 1993).

Our studies on magnetic field effects on fish early in their ontogenesis, carried out for several years, allow to conclude that both fish eggs and sperm, when placed in a constant magnetic field of a certain value, retain their viability and ability to fertilise and get fertilised for a longer time. The proportion of activated spermatozoa increases and duration of their motility becomes significantly extended after contact with water. As a result, effects of fertilisation (the eggs are longer prone to become fertilised) are significantly better on exposure to magnetic field than without it (Formicki et al. 1990, 1991; Formicki and Winnicki 1993).

Those promising results have prompted the authors to investigate a possibility of applying a constant magnetic field to transportation of fish gametes and fertilised eggs.

Results of the 2-year-long period of studies confirmed the earlier laboratory findings and seem to demonstrate the usefulness of the method. Application of a magnetic transporting device significantly increases fertilisation rate and reduces losses, usually incurred during transportation of fertilised eggs (Winnicki et al. 1994). Moreover, the device provides an opportunity to store, for up to several days, the viable spermatozoa without a necessity to use other, more complicated procedures, e.g., deep freezing (Legendre and Billard 1980).

To translate the observations into practical use, there arose a need to construct a special device which would make it possible to carry out comprehensive studies on behaviour of gametes, embryos at different developmental stages, and larvae, during transportation in magnetic field with adjustable induction value and spatial characteristics, and to find optimal parameters for different species.

Thus the present paper describes a device for transporting fish eggs and sperm, or other biological material, exposed to constant magnetic field of induction value adjustable within 3–9 mT.

DESCRIPTION OF THE DEVICE

The device (Fig. 1) consists of two magnetic circuits: external and internal (with a working space to house a container with biological material to be transported) and two sets of magnets.

The external magnetic circuit consists of four pipes connected with elbows, which form the transporter frame (1); two trestles for mounting the pipes (2); distance adjusters attached to the trestles (3); and external magnet poles (4).

The internal magnetic circuit consists of two sets of internal magnetic poles with expanders (5) and two working space poles (6). The constant distance between the working space poles is secured by brass distancers (7), one of which forms a pivot for the thermally insulated cylindrical container (8) for storing biological material. The container is made of a brass plate. Thermal insulation is provided by a layer of styrofoam, covered with a thin aluminium sheet. Inside, the container consists of two parts: a 220 mm diameter, 40 mm high lower compartment for live material, and an upper, wider, compartment, 250 mm in diameter and 50 mm high for coolant containers. When the latter are not necessary, the live material may occupy the entire inner space of the container.

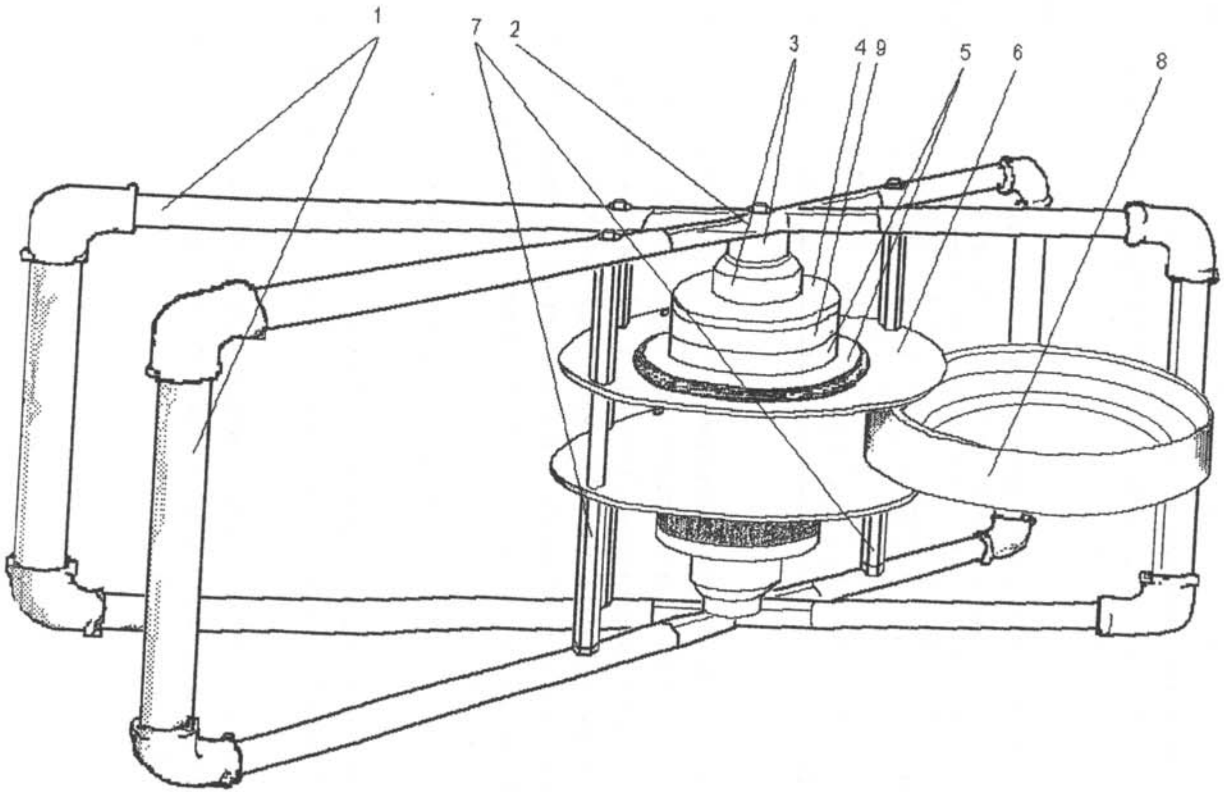

Fig. 1. Magnetic transporter (1—transporter frame; 2—trestles; 3—distance adjusters; 4—external magnet poles; 5—two sets of internal magnetic poles with expanders; 6—two working space poles; 7—brass distancers; 8—insulated container)

Magnetic field is produced by two sets of stable ferritic magnets (120, 12, and 17.6 mm diameter) made of FB-27 and magnetised along the symmetry axis (9). Magnetic field adjustment within the magnetic circuit working space (container) occurs by placing an appropriate amount of magnetic cramps onto the magnetic poles circuit. In this way a sizeable part of the magnetic flux is arrested. The adjustment is achieved by placing equal numbers of regular cramps (out of a total number of 10 pairs) and arch cramps (out of a total number of 3 pairs) on each pole. Changes in the magnetic field as a function of number of cramps are shown in Tab. 1.

Table 1

Magnetic field induction in the magnetic transporter working space as function of type and number of cramps applied

Number of regular cramps	0	1	2	3	4	5	6	7	8	9	10
Magnetic field induction (mT) without arch cramps	8.9	8.4	7.9	7.3	6.8	6.2	5.7	5.2	4.7	4.4	4.1
Magnetic field induction (mT) with arch cramps	4.8	4.5	4.2	4.0	3.9	3.7	3.6	3.4	3.3	3.1	3.0

The magnetic field induction was measured in the central part of the working space, intended for the biological material container where average field values occur. Magnetic induction in that space changes within ± 2.5% only (Fig. 2), which is quite satisfactory in view of a relatively small size (850 × 335 × 460 mm) and weight (50 kg) of the device. The large weight, relative to the volume of the space housing the biological material, has resulted from efforts to provide the most uniform magnetic field in that space (the magnetic circuit is enclosed by the frame).

Corrosion of all the corrosion-prone parts of the device has been prevented by zincifying.

The design of the transporter prevents dissipation of the magnetic field around the device. The magnetic field induction at a distance of 1 m is comparable with the geomagnetic field.

Preliminary results of field and laboratory tests will form a basis for future designs of simple "magnetic transporters", useful—in our view—in practice, i.e., for storage and transport of fish gametes and fertilised eggs.

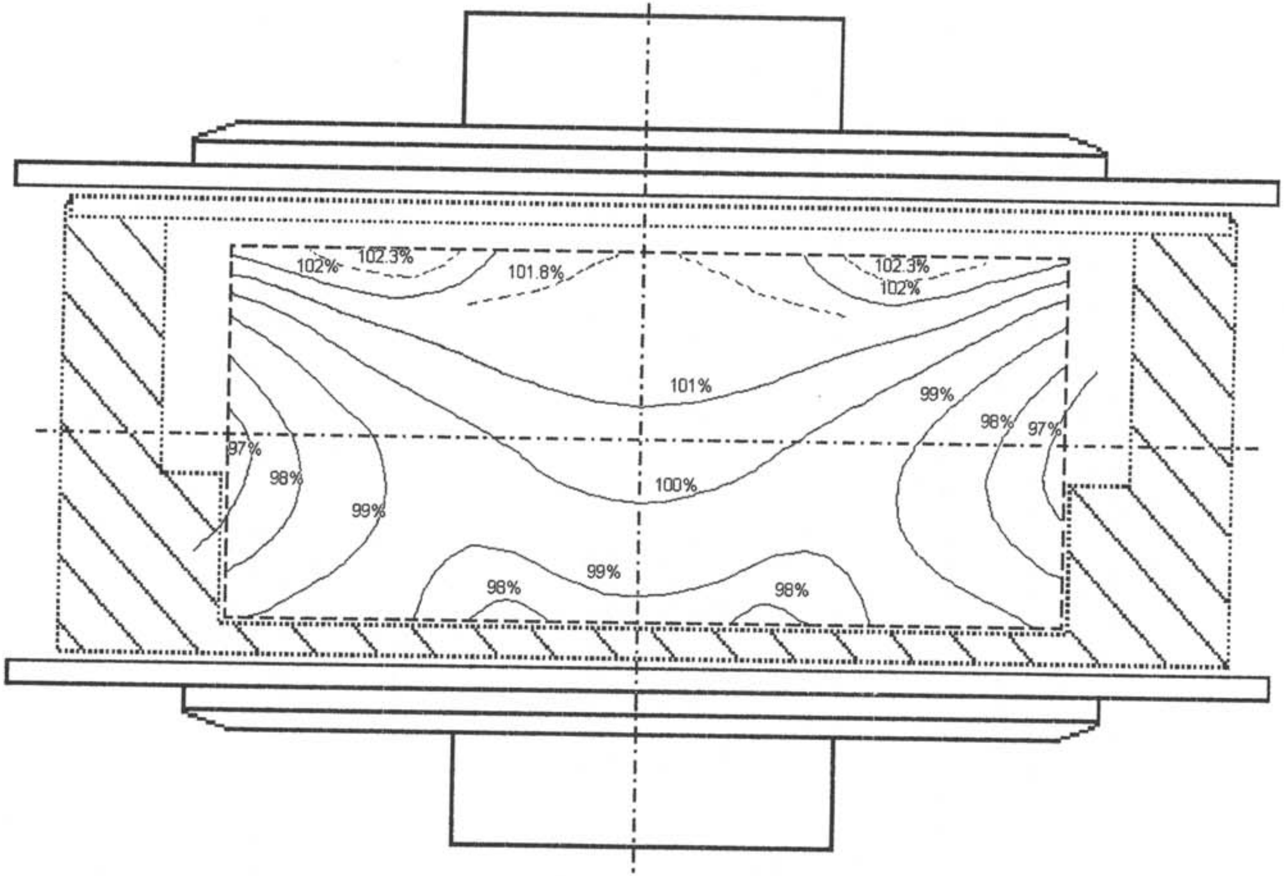

Fig 2. Distribution of magnetic field induction in transporter container (induction values are expressed as % of average)

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URZĄDZENIE DO TRANSPORTU GAMET I ZAPŁODNIONYCH JAJ RYB W POLU MAGNETYCZNYM

STRESZCZENIE

Skonstruowano urządzenie do transportu i przetrzymywania gamet, rozwijających się jaj ryb oraz wylęgu w stałym, o regulowanej wartości (3–9 mT), polu magnetycznym. Urządzenie posiada stosunkowo dużą, izolowaną termicznie przestrzeń na materiał biologiczny, o wysokiej jednorodności pola (± 2,5%).

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Parasitology

DACTYLOGYRUS TRIAPPENDIXIS SP. N. (MONOGENEA) PARASITE OF THE TENCH, *TINCA TINCA* (L.)

DACTYLOGYRUS TRIAPPENDIXIS SP. N. (MONOGENEA) PASOŻYT LINA, *TINCA TINCA* (L.)

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The present paper provides a detailed description of a new species of Monogenea—*Dactylogyrus triappendixis*, parasitic on gills of tench, *Tinca tinca* (L.) from the lakes of north-west Poland. The above-mentioned species differs from its congeners, parasitizing the tench, with details of the male copulatory organ and armament of the vagina. It is a frequent parasite of the tench in the water bodies studied, at the temperature of about 18°C.

INTRODUCTION

It can be concluded from the data provided by the "Catalogue of parasite fauna of Poland" (Grabda 1971) that a total of four species representing the *Dactylogyrus* Diesing, 1850 have been recorded from the tench *Tinca tinca* (L.) in the area studied, namely: *D. amphibothrium* Wagener, 1857, *D. anchoratus* (Dujardin, 1845), *D. macracanthus* Wegener, 1909, and *D. vastator* Wegener, 1909. According to the most recent literature (Gusev 1985) this host is parasitized by only two host-species: *D. macracanthus* and *D. tincae* Gusev, 1965. The former— *D. macracanthus* was more frequently listed in published works (Markevič 1951; Ergens and Lom 1970; Grabda 1971; Gusev 1965, 1985). On the other hand *D. tincae* was recorded in the drainage basin of the Danube and Elbe rivers on the territory of the former Czechoslovakia (Gusev 1965, 1985; Ergens and Lom 1970). In addition to that, this species was recorded by Puciłowska (1973) from Zegrzyński Reservoir (Poland). This author did not provide the name of the fish hosts, stating generally their systematic position on the family level (among others—Cyprinidae). Some time later Baturo (1978) recorded the presence of *Dactylogyrus* sp. on the tench fry in the ponds of north-eastern Poland.

Presently found parasites differed morphologically from currently known species of the genus *Dactylogyrus*. It prompted us to describe the specimens recovered, as a new species which we named *Dactylogyrus triappendixis* sp. n.

MATERIAL AND METHODS

The fish originated from lakes: Ińsko, Woświn, and Mielno located in the north-western part of Poland in the province of Szczecin. The material was collected between 14 May and 26 June 1996. A total of 32 tench, *Tinca tinca* was studied. Their total length ranged from 15.5 to 38.0 cm and their weight—from 45 to 790 g. Out of the studied fish, 21 came from Ińsko Lake, 6—from Woświn Lake, and 5—from Mielno Lake.

The fish were transferred in tanks filled with water from the lakes to laboratory, where they were kept in aquaria. The necropsies were performed on fresh fish, killed following their anesthetizing with Propiscin. The matter scrapped off the gill arches was examined under a dissecting microscope. The recovered parasites of the genus *Dactylogyrus* were studied fresh of fixed and cleared with lactophenol after being flattened on a glass slide. The drawings published in the present paper were prepared using a drawing tube. The measurements were based on 20 specimens.

The type specimens were deposited in the Museum of Natural History, Wrocław University (MNHW), Poland (cat. No MP667).

RESULTS

Dactylogyrus triappendixis sp. n.

Parasitizing gills of tench, *Tinca tinca* (L.). Relatively big; body length reaching 2.5 mm and body width—0.4 mm. Anterior end of body equipped with 4 pigmented-lined eye-spots. First pair smaller, measuring 4–9 μm. Second pair bigger—9–14 μm. Below eye-spots—muscular pharynx, measuring 68–122 × 108–130 μm. Posterior end of body with opisthohaptor armed with two hamuli, 14 marginal hooks, and two connecting bars (Fig. 1).

Central hamuli of opisthohaptor broad, with particularly widened bases of roots. Roots thick and not very long. Inner root distinctly longer than outer (Fig. 1). Total length of hamulus—50–59 μm, basal part—38–46 μm, inner root—17–28 μm, outer root—6–17 μm, blade—18–26 μm.

Marginal hooks having relatively long and wide handles (Fig. 1). Total length of hooks—33–39 μm.

Connecting bar (dorsal) slightly narrower in central part. Lateral ends wider and rounded (Fig. 1). Length of bar—10–17 μm, width—45–56 μm.

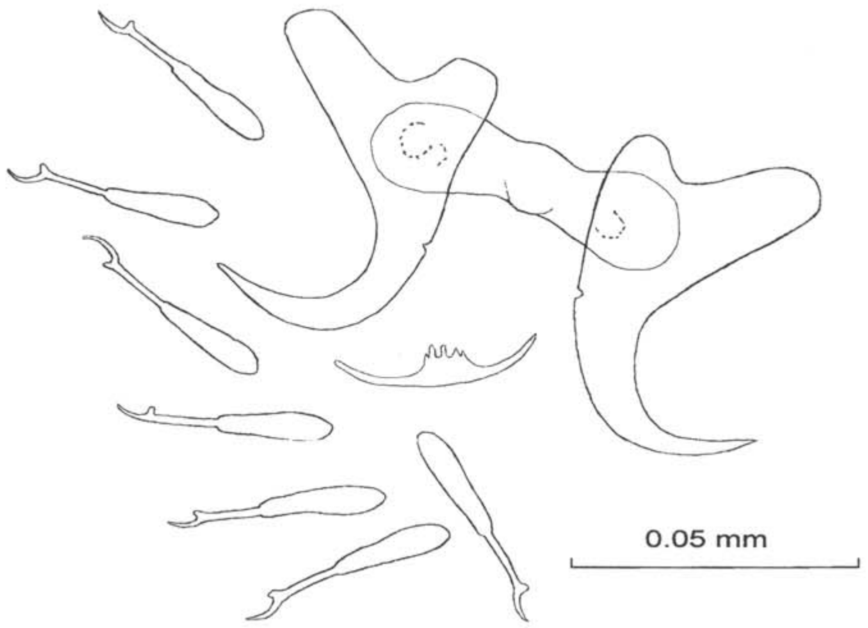

Fig. 1. Elements of attachment apparatus of *Dactylogyrus triappendixis* sp. n.

Supplementary bar (ventral) substantially smaller, thin, delicate, slightly arched, widened indistinctly in its central part and difficult to observe. Medial margin with 4–6 very small processes at widened area (Fig. 1). Dimensions of bar: 3–7 × 24–37 μm.

Armament of male copulatory organ of complex structure (Figs. 2–6). Support part consisting of lower, elongated, wide shaft and three upper processes. First of them robust, long, terminating with hook. Two small swellings on its hook-like bend, visible only a certain angle. Outgrowth terminating as robust, wide hook, which partly embraces copulatory tube branching from base of this process. Second process, running parallel to first one, also long, although substantially delicate, with very thin end reaching tip of copulatory tube. On some preparations, distinct branching visible on end of this process (Figs. 4, 5). Third process shorter and reaching almost to mid-length of first process. Fine denticulation occurring on its lateral margin (Fig. 2). Second and third processes not always clearly visible. Wide base of copulatory tube located in anterior part of shaft. Tube proper slightly narrower, arched and directed towards hook-like process of support part. Total length of copulatory organ of mature specimens: 105–126 μm, while in slightly younger individuals—84–96 μm.

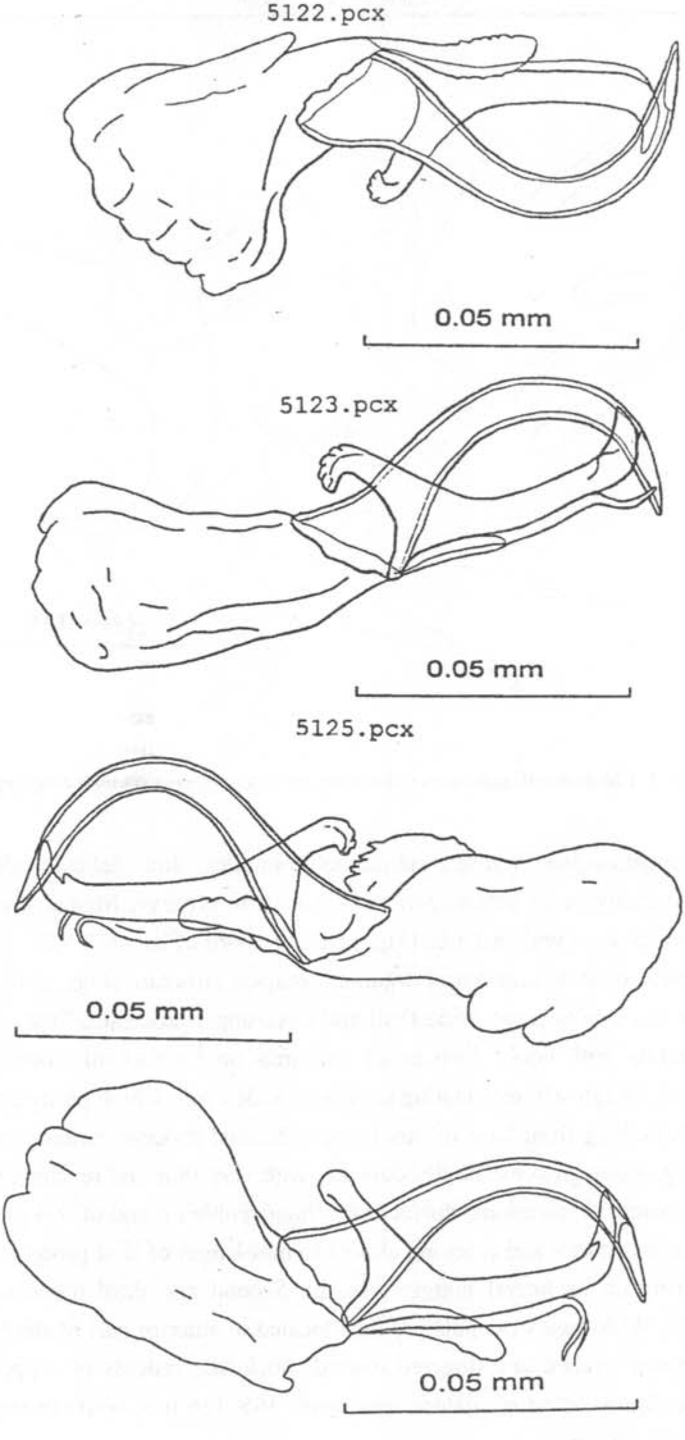

Fig. 2–5. Male copulatory organs of adults *Dactylogyrus triappendixis* sp. n.

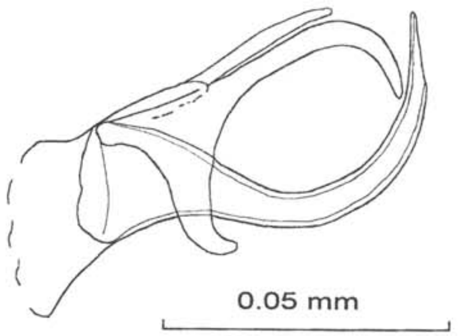

Fig. 6. Male copulatory organs of younger specimen of *Dactylogyrus triappendixis* sp. n.

Armament of vagina, as distinctly marked oval ring located on surface of very fine plate. This ring situated always next to, well visible, arched edge (Fig. 7). Dimensions of ring: 6–9 × 9–13 μm (ring with distance from arched edge—9–16 μm.

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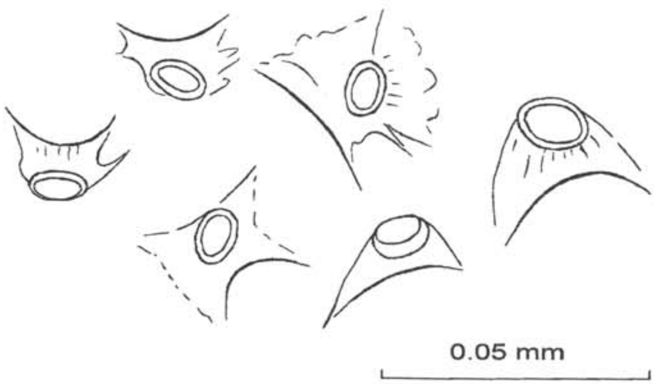

Fig. 7. Vaginal armament of *Dactylogyrus triappendixis* sp. n.

Eggs (Fig. 8) measuring 60–117 × 43–83 μm, filament—up to 17 μm.

The prevalence of this species infecting the tench was 4.76% in Ińsko Lake, 83,33% in Woświn Lake, and 100% in Mielno Lake. Single fish yielded from 6 to 50 specimens of the parasite. In May, at the water temperature in the lake about 12°C, only single individu-

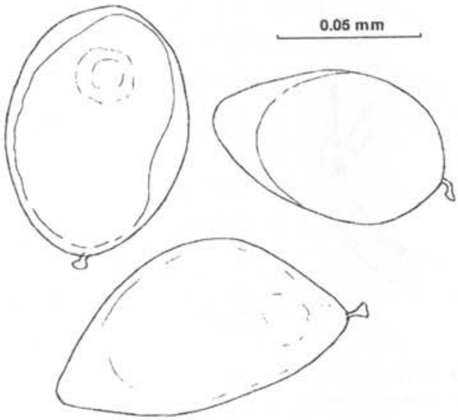

Fig. 8. Eggs of *Dactylogyrus triappendixis* sp. n.

als were encountered. By the end of June, however, while the water temperature reached some 18°C, the parasites intensively reproduced. At that time, numerous adult specimens, their eggs, as well as the juveniles, were found on the gills.

DISCUSSION

Comparing the presently found parasites with the descriptions of the currently known species of the genus *Dactylogyrus* we have found the closet similarity with *D. tincae*. Gusev (1965) describing the latter species provided detailed measurements of the parasites, as well as the drawings of the opisthohaptor and male copulatory organ. Subsequently Ergens and Lom (1970) supplemented this description publishing additional illustrations of the vaginal plate.

Despite the close similarity of the morphological and morphometric characters of *D. tincae* and the newly described species, there are very distinct differences between them. They concern the structure of the male copulatory organ and the armament of the vagina. *D. tincae* has one robust process with and outgrowth in the support area of the copulatory organ (Gusev 1965, 1985; Ergens and Lom 1970). In the presently found material, the specimens were equipped with three different processes in the support part of this organ. In addition to the robust process with outgrowth, almost identical as it was in *D. tincae*, they had two additional processes (long and short), branching from the base of the support part of the copulatory organ (Fig. 2–6). The armament of the vagina of *D. tincae* consisted of the ring and the plate, the latter approximating triangle with fuzzy edges (Ergens and Lom 1970). The parasites in our own material had the ring and a delicate not very regular plate (Fig. 7), the latter always different from that of *D. tincae*.

In the presently studied material we have not encountered the second species specific for the tench—*D. macracanthus*. It differs very distinctly from *D. triappendixis* sp. n. and *D. tincae* with the structure of the male copulatory organ, with the smaller dimensions of this organ and with the lack of a vaginal armament.

RECAPITULATION

1. The parasites of the genus *Dactylogyrus*, presently recovered from the gills of the tench, differ substantially from the hitherto described species with the structure of their male copulatory organ and with the structure of the vaginal plate.
2. The above differences permit us to conclude that the presently found parasites represent a separate species, which was described in this paper as *Dactylogyrus triappendixis* sp. n.
3. It is a frequent parasite of the tench in the studied water bodies at the water temperature of about 18°C.

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DACTYLOGYRUS TRIAPPENDIXIS SP. N. (MONOGENEA)
PASOŻYT LINA, *TINCA TINCA* (L.)

STRESZCZENIE

Podano szczegółowy opis nowego gatunku Monogenea *Dactylogyrus triappendixis* pasożytującego na skrzelach lina, *Tinca tinca* (L.) z jezior północno-zachodniej Polski. Gatunek ten różni się elementami budowy męskiego narządu kopulacyjnego i uzbrojeniem pochwy od innych pasożytów rodzaju *Dactylogyrus* występujących u lina. *D triappendixis* sp. n. jest częstym pasożytem lina w badanych zbiornikach przy temperaturze wody około 18°C.

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