Activity of selected hydrolases in secretory-excretory products and extracts from juveniles of *Contracaecum rudolphii* Hartwich, 1964 (Ascaridida: Anisakidae).

Janina Dziekońska-Rynko[1] and Jerzy Rokicki[2]

[1] Department of Zoology, Faculty of Biology, University of Warmia and Mazury, Oczapowskiego St. 5, 10-957 Olsztyn, Poland; e-mail:jdr@uwm.edu.pl
[2]Department of Invertebrate Zoology, Gdańsk University, Al. M. Pilsudskiego 46, 81-378 Gdynia, Poland

Accepted for publication 23 August 2010

Summary. An API ZYM test was applied to determine the activity of hydrolases in excretory-secretory products and in extracts from the second- (J2), third- (J3) and fourth- (J4) stage juveniles of *Contracaecum rudolphii*. The J2s were obtained by hatching from eggs, whereas the J3s were obtained from experimentally infected fish and the J4s were isolated from stomachs of cormorants. The highest number of active hydrolases was determined in the ES products (16) and extracts from J3 (19). Analyses of ES products from J2 and J4 showed the activity of eight and ten hydrolases, respectively. In the extracts from J2, the number of active hydrolases accounted for 15, whereas in the extracts from J4 it was 10.
Key words: anisakids, bird parasites, excretory-secretory enzymes.

Like the majority of nematodes in the Anisakidae family, *Contracaecum rudolphii* is characterised by a complex developmental cycle including intermediate and paratenic hosts. The first intermediate host may be freshwater fish and sea copepods or benthos invertebrates (gammaruses, juveniles of aquatic insects). These animals are infected with free-swimming second-stage juveniles released from eggs. Once crustaceans were infected experimentally, juveniles were found first in the intestine and after a few hours in the haemocoel, antennae, limbs and abdomen (Huizinga, 1966; Bartlett, 1966; Mosgovoy *et al*., 1968; Dziekońska-Rynko & Rokicki, 2007). The second intermediate host or paratenic host is mainly plantkivorous fish. Encysted juveniles were found most often in the intestinal wall and in intestinal mesentery, but also in the area of the liver and bile ducts (Torres & Cubillos, 1987; Kvach, 2005; Pronkina & Belofastowa, 2005; Szostakowska & Fagerholm, 2007; Dziekońska-Rynko *et al.*, 2008; Dziekońska-Rynko *et al*., 2010; Moravec, 2009). The final hosts of these juveniles are piscivorous birds and in Poland these are mainly cormorants (Kanarek, 2007; Dziekońska-Rynko & Rokicki, 2008). In the bird stomach the juveniles moult twice, grow and reach sexual maturity. Adults inhabit the lumen of the stomach, whereas juveniles are anchored in the wall, thus evoking ulceration and inflammatory states which, in turn, lead to a deterioration of the bird's condition (Huizinga, 1971; Abollo *et al*., 2001).

One of the factors that allow a parasite to colonise a host are penetrating enzymes contained in the excretory-secretory products (ES). Proteases contained therein facilitate the intra-tissue migration of the juveniles through the degradation of a tissue barrier, inhibit the coagulation process of the host's blood, protect the parasite against the immune response of a host organism, facilitate processes of juvenile hatching and moulting as well as serve an important function in their feeding (Sajid & McKerrow, 2002). A similar role may also be played by other enzymes occurring in the ES products of parasites, including hyaluronidase (Hotez *et al*., 1994), acetylcholine esterase (Opperman & Chang, 1992; Lee, 1996) and glycosidases (Irwin *et al*., 2004).

No reports were found in the available literature on the presence of enzymes in ES products or extracts from juveniles of *C. rudolphii* nematode. Therefore, the objective of this study was to determine the activity of selected hydrolases in ES products and extracts from J2, J3 and J4 juveniles.

MATERIAL AND METHODS

Adult nematodes were isolated from the gastrointestinal tract of cormorants obtained in the area of Lake Selment Wielki, Poland. The embryonic development was conducted following the methodology described by Dziekońska-Rynko & Rokicki (2007).

Collection of juveniles. The second-stage juveniles (J2) released from eggs were separated from post-hatching residues using the Baermann method, *i.e.* by placing a thin layer of cotton-wool on the bottom of a funnel. The third-stage juveniles (J3) were recovered from experimentally-infected goldfish (*Carassius auratus*) by a standard pepsin digestion procedure (1% pepsin solution pH 2) at 30th day post infection (Jackson *et al*., 2001). The experiment was reviewed and approved by the University Research Ethics Committee (permission number 06/2006). The J4s were isolated from stomachs of the cormorants. The juvenile stages were identified following Torres *et al*. (2000) and Kanarek & Bohdanowicz (2009).

Collection of excretion-secretion products from juveniles. After repeated rinsing in a solution of antibiotics and fungicidal agents (penicillin: 100 U ml^{-1}, streptomycin: 100 µl ml^{-1}, nystatin: 100 U ml^{-1}), the juveniles (*ca* 10,000 J2 individuals, 15 J3 individuals, 15 J4 individuals) were placed in 2 ml 0.7% sterile solution of NaCl (J2s and J3s) and incubated for 24 h at room temperature (*ca* 20°C). In the case of the J4s, they were placed in 2 ml 0.9% solution of NaCl and incubated at a temperature of 40°C. Afterwards, the solutions containing the excretory-secretory products (ES) were collected, dialysed at 4°C against distilled water for 24 h and lyophilized. Before the determinations, the lyophilizates of ES products from J2s and J3s were dissolved in a 0.7% solution of NaCl and those from J4s were dissolved in 0.9% of NaCl.

Preparation of juvenile crude extracts. Extracts were obtained by homogenisation of the juvenile in a glass homogeniser with a 0.7% NaCl solution (J2s and J3s) or 0.9% NaCl (J4). The homogenates were centrifuged for 10 min at 3000 *g*. The supernatant obtained was then used for the determination of the enzymatic activity.

The protein contents in all samples tested were determined using Bradford's (1976) method. The supernatants were standardized by dilution with the physiological salt solution.

Determination of enzymatic activity. The assays of the enzymatic activity in the ES products and homogenates were carried out using an API ZYM test kit (Bio Merieux S.A., Lyon, France), containing substrates to enable determination of the activity of 19 hydrolases (Table 1). In brief, 65-µl portions of each solution examined were applied onto wells containing respective substrates and incubated for 4 h at a temperature of 37°C. The results were read following the manufacturer's instructions. The activity of hydrolases was determined in nanomoles (nmol) of the hydrolysed substrate.

RESULTS

Results referring to the activity of hydrolases in the ES products and extracts from successive juvenile stages of *C. rudolphii* are presented in Table 1.

In the ES products of J2s, analyses demonstrated the activity of eight hydrolases. The highest activity was determined for acid and alkaline phosphatase (30 and 20 nmol), whereas the activity of the other hydrolases was substantially lower.

The highest number of active hydrolases was determined in the ES products of J3s (16), whereas in the ES products of J4s the assays showed the activity of 10 hydrolases. The highest activity levels were displayed by acid phosphatase, naphthol-AS-BI-phosphohydrolase, esterase, esterase lipase and α-glucosidase. The activities of N-acetyl-β-glucosaminidase, cystine and valine arylamidase, α-chymotrypsin, lipase, β-glucuronidase and α-fucosidase were determined only in the ES products of J3s, whereas those of β-glucosidase and α-galactosidase were determined only in the ES products of the J4s.

In the J2s extracts, analyses demonstrated the activity of 15 hydrolases. The highest activity was with acid and alkaline phosphatase, napthol-AS-BI-phosphohydrolase and N-acetyl-β-glucosaminidase (40 nmol), whereas the activity of the other hydrolases was substantially lower.

In the J3s extracts, the enzymatic activity was shown for all hydrolases examined, whereas in the extracts of J4s the enzymatic activity was shown for 10 hydrolases. In the extracts from J3s and J4s, the highest activity was reported for the enzymes belonging to a subclass of esterases and for leucine arylamidase, N-acetyl-β-glucosaminidase, α-glucosidase and β-glucuronidase. In the case of the other hydrolases, the activity was remarkably lower.

DISCUSSION

In this study, the lowest activity in both ES products and extracts from all juvenile stages analysed was determined for enzymes belonging to a subclass of the proteases. Their activity was detected in the ES products of multiple parasites (Sajid & McKerrow, 2002).

Table 1. Activity of enzymes from excretion-secretion products (ES) and extracts (EX) of second- (J2), third- (J3) and fourth- (J4) stage juveniles of *Contracaecum rudolphii.*

	Enzyme	CLASS-IFICATION	**substrate**	**pH**	Activity in nmoles of hydrolysed substrate					
					J2		**J3**		**J4**	
					ES	**EX**	**ES**	**EX**	**ES**	**EX**
PROTEASES	Leucine arylamidase	3.4.11.14	L-leucyl-2-naphthylamide	7.5	5	10	5	40	10	40
	Valine arylamidase	3.4.11.14	L-valyl-2-naphthylamide	7.5	5	5	5	5	0	0
	Cystine arylamidase	3.4.11.14	L-cystyl-2-naphthylamide	7.5	0	5	5	5	0	0
	Trypsin	3.4.4.4	N-benzoyl-DL-arginine-2-naphthylamide	8.5	5	5	5	5	0	0
	α- chymotrypsin	3.4.4.5	N-glutaryl-phenylalanine-2-naphthylamide	7.5	0	5	5	5	0	0
ESTERASES	Alkaline phosphatase	3.1.3.1	2-naphtyl phosphate	8.5	20	40	5	40	5	30
	Acid phosphatase	3.1.3.2	2- naphthyl phosphate	5.4	30	40	40	40	40	40
	Naphthol-AS-BI-phosphohydrolase	3.1.3.31	Naphthol-AS-BI-phosphate	5.4	0	40	40	40	30	40
	Esterase (C 4)	3.1.1.6	2-naphthyl butyrate	6.5	0	5	40	30	30	5
	Esterase lipase (C 8)	3.1.1.3	2-naphthyl caprylate	7.5	5	5	30	40	10	5
	Lipase (C 14)	3.1.1.3	2-naphthyl myristate	7.5	0	0	5	5	0	0
GLUCOSIDASES	α-galactosidase	3.2.1.22	6-Br–2-naphtyl-αD-galactopyranoside	5.4	0	5	0	5	5	0
	β-galactosidase	3.2.1.23	2-naphthyl- βD-galactopyranoside	5.4	0	5	5	5	5	10
	β-glucuronidase	3.2.1.31	Naphthol-AS-BI-βD-glucuronide	5.4	0	0	5	30	0	5
	α-glucosidase	3.2.1.20	2-naphthyl-αD-glucopyranoside	5.4	5	10	10	40	10	10
	β-glucosidase	3.2.1.21	6-Br-2-naphthyl-βD-glucopyranoside	5.4	5	10	0	5	5	0
	N-acetyl-β-glucosaminidase	3.2.1.50	1-naphthyl-N-acetyl-βD-glucosaminide	5.4	0	40	20	20	0	30
	α-mannosidase	3.2.1.24	6-Br-2-naphthyl-αD-mannopyranoside	5.4	0	0	0	5	0	0
	α-fucosidase	3.2.1.51	2-naphthyl-αL-fucopyranoside	5.4	0	0	5	10	0	0

According to Criado-Fornelio *et al.* (1992), proteases contained in the ES of *Trichinella spiralis* juveniles are linked with processes of juvenile moulting and their penetration into enterocytes, whereas proteases occurring in the extracts are digestive enzymes. The low activity of proteinases determined in the present study may have been due to the application of synthetic substrates for determinations, which are characterised by the considerably shorter peptide chains as compared with the natural substrates. Proteases of adult individuals and juveniles of *T. spiralis* display a substantially higher activity when natural substrates are used for determinations, in comparison with synthetic substrates (Moczoń & Wranicz, 1999; Ros-Moreno *et al*., 2000). Polzer & Taraszewski (1993) also reported that proteases contained in extracts of juveniles and adult individuals of *Anguillicola crassus* exhibited different substrate preferences. The enzymes isolated from J2s displayed the highest activity in the case of azocol and the highest activity of the enzymes isolated from J3s was obtained in the case of creatine, whereas proteases from adult individuals digested only hemoglobin. According to the authors, enzymes of the juvenile stages are penetrating

enzymes, whereas those of the adult forms are digestive enzymes. In the present work, a high level of activity of all enzymes belonging to a subclass of proteases was determined only for leucine arylamidase. Aminopeptidases occurring in parasites are potential activators of hormones and proenzymes linked with the process of hatching and moulting of juveniles (Rhoads *et al.,* 1997; Young *et al.*, 1999). The addition of inhibitors to a hatching medium resulted in the inhibition of the hatching process of *Ascaris suum* juveniles (Rhoads *et al.,* 1998). The high activity of leucine aminopeptidase, determined in the reported study both in the ES products and extracts from all juvenile stages, is most likely linked with the preparation of the juveniles for the subsequent moulting process.

In the ES products and extracts from all juvenile stages, the highest activity was determined for enzymes belonging to a subclass of esterases. It is enzymes that play a significant role in the regulation of metabolic processes. Acidic phosphatase (a lysosome marker) indirectly provides information on the process of intracellular digestion. According to Skotarczak (1987), phosphatases play very important roles in metabolic processes of *A. suum* embryos, and their activity depends on the intensity of metabolic processes. An especially high activity of those enzymes was assayed by the above author at the early stages of embryonic development (cleavage and gastrulation) when the demand for energy was very high. For most parasites, the presence of phosphatases is claimed to be an indicator of sites responsible for the secretory and excretory activity and the absorption of nutrients (Maki & Yanagisawa, 1980). A high activity of phosphatases was also determined in the extracts from J3s and J4s of *Anisakis simplex* (Dziekońska-Rynko *et al.*, 2003; Łopieńska-Biernat *et al.,* 2004), in adults of *Hysterothylacium aduncum* (Żółtowska *et al.*, 2007) as well as in cuticle, sub-cuticle cells and parenchyma of tapeworms (Arme, 1966; Niemczuk, 1993). Amongst enzymes belonging to a subclass of esterases, activities of esterase and esterase lipase were determined in all samples, whereas the activity of lipase was determined only in the ES products and extracts from J3s. The term 'non-specific esterase' is used to describe a group of enzymes splitting esters of a fatty acid with a chain no longer than eight atoms of carbon with particularly efficient 2–4 carbon atom chain fatty acids. The activity of esterases determined in the presented study in the ES products and extracts of J3 was several times higher than determined for J2s and J4s, which may be linked with the process of degradation of host tissues during juvenile migration.

For most parasitic organisms, the major source of energy is carbohydrate metabolism (Barrett, 1981). The activity of all hydrolases from a subclass of glycosidases was determined only in the extracts of J3s, whereas in both extracts and ES products from J2s and J4s the number of active glycosidases was definitely lower. A part of enzymes from the extracts is represented by typical digestive enzymes (α-glucosidase and β-galactosidase). Irwin *et al.* (2004) had shown the presence of activity of eight different glycosidases (α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, β-N-acetylhexosaminidase, α-mannosidase, α-fucosidase, neuraminidase) in the ES products from *Fasciola hepatica* except for proteases According to the authors, by digesting glycoproteins these enzymes facilitate the penetration of a host epithelium. In the reported experiment, a high number of active hydrolases was found mainly in the ES products of J3s. Migration time of J3s in the intermediate host is quite long. Encysted juveniles were found in the brush border, peritoneum around the liver and bile ducts (Torres & Cubillos, 1987; Kvach, 2005; Pronkina & Belofastowa, 2005; Dziekońska-Rynko *et al.*, 2010). The extracts from all juvenile stages were characterised by a high activity of N-acetyl-β-glucosoaminidase. It is known that this enzyme plays an important role in the hydrolysis of degradation products of the hyaluronic acid, one of the major constituents of the connective tissue. A similarly high activity of enzymes in a glycosidases subclass was also found in the extracts from *A. simplex* juveniles and adult individuals of *C. rudolphii* and *H. aduncum* (Dziekońska-Rynko *et al*., 2003; Łopieńska-Biernat *et al*., 2004; Dziekońska-Rynko & Rokicki, 2005; Żółtowska *et al.*, 2007). Łopieńska *et al*. (2001) reported that the activity of α-amylase in J4s of *A. simplex* was almost twice higher than in the J3s and that changes in the activity were also accompanied by the reconstruction of endogenous storage carbohydrates. According to the authors, it is associated with changes in metabolism in the process of host passage from ectothermal into hematothermal environment. In the current study we found the highest activity of glycosidases in the J3s extracts which may occur due to the preparation for the host changing from being ectothermal to hematothermal.

The results obtained suggest that the migration of *C. rudolphii* juveniles in the body of an intermediate host (crustaceans, fish) is intensified by hydrolytic enzymes presented in the ES products. It seems that these enzymes are most likely responsible for the damage to stomach walls observed in a final host (piscivorous birds). The high activity of leucine

arylamidase in the ES products and extracts from all stages is associated with the moulting process of juveniles which continues in the intermediate and final (the last two moults) host. .

REFERENCES

ABOLLO, E., GESTAL, C., PASCUAL, S. 2001. Anisakid infection in the European shag *Phalacrocorax aristotelis aristotelis*. *Journal of Helminthology* 75: 209-214.

ARME, C. 1966. Histochemical and biochemical studies on some enzymes of *Ligula intestinalis* (Cestoda: Pseudophyllidea). *Journal of Parasitology* 52: 63-68.

BARRETT, J. 1981. Biochemistry of Parasitic Helminths, Macmillan Publishers LTD, London. 308 pp.

BARTLETT, C.M. 1996. Morphogenesis of *Contracaecum rudolphii* (Nematoda: Ascaridoidea), a parasite of fish-eating birds, in its copepod precursor and fish intermediate hosts. *Parasite* 4: 367-76.

BRADFORD, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248-254.

CRIADO-FORNELIO, A., DE ARMAS-SERRA, C., GIMÉNEZ-PARDO, C., CASADO-ESCRIBANO, N., JIMÉNEZ-GÓNZALEZ, A., RODRIGUEZ-CAABEIRO, F. 1992. Proteolytic enzymes from *Trichinella spiralis* larvae. *Veterinary Parasitology* 45: 133-140.

DZIEKOŃSKA-RYNKO, J., ROKICKI, J. 2005. Activity of selected hydrolases in excretion-secretion products and extracts of adult *Contracaecum rudolphii*. *Wiadomości Parazytologiczne* 51: 227-231.

DZIEKOŃSKA-RYNKO, J., ROKICKI, J. 2007. Life cycle of the nematode *Contracaecum rudolphii* Hartwig, 1964 (sensu lato) from northern Poland under laboratory conditions. *Helminthologia* 44: 95-102.

DZIEKOŃSKA-RYNKO, J., ROKICKI, J. 2008. Infestation of the black cormorants (*Phalacrocorax carbo sinensis* L. 1758) from the colony on the Selment Wielki, with the nematode *Contracaecum rudolphii* Hartwich, 1964 (Nematoda, Anisakidae). *Oceanological and Hydrobiological Studies* 37: 1-11.

DZIEKOŃSKA-RYNKO, J., ROKICKI, J., GAMUŁKA, P. 2010. Development of larval *Contracaecum rudolphii* Hartwich, 1964 (Ascaridida: Anisakidae) in experimentally infected goldfish (*Carassius auratus* L., 1758). *Journal of Helminthology* 84: 234-240.

DZIEKOŃSKA-RYNKO, J., ROKICKI, J., JABŁONOWSKI, Z. 2003. Activity of selected hydrolases in excretion-secretion products and homogenates from L_3 and L_4 larvae of *Anisakis simplex* (Nematoda: Anisakidae) parasitizing herring. *Acta Ichthyologica et Piscatoria* 33: 125-135.

DZIEKOŃSKA-RYNKO, J., ROKICKI, J., WZIĄTEK, B. 2008. Experimental infection of *Carassius auratus* (L., 1758) with the second stage larvae of the nematode *Contracaecum rudolphii* Hartwich, 1964. *Wiadomości Parazytologiczne* 54: 339-341.

HOTEZ, P.J., CAPPELLO, M., HAWDON, J., BECKERS, C., SAKANARI, J. 1994. Hyaluronidases of the gastrointestinal invasive nematodes *Ancylostoma caninum* and *Anisakis simplex*: possible functions in the pathogenesis of human zoonoses. *Journal of Infectious Diseases* 170: 918-926.

HUIZINGA, H.W. 1966. Studies on the life cycle and development of *Contracaecum spiculigerum* (Rudolphi, 1809) (Ascaroidea: Heterocheilidae) from marine piscivorous birds. *The Journal of the Elisha Mitchell Society* 82:181-195.

HUIZINGA, H.W. 1971. Contracaeciasis in pelicaniform bird. *Journal of Wildlife Diseases* 7: 198-204.

IRWIN, J.A., MORRISEY, P.E.W., RYAN, J.P., WALSCHE, A., O'NEILL, S.M., CARRINGTON, S.D., MATTHEWS, E., FITZPATRICK, E., MULEAHY, G., CORFIRLD, A.P., DALTON, J.P. 2004. Glycosidase activity in the excretory-secretory products of the liver fluke, *Fasciola hepatica*. *Parasitology* 129: 465-472.

JACKSON, G.J., BIER, J.W., PAYNE, W,L., MCCLURE., F.D. 1981. Recovery of parasitic nematodes from fish by digestion or elution. *Applied and Environmental Microbiology* 41: 912-914.

JENNINGS, J.B., VAN DER LANDE, V. 1967. Histochemical and bacteriological studies on digestion in nine species of leech. *Biological Bulletin* 33: 166-183.

KANAREK, G. 2007. [Helminth fauna of the Great cormorant (*Phalacrocorax carbo* L. 1758) in northern Poland.} *Doctoral Thesis*, University of Gdańsk. 145 pp.

KANAREK, G., BOHDANOWICZ, J. 2009. Larval *Contracaecum sp*. (Nematoda: Anisakidae) in the Great cormorant [Phalacrocorax carbo (L., 1758)] from north-eastern Poland: A morphological and morphometric analysis. *Veterinary Parasitology* 166: 90-97.

KVACH, Y. 2005. A comparative analysis of helminth faunas and infection parameters of ten species of gobid fishes (Actinopterygii: Gobiidae) from the north-western Black Sea. *Acta Ichthyologica et Piscatoria* 35: 103-110.

LEE, D.L. 1996. Why do some nematode parasites of the alimentary tract secrete acetylcholinesterase?. *International Journal for Parasitology* 26: 499-508.

ŁOPIEŃSKA, E., ŻÓŁTOWSKA, K., ROKICKI, J. 2001. [The coparison of properties α-amylase from the third and fourth stage of *Anisakis simplex* larvae.] *Wiadomości Parazytologiczne* 47: 323-327.

ŁOPIEŃSKA-BIERNAT, E., ŻÓŁTOWSKA, K., ROKICKI, J. 2004. The activity of hydrolases of larval stages of *Anisakis simplex* (Nematoda). *Wiadomości Parazytologiczne* 50: 503-507.

MAKI, J., YANAGISAWA, T. 1980. Acid phosphatase activity demonstrated in the nematodes, *Dirofilaria immitis* and *Angiostrongylus cantoniensis* with special reference to the characters and distribution. *Parasitology* 80: 23-38.

MOCZOŃ, T., WRANICZ, M. 1999. *Trichinella spiralis*: proteinases in the larvae. *Parasitology Research* 85: 47-58.

MORAVEC, F. 2009. Experimental studies on the development of *Contracaecum rudolphii* (Nematoda: Anisakidae) in copepod and fish paratenic hosts. *Folia Parasitologica* 56: 185-193.

MOSGOVOY, A.A., SHAKHMATOVA, W.I., SEMENOVA, M.K. 1968. [Life cycle of *Contracaecum spiculigerum* (Ascaridata: Anisakidae), a parasite of domestic and game birds.] *Trudy Gelmintologicheskoi Laboratorii* 19: 129-136.

NIEMCZUK, W. 1993. [Localization and activity of several enzymes in tissues of *Bothriocephalus acheiloghathi* and *Khawia sinensis* tapeworms parastizing carp.] *Wiadomości Parazytologiczne* 39: 189-197.

OPPERMAN, C.H., CHANG, S. 1992. Nematode acetylocholonesterases: Molecular forms and their potential role in nematode behavior. *Parasitology Today* 8: 406-411.

POLZER, M., TARASZEWSKI, H. 1993. Identification and characterization of the proteolytic enzymes in the developmental stages of the eel-pathogenic nematode *Anguillicola crassus*. *Parasitology Research* 79: 24-27.

PRONKINA, N.V., BELOFASTOVA, I.P. 2005. New data on nematodes of the golden grey mullet *Liza aurata* (Pisces: Mugilidae) from Black Sea. *Ekologia Morja* 68: 77-82 (in Russian with Engl. summ.).

RHOADS, M.L., FETTERER, R.H., URBAN, J.F. 1997. Secretion of an aminopeptidase during transition of third- to fourth- stage larvae of *Ascaris suum*. *Journal of Parasitology* 83: 780-784.

RHOADS, M.L., FETTERER, R.H., URBAN, J.F. 1998. Effect of protease class-specific inhibitors on in vitro development of the third- to fourth-stage larvae of *Ascaris suum*. *Journal of Parasitology* 84: 686-690.

ROGERS, W.P. 1982. Enzymes in the exsheathing fluid of nematodes and their biological significance. *International Journal for Parasitology* 12: 495-502.

ROS-MORENO, R.M., VÁZQUEZ-LÓPEZ, C., GIMÉNEZ-PARDO, C., DE ARMAS-SERRA, C., RODRIGUEZ-CAABEIRO, F. 2000. A study of proteases throughout the life cycle of *Trichinella spiralis*. *Folia Parasitologica* 47: 49-54.

RUITENBERG, E.J., LOENDERSLOOT, H.J. 1971. Enzym-histochemische onderzoek van *Anisakis sp*. larwen. *Tijdschrift voor Diergeneeskunde* 96: 247-260.

SAJID, M., MCKERROW, J.H. 2002. Cysteine proteases of parasitic organisms. *Molecular & Biochemical Parasitology* 120: 1- 21.

SAKANARI, J.A., MCKERROW, J.H. 1990. Identification of the secreted neutral proteases from *Anisakis simplex*. *Journal of Parasitology* 76: 625-630.

SKOTARCZAK, B. 1987. Histochemical studies on eggs of *Ascaris suum* during emryogenesis. I. Phosphatase. *Wiadomości Parazytologiczne* 33: 681-685.

SZOSTAKOWSKA, B., FAGERHOLM, H.P. 2007. Molecular identification of two strains of third-stage larvae of *Contracaecum rudolphii* sensu lato (Nematoda:Anisakidae) from fish in Poland. *Journal of Parasitology* 93: 961-64.

TORRES, P., CUBILLOS, V. 1987. Infection with larvae of *Contracaecum* (Nematoda, Anisakidae) in salmonids acclimatized in Chile. *Journal of Veterinary Medicine*, Series B 34: 177-182.

TORRES, P., VALDIVIESA, J., SCHLATTER, R., MONTEFUSCO, A., REVENGA, J., MARIN, F., LAMILLA, J., RAMALLO, G. 2000. Infection by *Contracaecum rudolphii* (Nematoda: Anisakidae) in the Neotropic cormorant *Phalacrocorax brasilianus*, and fishes from the estuary of the Valdivia River, Chile. *Studies of Neotropical Fauna and Environments* 35: 1-9.

YOUNG, A.R., MANCUSO, N., BOWLES, V.M. 1999. Biochemical aspects of egg hatch in endo- and ectoparasites: potential for rational drug design. *International Journal for Parasitology* 29: 861-867.

ŻÓŁTOWSKA, K., DMITRYJUK, M., ROKICKI, J., ŁOPIEŃSKA-BIERNAT, E. 2007. Hydrolase of *Hysterothylacium aduncum* (Nematoda). *Wiadomości Parazytologiczne* 53: 91-95.

J. Dziekońska-Rynko, J. Rokicki. Активность некоторых гидролаз в экскреторно-секреторных продуктах и экстрактах личинок *Contracaecum rudolphii* Hartwich, 1964 (Ascaridida: Anisakidae).

Резюме. Тест API ZYM был использован для определения активности гидролаз в экскреторно-секреторных продуктах (ЭС) личинок 2-й (J2), 3-й (J3) и 4-й (J4) стадий *Contracaecum rudolphii,* которых получали вылуплением из яиц, из промежуточных хозяев-рыб и из желудка бакланов. Наивысший уровень гидролаз выявлен в ЭС (16) и экстрактах J3 (19). В ЭС продуктах J2 и J4 выявлена активность соответственно 8 и 10 гидролаз. В экстрактах J2 насчитывается 15 активных гидролаз, тогда как в экстрактах J4 - 10.