FOLIA PARASITOLOGICA 40: 313–318, 1993

Redial productivity of *Clinostomum complanatum* (Trematoda: Clinostomatidae) within the snail host

X. H. Liao

Ichthyology Laboratory, Zhongshan University, Guangzhou, People's Republic of China

Key words: *Clinostomum*, redial productivity, "mixed" rediae, cercarial emergence, zigzag pattern

Abstract. Studies have been conducted on the rediae of *Clinostomum complanatum* Rud., including the problem of intramolluscan productivity, their population structure and the nature of rediae of different generations. It has been found that the reproduction of rediae is marked by their mass multiplication in the first two generations: this ensures the establishment of a population of considerable size. The mother redia is the sole parent redia which produces daughter rediae. The longevity of the mother redia is about 12 days, with a production period of 5 days. Rediae of the second generation which appear on the 7 th–8 th day start to propagate 3 days later. The peak of production of the second generation lasts for 3 days and then shifts to an entirely different phase, to that of cercarial production. The majority of the third generation are cercarial producers, with only a few "mixed" rediae which are important in maintaining the redial population. The size of redial population maintains a level of over 1,000–2,500 individuals during a period from 39–59 days. The polyembryony and the changes in nature in the production of rediae are considered to be attributable to their adaptation to the parasitic life of the digenetic trematodes.

The intramolluscan asexual phases of reproduction in digenea, in which a single miracidium multiplies to produce numerous offspring, have aroused great interest among helminthologists. However, no clear picture has yet been sketched about the pattern of reproduction and the potential productivity of any species of digenea to exemplify the polyembryony within the snail hosts. Edney (1950), working on the productivity of *Clinostomum complanatum* (Rudolphi, 1819)(syn. *marginatum*), found that rediae of different stages of development were present in snails and the infection was maintained by "continuous production of rediae by rediae." Cort et al. (1950) studied the germinal development of *Clinostomum complanatum* and noticed that "these germinal cell groups must be dividing very rapidly to produce the large numbers of embryos that develop in the rediae." Working on the succession of redial generations of *Fasciola gigantica* Cobbold in *Lymnaea natalensis caillaudi* (Bourguignat) and the development of *Paramphistomum sukari* Dinnik in *Biomphalaria pfeifferi* respectively, Dinnik and Dinnik (1956, 1957) were able to trace the characteristics of rediae. They found in both species that the "rediae of the first generation commence with the production of daughter rediae then enter the second phase of their productivity to produce cercariae" and rediae. "Rediae of subsequent generations also show this alternating development of daughter rediae and cercariae in their cavities." Zischke (1967) examined the redial populations of *Echinostoma revolutum* developing in snails *(Stagnicola palustris)* of sizes 2–20 mm long when exposed to either one or five miracidia. The results showed that "in both types of infection, the number of mother rediae was proportional to the size of the snail, and the populations were larger when snails were exposed to five miracidia." Later Lim and Lie (1969) conducted researches into the redial population of *Paryphostomum segregatum* in the snail *Biomphalaria glabrata.* Their results agreed with those of Zischke (op.cit.) in that the number of rediae was proportional to the size of the snail host, but they found that "the maximum size of the redial population in snails of similar size remained approximately the same whether the snails were exposed to 1, 10, or 100 miracidia each." Dönges (1971) and Dönges and Götzelmann (1988) developed a technique of "chain transplantation" of rediae of *Isthmiophora melis* from infected to uninfected snails, *Lymnaea stagnalis.* The experiment demonstrated that a great number of generations of rediae can proceed "ad infinitum", and thus "there appears to be no genetically programmed end to redial multiplication."

The above shows that there is a lack of systematic studies on the productivity of digenetic trematodes. That is probably due to the complexity of redial generations which are too massive to be traced one generation after another. The present work is an approach to the study of the reproduction and productivity of redial generations of *Clinostomum complanatum.* It is expected that through a systematic study of the characteristics of different generations and the size distribution of the redial population, some light may be thrown on the pattern of intramolluscan reproduction of rediae.

MATERIALS AND METHODS

Laboratory-bred pond snails, *Radix swinhoe* (H. Adams), ranging from 10 to 12 mm in shell length were each exposed to a single miracidium in a capsule containing 2 ml sterilized rain water at 27°–28°C for no more than one hour. The infected snails were then maintained at a temperature of 27°–29°C and fed with lettuce and rape. The water was changed every day. During the time of observation, one or two snails were dissected daily after infection. From the 7th day onward all the rediae harboured in the hepatopancreas of the hosts were counted. A random sample of 100 rediae were anaesthetized with 3% alcohol. All rediae within the sample were measured and dissected to determine the number of daughter rediae or cercariae within each redia.

Different methods were used to find the best fit to the given data of the redial productivity within the snail hosts:
The models used were:

(1) Logistic curve (Wan and Liang 1983). The general equation of the model is:

$$y = \frac{L}{1 + e^{a-bt}}$$

where y = the productivity of rediae on date t
t = the dates from the day when the mother redia starts to reproduce
L = the theoretical maximum of productivity in one day
a and b are paremeters.

(2) Gompertz growth curve (Winsor 1932). The general equation is:

$$y = La^{b^t}$$

where what y, L, t, a, b stand for are the same as in method (1).

(3) Mixed model of combining Gompertz growth curve and the AR model (the autoregressive equation, Yang and Gu, 1986) of the residual errors of the former.

The number of cercariae which emerged daily fom four *Radix* hosts with shell lengths ranging 10–13 mm was couted. The snails were each infected with a single miracidium, kept in a 100 ml beaker and fed with lettuce and rape. The water in each beaker, that contained cercariae, was collected separately once every day at 18.00 hr until the death of the host. The cercariae were fixed with 7% formaldehyde solution and were counted. The data of every two days were pooled and the averaged number was plotted against time.

RESULTS

The miracidim, after penetrating into the tissue of the mantle of the host, developed into a sporocyst and on the 4th day developed into a single mother redia. The newly emerged mother redia was transparent, cylindrical in shape and measured 0.16 mm in body length. Between the 7th and the 8th day it was detected that after infection a mother redia had migrated to the hepatopancreas and had grown rapidly to 0.900 mm. It gave birth to the first daughter redia of the second generation (Fig. 1). Meanwhile, in the body cavity of the mother redia, there were 10–19 rediae of different stages of development as well as embryos. On the 10th day a mother redia was found to have grown to 1.400 mm with 18 young rediae in the body cavity. The daughter rediae recovered from the hepatopancreas were 8–54 in number. The range of their body lengths, 0.15–1.02 mm, suggested that their births were continuous. By this time, the rediae of the second generation were already in the state of propagation, and 1–9 developing young rediae of the third generation were detected in their body cavities. The number of these young rediae correlated with the body length of the parent redia. On the 11th and the 12th day, the number of rediae in the hepatopancreas of the host increased to about 100 as a result of the continuous delivery of the daughter rediae by the mother redia and also because, by then, the second generation had begun to reproduce. The rediae, 0.15–0.35 mm in length, constituted 59.8–61.5% of the total population. At this time the mother redia showed signs of loss of vitality; its body wall became soft and torpid, brownish and dark grey in colour and it carried very few or no daughter rediae within its body cavity. The population now contained three distinct generations, the mother redia and the daughter rediae of the second and the third generation, with the sizes of the latter two groups overlapping each other.

The numbers of daughter rediae, mainly the third generation, increased considerably on the 13th day, the size class less than 0.30 mm constituting 44.4% of the total population. On the following day, the reproduction of daughter rediae showed a rapid increase and continued until the population increased 15.2 times the number produced on the 9th day. This rapid increase of the population of rediae was attributed mainly to the breeding activity of the second generation. When cercariae began to develop, the population was maintained by but a small number of "mixed" rediae. The "mixed" rediae contained 3–12 energetic rediae and 1–2 cercarial embryos which were recognizable by the pattern of their excretory system. By the 15th day the cercariae had nearly replaced the daughter rediae, the cercariae embryos having entered their later stages of development, with complete tail stems and furcae as well as brownish eye spots. Rediae less than 0.54 mm in length carried only undifferentiated embryos. On the 16th day, the number of cercariae increased conspicuously in the body cavity of the rediae whose length exceeded 0.80 mm, some of which were active with contractile bodies and typical jerking of the tail stems. A single redia of 1.0–1.3 mm in length might contain 17–43 well-developed cercariae.

From the 18th day onward, the succession of the redial population within the snail host was sustained by "mixed" rediae, even though they formed less than 1% of the population. One "mixed" redia measuring 1.10 mm in length contained 10 cercariae and a single redia. Other rediae above 0.40 mm in length, apparently rediae of the third generation, contained recognizable fish-shaped cercarial embryos.

The first cercariae were seen emerging from the host on the 19th day. Meanwhile, the rediae of the second

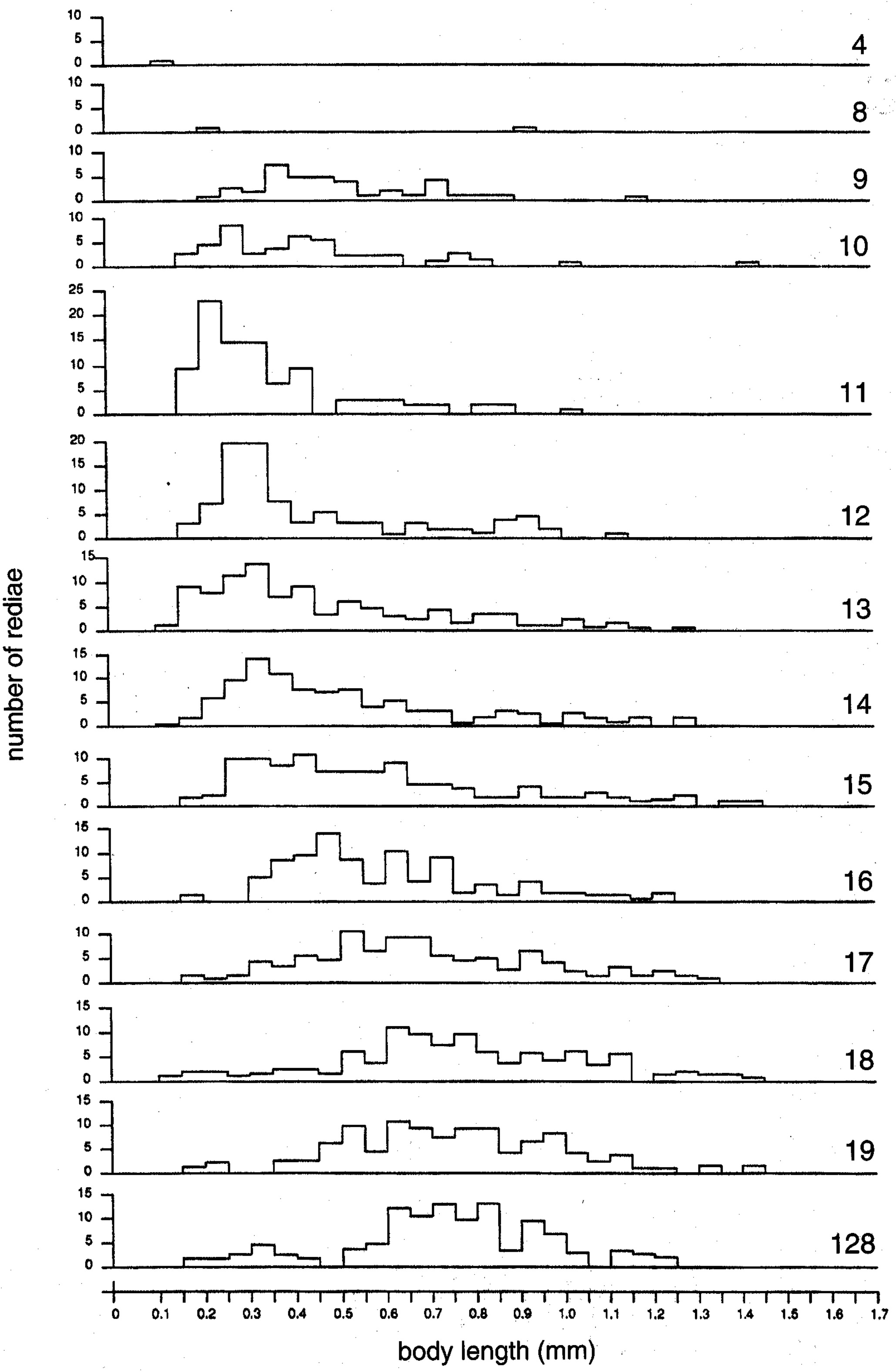

Fig. 1. The size distribution of rediae at 27°–29°C during the period between the occurrence of the mother redia (day 4), the first emergence of cercariae (day 19), and day 128 of observation. On the 4th, 8th, 9th and 10th days of observation the total number of individuals in the population was less than 50, the actual number of rediae being represented in the histograms. From the 11th day onward, a sample of about 100 individuals was measured and the relative number of rediae is represented as percentage (%).

generation continued to increase in size and the number of cercariae in their body cavities increased as well. One redia measuring 1.56 mm in length contained 59 well-developed cercariae. Rediae of a smaller size measuring 0.30 mm in length carried cercariae of different stages of development. At this stage, a few young daughter rediae were found in the population.

Dissection of infected sample snails was done every three days after the 20th day. Small numbers of newly emerged rediae were continuously recovered from the sample after the 26th day. The progeny of successive generations of rediae overlapped in size and it was almost impossible to distinguish the age groups by length. The propagative rediae became smaller and smaller in relation to time. The rediae of minimal size that produced cercariae measured 0.82 mm on the 18th day, 0.52 mm on the 26th day, 0.45 mm on the 39th day, and 0.31 mm on the 57th day. The last snail of this series was dissected on the 128th day after experimental infection. The size distribution of the rediae found in this snail is illustrated in the lowermost histogram in Fig. 1. In this snail, the number of cercariae in 34 rediae averaged 12.4.

The Gompertz curve has been used in the following works to express the gastric evacuation in the sandbar shark (Medved 1985), the secondary production of a mussel population (Craeymeersch et al. 1986), the seasonal functions of growth in length of the haddock (Sager et al. 1987) and the growth of juvenile Atlantic mackerel (D'Amours et al. 1990). In the present paper the Gompertz growth curve is fitted to the data of the productivity of the rediae within the snail hosts. The result is:

$$\hat{y}_t = 2165 \times 0.0292^{0.9419^t}$$

The error series [$\varepsilon t : \varepsilon t = y_t - \hat{y}_t,\ t = 0, 1, 2, \dots 53$] is used to create the AR model. t = 0, where 0 indicates the 8th day when the mother redia starts to reproduce. The result is:

$$\varepsilon t = 7.51 + 1.13\ \varepsilon_{t-1} + 0.27\ \varepsilon_{t-2}$$

The combination of the results yields the following mixed model:

$$y_t = 2165 \times 0.0292^{0.9419^t} + 7.51 + 1.13\ \varepsilon_{t-1} + 0.27\ \varepsilon_{t-2}$$

This model proved to be the best fitting among the different methods tried (Pan and Cai 1986; see Materials and Methods).

The number of cercariae emerged from four *Radix* hosts, as shown in Fig. 2, revealed a zigzag pattern. Cercariae averaged less than 370 on the first two days, increased steadily from the third day onward, and reached a peak over 3,000 for a brief period from the 5th to the 8th day. The highest record of cercariae emerging from a single snail was 6,025 on the 14th day. The number dropped to a level below 1,000 between the 44th day and the 60th day. This phenomenon coincided with the reproductive capacity of the second generation rediae during that period. The life span of *Radix* hosts, when confined to the conditions of a small container, lasted 39–78 days and the total number of cercariae produced by a snail ranged from 71,275 to 117,766.

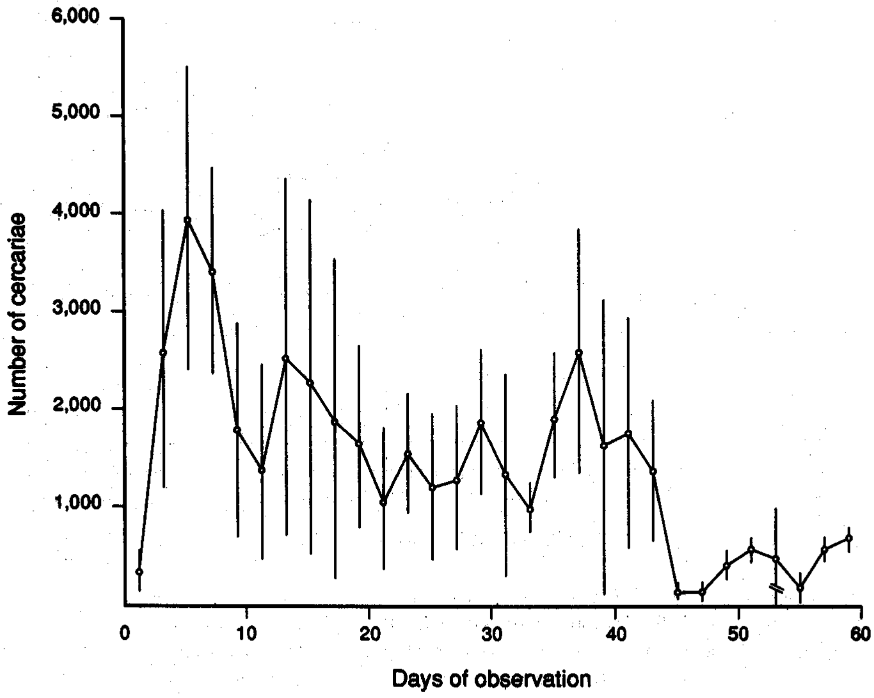

Fig. 2. Average emergence of cercariae per snail under confined laboratory conditions (N=4). Bars represent standard deviations.

DISCUSSION

The complexity of intramolluscan reproduction in digenea in which a single miracidium multiplies to produce large numbers of offspring, is generally acknowledged, and it is considered impossible to determine the generation number by direct observation (Dönges 1971). To solve the problem, Dönges developed the "chain-transplantation" technique to transplant single rediae of known generation number into uninfected snails. This technique enabled him to obtain rediae that passed through "a minimum of 42, but possibly 102, successive generations." The results tend to show that "there is no genetically programmed end to redial multiplication." However, observations carried out in the present experiment showed that these results do not give a true picture of the intramolluscan reproduction of digenetic trematodes under natural conditions. Continuous redial reproduction of successive generations in the same host inevitably increases the parasite burden of the host. Overcrowding may therefore result in competition for space and subsequently decrease natality. Hence reproduction is density-dependent (Kennedy 1977). The limited spacial capacity and nutrient resources of the host restrain to some extent the reproduction of the rediae and thus, the redial generation potential is not "*ad infinitum*". Self-regulating mechanisms may operate upon the reproductivity of the rediae and consequently the redial infra-population within the host is brought to a stable equilibrium.

As to the patterns of intramolluscan reproduction of digenetic trematodes, they are manifold: some consist of more than two generations of sporocysts and some of successive generations of rediae. Lin (1987), working on the life history of *Centrocestus formosanus* Nishigori (Trematoda: Heterophyidae), and Lü (1990), working on the life history of diplostomids, found that the reproduction of these trematodes followed the pattern of more than two generations of sporocysts. Though they did not follow up the actual number of generations of intramolluscan reproduction, their studies indicate that the sporocysts of these two trematodes form the key stage for the production of successive generations.

The present study shows that the reproduction of *Clinostomum complanatum* follows the pattern of successive generation of rediae. Like most digenean trematodes, it is characterized by polyembryony. The miracidium, after establishing its infection in an intermediate host, multiplies asexually into numerous offspring. The stake of intramolluscan reproduction lies in the multiplication of embryos within the rediae. It was noticed that a miracidium developed into a single mother redia through the sporocyst stage. It was the mother redia, instead of sporocysts, that produced solely daughter rediae. At first, the daughter rediae of the second generation produced rediae of the third generation to ensure the establishment of a redial population, and then some turned into "mixed" rediae which contained mainly young rediae and 1–2 cercarial embryos. From the third generation onward, there occurred "mixed" rediae of an entirely different nature, in which the body cavity of these rediae contained mainly cercariae and 1–2 young daughter rediae, with a few possessing as many as 5-8. This particular mode of reproduction is characterized by self-regulation and it plays an essential role in the long term in making it possible for the rediae to attain a state of stability through affecting their population processes of natality and mortality. Though the "mixed" rediae show a very low potentiality in producing rediae, they produce large numbers of cercariae on the one hand and maintain the succession of redial population on the other as long as the infected intermediate host survives.

The daily emergence of cercariae from a snail host occurred in a zigzag pattern. As shown in Fig. 2, after the first emergence of cercariae a major peak occurred on the 6th day and several minor peaks fell on the 14th, 16th, 24th, 29th and 38th days respectively. The first batch of cercariae were released by the first rediae of the second generation which harboured the maximal number of cercariae. The peak of cercarial emergence declined gradually to a valley of low emergence on the 11th day. The second peak, a minor one, seemed to coincide with the cercarial production by the daughter rediae of the third generation. Other peaks of cercarial emergence after the 24th day could not be traced with respect to redial reproduction. The progeny of several generations of rediae might overlap, so it was impossible to distinguish them from one another with certainty. Between the 44th and the 60th day, the number of cercariae dropped to a level below 1,000. The decline indicates the inadequacy of nutrient resources provided by the hosts. It also shows that the size of rediae that produced cercariae was getting smaller and smaller until 56 days later when a great majority of the experimentally infected hosts died.

Acknowledgement. I would like to thank Professor Claude Combes of the University of Perpignan for reading my manuscript, helpful comments, and especially for his kindness in redrawing the figures.

REFERENCES

CORT W. W., AMEEL D. J., VAN DER WOUDE A. 1950: Germinal material in the rediae of *Clinostomum marginatum* (Rudolphi). J. Parasitol. 36: 157–163.

CRAEYMEERSCH J. A., HERMAN P. M. J., MEIRE P. M. 1986: Secondary production of an intertidal mussel *(Mytilus edulis* L.) population in the Eastern Scheldt (S. W. Netherlands). Hydrobiology 133: 107–115.

D'AMOURS D., LANDRY J. G., LAMBERT T. C. 1990: Growth of juvenile (0-group) Atlantic mackerel *(Scomber scombrus)* in the Gulf of St. Lawrence. Can J. Fish. Aquat. Sci. 47: 2212–2216.

DINNIK J. A., DINNIK N. N. 1956: Observations on the succession of redial generations of *Fasciola gigantica* Cobbold in a snail host. Z. Tropenmed. Parasitol. 7: 397–419.

DINNIK J. A., DINNIK N. N. 1957: Development of *Paramphistomum sukari* Dinnik, 1954 (Trematoda: Paramphistomidae) in a snail host. Parasitology 47: 209–216.

DÖNGES J. 1971: The potential number of redial generations in Echinostomatids (Trematoda). Int. J. Parasitol. 1: 51–59.

DÖNGES J., GÖTZELMANN M. 1988: Digenetic trematodes: multiplication of the intramolluscan stages in some species is potentially unlimited. J. Parasitol. 74: 884–885.

EDNEY J. M. 1950: Productivity in *Clinostomum marginatum* (Trematoda: Clinostomatidae). Trans. Am. Microscop. Soc. 69: 186–188.

KENNEDY C. R. 1977: The regulation of fish parasite population. In: C. W. Esch (Ed.), Regulation of Parasite Populations. Academic Press, Inc., New York, London, San Francisco, pp. 63–109.

LIM H. K., LIE K. J. 1969: The redial population of *Paryphostomum segregatum* (Trematoda: Echinostomatidae) in the snail *Biomphalaria glabrata.* Z. Parasitenk. 32: 112–119.

LIN K. B. 1987: Studies on the life history and bionomics of *Centrocestus formosanus* (Nichigori, 1924). M. Sc. Thesis, Zhongshan University, pp. 23–25. (In Chinese, with English Summary.)

LÜ J. Y. 1990: Studies on the life history of *Diplostomum neidashui* Pan et Wang and *D. hupehensis* Pan et Wang. I. Description of the early larvae and adults. Arch. Hydrobiol. 14: 265–273.

MEDVED R. J. 1985: Gastric evacuation in the sandbar shark, *Carcharhinus plumbeus.* J. Fish Biol. 26: 239–253.

PAN X. H., CAI W. P. 1986: The curve-fitting of redial productivity of *Clinostomum complanatum* (Rud.) within snail hosts. B. Sc. Thesis, Zhongshan University, pp. 1–12.

SAGER G., BERNER M., SAMMLER R. 1987: A comparison of seasonally modified functions of growth in length demonstrated for the haddock *(Melanogrammus aeglefinus* Gill) of the south-eastern North Sea after data series from Thompson (1922-1927). Fisch.-Forsch. 25: 14–19.

WAN C. X., LIANG Z. Y. 1983: A method fitting the logistic curve. Acta Ecol. Sin. 3: 288–296. (In Chinese, with English Summary.)

WINSOR C. P. 1932: The Gompertz curve as a growth curve. Proc. Nat. Acad. Sci. 18: 1–7.

YANG W. G., GU F. 1986: Time series analysis and modeling of dynamic data. Beijing Industry Institute Press, pp. 117–126. (In Chinese.)

ZISCHKE J. A. 1967: Redial population of *Echinostoma revolutum* developing in snails of different sizes. J. Parasitol. 56: 1200–1204.

Received 21 September 1992

Accepted 17 November 1993