PRODUCTION AND MAINTENANCE OF LARGE NUMBERS OF *DUGESIA TIGRINA* (TURBELLARIA: TRICLADIDA) FOR THE CONTROL OF MOSQUITOES IN THE FIELD

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ABSTRACT. Methods to increase and preserve *Dugesia tigrina* for mosquito control purposes through cocoon production, mechanical sectioning and cold storage were examined. Cocoon production was an ineffective and unreliable means of increasing planarian numbers, with an average of only 0.19 cocoons containing 0.74 young produced per adult per week. In comparison, mechanical sectioning proved to be more appropriate for increasing the number of planaria. One hundred planaria averaging 8 mm in length could be sectioned into 600 segments in approximately 18 min; regeneration was achieved by approximately 94% of these segments, usually within 8 days after sectioning. Techniques were developed to store planaria at 10°C until needed for field release.

INTRODUCTION

Planaria (*Dugesia* species) have demonstrated their ability to control pestiferous mosquitoes in a wide variety of habitats (Legner and Medved 1974, Levy and Miller 1978, Ali and Mulla 1983, George et al. 1983). At times, these populations of planaria need to be artificially increased so that mosquito control can be achieved (Legner et al. 1975, Nelson 1979). Because producing and maintaining large numbers of planaria in the laboratory for later field release is typically very time consuming, an important objective would be to increase planarian numbers while reducing the amount of care involved.

Legner et al. (1975) reported that planaria cocoons (which contain up to 20 eggs) "can be stored and disseminated with the convenience currently possible with insecticides." Nelson (1979) felt that mechanical sectioning would be a viable means if several hundred planaria sections could be produced per hr. *Dugesia dorotocephala* (Girard) placed in cold storage at 10°C for 6 months experienced little mortality but had a large decrease in body length from an average of 12.4 to 5.1 mm (Legner and Tsai 1978).

The purpose of this study was to evaluate and modify existing methods and develop new ones to increase the feasibility of using planaria in the biological control of mosquito larvae. Three means of achieving this goal were examined: 1) cocoon production, 2) mechanical sectioning and 3) cold storage of planaria.

MATERIALS AND METHODS

Dugesia tigrina for a laboratory colony were collected from water lettuce (*Pistia stratiotes* L.) and water hyacinth (*Eichhornia crassipes* (Mart.) Solms.) in unreclaimed phosphate pits in Polk County, Florida from November 13, 1986 to January 14, 1987. They were placed in 33 × 22 × 5 cm enamel pans in tap water aged for 24 hr. Styrofoam packing chips were placed in the pans for planaria resting sites and cover. Plywood pieces (30 cm^2) were placed on top of the pans to darken the interior. Twice a week, the planaria were allowed to feed on beef liver for 2 hr, after which they were transferred to pans with fresh water.

Cocoons: From February 11 to 25, 1987, large, thick-bodied planaria deemed most apt to be the cocoon-producing, sexual variety [based on the description by Jenkins (1974)] were removed from the above collection. These planaria (F_1) were isolated in a separate enamel pan with styrofoam chips. Twice each week, beginning February 25, 1987 and ending April 22, 1987, the chips containing the attached cocoons were removed and isolated, according to collection date, in 150 ml polystyrene cups with darkened lids. Young planaria (F_2) which emerged from the cocoons were retained in the cup until mature. Twice each week, they were allowed to feed for 2 hr and then transferred to cups with fresh water.

On May 4, 1987, all F_2 planaria were combined into one enamel pan. This group of planaria produced a large number of cocoons and several young planaria (F_3). The F_1, F_2 and F_3 planaria were combined into one pan on August 1, 1987 and served as the sexual variety stock culture.

Cocoon production declined for several months. When it resumed in early autumn, the cocoons were isolated in one enamel pan and counts made of the emerging young until March 3, 1988. Although cocoon production continued, the study was terminated.

Mechanical Sectioning: Sectioning experiments were conducted to determine 1) the percentage of individuals that could achieve com-

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plete regeneration, 2) the development rate of the different segments, 3) the frequency with which planaria could be sectioned and 4) the number of planaria segments that could be easily produced per hr.

Asexual *D. tigrina* greater than 8 mm in length were used for the sectioning studies. These planaria were not fed for at least 48 hr prior to sectioning to reduce the chance of infection and increase viability (Coward et al. 1964, Montgomery and Coward 1974, Cowden 1982). The sterile conditions/procedures of Coward et al. (1964) and Montgomery and Coward (1974) were not imposed in these studies, in order to further reduce labor. Three days after sectioning, food was offered to all groups twice each week for 2 hr at a time, after which the planaria were transferred to cups with fresh water. The sectioned and unsectioned planaria were kept at room temperature, which ranged from 22 to 27°C during these studies.

Planaria were collected from the asexual stock pan with a plastic pipet and placed in groups of 10–20 in a small pool of water on a 25 cm^2 sheet of Plexiglas. The planaria were sectioned with a square cover slip (No. 0) as they glided around in the water, then rinsed into another pan with aged tap water.

The sectioning process was easily achieved by simply placing an edge of the cover slip on the planaria and gently pressing down; a slicing motion was not necessary. When sectioning into 3 or more pieces, it was easier to make the initial cut nearer the tail, since a cut nearer the head end caused the remaining tail section to temporarily curl up and momentarily impede the next cut.

The development of 2 eyespots was used as evidence of the completion of normal development or total regeneration of a segment into an individual planarian (Montgomery and Coward 1974).

The first experiment, conducted for 59 days, had 4 replicates each of 4 head segments, 4 tail segments, 2 head and 2 tail segments and 2 unsectioned *D. tigrina* per cup. The sectioned and unsectioned planaria were isolated in 150 ml of aged tap water in 200 ml capacity polystyrene cups with darkened lids and styrofoam chips.

In the second experiment, three replicates of 50 *Dugesia tigrina* were sectioned into 2 segments each (group 1). After 33 days, 50 of the newly formed planaria from each group 1 replicate were sectioned into 2 segments each (group 2). After 16 days, 50 of the regenerated planaria from each replicate in group 2 were sectioned into 2 segments each (group 3). Eight days later, 50 of the planaria from each replicate of group 3 were sectioned into 2 segments each (group 4). Four days after the final sectioning, the group 4 planaria were examined for completion of regeneration.

In the third experiment, 100 *D. tigrina* (12–18 mm in length) in 8 replicates were each cut transversely into 3 segments. An equal number of planaria were sectioned into 6 pieces each. The amount of time required to complete sectioning of each group was recorded and the average number of planaria sections that could potentially be produced in 1 hr was calculated.

Cold Storage: In the first experiment, 3 replicates of 10 asexual *Dugesia tigrina* 10+ mm in length were maintained at 10 ± 2°C in an incubator in loosely covered cups of aged tap water. Planaria were removed from cold storage ca. every 33 days, warmed to room temperature, fed, cleaned, counted and then returned to cold storage. Seven such cycles were completed between March 13 and November 4, 1987 (231 days).

In the second experiment, planaria were held in the incubator for 54 days, then removed and their condition evaluated. After an additional 99 days in cold storage, they were removed and examined again. For this 153-day study, 3 replicates of 25 *D. tigrina* 15+ mm in length, 4 replicates of 25 *D. tigrina* 5–15 mm in length and 2 replicates of a mixed group of 25 head and tail segments from planaria greater than 15 mm in length were used.

Analysis: Statistical analyses were conducted on an IBM PC XT using the SPSS/PC+ statistical program (Norusis 1986). Log transformations were used to normalize the data prior to analysis of variance (ANOVA) at the $P \leq 0.05$ level.

RESULTS AND DISCUSSION

Cocoons. Cocoon production by the F_1, F_2 and F_1-F_2-F_3 groups was fairly constant throughout the year, with the highest rate (0.23 cocoons/planaria/week) occurring from early November to early March (Table 1). However, during this same period, the number of young emerging per cocoon was at its lowest level (0.13 young/cocoon). In comparison, the number of young produced per cocoon was almost 15 times greater during late February through late April; the survival rate of the offspring produced during this period was also high (Table 1). Although some asexual reproduction occurred throughout the year, the greatest number of fission products were found during September and October, when sexual production was at its lowest level.

Dugesia tigrina can reproduce both sexually and asexually (Pennak 1978, Benazzi and Gremigni 1982), depending on the genetic or physiological strain and the prevailing ecological conditions. One physiological variety reproduces

Table 1. Cocoon production by the three groups of *Dugesia tigrina*.

	Groups		
	F_1	F_2	F_1-F_2-F_3
Period	Late Feb.–late April	Early May–late June	Early Nov.–early March
No. of weeks	8.0	12.6	12.0
No. of planaria	17	36	61*
No. of cocoons produced	23	74	171
Mean no. cocoons/planaria/week	0.17	0.16	0.23
Mean no. offspring/cocoon	1.91	0.19	0.13
Offspring			
No. produced	44	14	22
Survival (%)	81.8	14.3	95.5

* Includes 2 F_1 and 4 F_2 planaria produced asexually.

only by asexual means. Another strain reproduces asexually during part of the year and sexually for the remainder; low water temperatures may cause sexual reproduction in this strain throughout the year. The third type reproduces sexually, regardless of the normal seasonal temperature variations. The *Dugesia* used in this study apparently belonged to the strain with seasonal alternation of sexual and asexual phases. The relatively low temperatures in the laboratory (averaging 25°C) apparently stimulated the almost year-round production.

Cocoon production does not appear to be an economical method for increasing *D. tigrina* numbers. During the cocoon-producing season, a certain percentage of cocoons may be sterile, especially those produced at the beginning and end of each cycle (Reynoldson 1968, Benazzi and Gremigni 1982); there was evidence of this occurring in this study. Each cocoon contains only a small number of eggs (2–20), and the development of the embryo in the egg is relatively slow, especially when compared to the potential of asexual reproduction. While the *D. tigrina* in this study produced an average of 0.19 cocoons per adult per week, Jenkins (1974) reported that *D. dorotocephala* produced up to 2.76 cocoons per adult per month, and *D. lugubris* produced approximately 0.94 cocoons/planaria/month.

It requires considerable effort to induce sexual reproduction, collect and store cocoons and disseminate them in known mosquito larval habitats, and the inability to predict the viability of the cocoons or the number of juvenile planaria that will successfully hatch severely restricts the applicability of this method. There is a very real possibility that mosquito control cannot be attained because of low numbers of planaria emerging from the cocoons.

Mechanical Sectioning: Planaria numbers increased from 48 head, tail and mixed head/tail segments to 120 completely developed planaria,

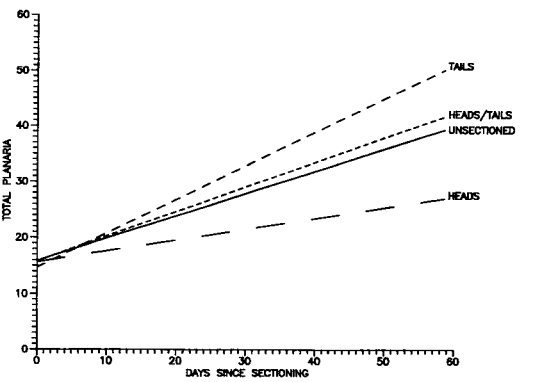

Fig. 1. Increase in total numbers of completely developed planaria produced by groups of unsectioned planaria and by groups of head segments, tail segments and combined head/tail segments. Linear regression equations used to plot best-fit lines are: Y = 0.196X + 15.661 (heads); Y = 0.444X + 15.802 (heads/tails); Y = 0.608X + 14.667 (tails); Y = 0.402X + 15.861 (unsectioned).

or a 150% increase. The number of planaria produced by the tail segments was the highest of all the sectioned groups, increasing 225% during the 59-day experiment (Fig. 1). Production by the head segments was significantly lower ($P = 0.005$) than that of the other 3 groups and less than half that of the tail segments. Nelson (1979) also found that the number of planaria developing from head sections was less than that produced by other sections.

As expected, the number of complete planaria produced by the group of combined head and tail segments was not significantly different ($P = 0.725$) from that produced by the unsectioned planaria, a conclusion also reached by Legner et al. (1976).

Regeneration of the segments into fully developed planaria was complete at the end of the 33-, 16-, and 8-day intervals. When the segments

in group 4 were examined only 4 days after sectioning, 69.7% of the segments had regenerated. Most of the head segments had fairly well defined tails, but many of the tail sections had poorly defined heads and few had eyespots. Eight days after group 4 was sectioned, 99.8% of the segments had completed development. Nelson (1979) also found that head sections developed into complete planaria at a faster rate than tail sections. These results indicate that planaria could be sectioned on a weekly basis. A regeneration rate of approximately 94% in these sectioning experiments agrees with Nelson (1979) and indicates that planaria sectioning need not be done under aseptic conditions.

The dissecting microscope used by Nelson (1979) and Cowden (1982) does not appear to be necessary for mass production purposes when planaria are sectioned into ca. 2 mm^2 pieces as done here. Fragments of *D. dorotocephala* as small as 0.08 mm^3 have been found to be capa- of complete regeneration (Montgomery and Coward 1974), but if smaller sections are desired, a dissecting microscope would be necessary.

An average of 16 min was required to section 100 planaria approximately 8 mm in length into 300 segments and approximately 18 min to section 100 planaria into 600 segments using a cover slip. At the higher rate, one person could produce almost 2,000 segments in 1 hr. Successful control of mosquitoes has occurred at stocking rates of 25 planaria/m^2 (101,175/acre) (Legner et al. 1975, Yu and Legner 1976, Legner 1977). In just 12.5 hr, which can easily be divided into 2–3 sessions each week, enough planarian segments can be produced for treatment of a 1,000 m^2 (0.25 acre) area of mosquito breeding habitat.

A biological filtration system (Legner and Tsai 1978) has been used to almost triple the reproductive rate of *D. dorotocephala*. This system could be used to rapidly produce large numbers of planaria, which could then be mechanically sectioned to further increase production. The numbers required to treat an area would thus be achieved at an even faster rate than possible with only natural fissioning, mechanical sectioning or the filtration system.

Cold Storage: There were only 2 (6.7%) deaths among the 30 planaria that were stored at 10°C for 231 days (Table 2). During this experiment, the planaria decreased in size from an average of 10.4 mm to approximately 5.6 mm in length. This 46.2% decrease compares favorably to the 58.9% decrease observed by Legner and Tsai (1978) in a 6-month storage study in which *D. dorotocephala* were warmed to room temperature every 2 months but were not fed. Feeding the planaria at monthly intervals may have helped reduce the decline in body size experienced by the *D. tigrina* in this study.

The larger planaria experienced a lower mortality rate and a lower percentage decrease in body length than the medium-sized planaria when stored for 153 consecutive days at 10°C (Table 3). In contrast to these 2 groups of unsectioned planaria, 92.0% of the segments died in storage. Results of this experiment are analogous to those reached by Reynoldson (1968) in which specimens of *D. lugubris* 15 mm in length survived without food for 41 weeks, whereas smaller specimens approximately 2.3 mm in length lived for only 15 weeks under similar conditions. This relationship between initial size and survivorship probably relates to nutrient and energy reserves; the reserves were not adequate to sustain the segments or to allow sufficient regeneration while they were in storage.

Table 2. Results of storing 30 *Dugesia tigrina* for 231 days (7 cycles of 31–42 days each) at 10 ± 2°C.

Phase	Total days in storage	Condition at end of phase: No. of live planaria	Condition at end of phase: Mean length (mm)
0	—	30	10.4
1	31	30	10.0
2	62	30	9.4
3	93	30	8.7
4	128	29	7.9
5	165	29	7.2
6	189	29	6.4
7	231	28	5.6

Table 3. Mortality and decrease in body length of *Dugesia tigrina* held for 153 days in an incubator at 10 ± 2°C.

	No. of planaria			Mean body length (mm)		
	Day 1	Day 153	Mortality (%)	Day 1	Day 153	Decrease (%)
Large (>15 mm) (3 reps)	75	73	2.7	17.2	7.5	56.4
Medium (5–15 mm) (4 reps)	100	92	8.0	12.7	5.5	56.7
Head and tail segments (2 reps)	50	4	92.0	8.4	3.8	54.8

Even though a large decrease in body length was experienced in both of the storage experiments, it was not deemed detrimental to a mass production program for several reasons. Meyer and Learned (1981) and George et al. (1983) determined that body size is not important in relation to the ability of a planarian to attack or consume mosquitoes. Small planaria are just as capable of reducing larval populations as the larger planaria. Secondly, after approximately 45 days at room temperature and twice-weekly feedings, the planaria in both experiments returned to 8+ mm in length. If larger planaria are desired for some reason, they can be developed without too much time and effort involved. Finally, Benazzi and Gremigni (1982) stated that: "Through starvation, adults may be reduced to the size of very young worms; and evidence has been obtained that they actually become 'young' again. Therefore, starvation may act like fissioning or artificial division of the body, which is a very effective method of increasing specimen populations."

CONCLUSION

Cocoon production by sexual strains of *Dugesia tigrina* does not seem to be an efficient method to increase planarian numbers. Although sexual strains of *D. tigrina* may be more prevalent than previously thought, the production, development and hatching of young from these cocoons is too sporadic to be useful. However, mechanical sectioning appears to be an excellent means of enhancing planaria population increases normally produced through natural fissioning. Coupled with the rapid rate of complete regeneration (ca. 8 days in this study) exhibited by *Dugesia*, a continuous supply of planaria for stocking purposes is practically ensured.

Preservation in cold storage also appears to be a useful tool for mass production purposes since large numbers of planaria can be produced and stored at low temperatures for several months until needed for stocking. While periodic feeding may help reduce declines in body length and mortality, this is offset by the time and effort required to feed the planaria. In fact, starvation is believed to enhance reproduction and may actually be the condition in which planaria should be maintained.

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