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# The Transmission of *Parafilaroides decorus* (Nematoda: Metastrongyloidea) in the California Sea Lion (*Zalophus californianus*)[1]


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ABSTRACT: *Parafilaroides decorus* (Dougherty and Herman, 1947) was found in 10 of 14 California sea lions (*Zalophus californianus* Lesson, 1828) examined over a 9 month period. Eight species of mollusc, one species of copepod, and a teleost fish (*Girella nigricans*) from contaminated pools at the San Nicolas Island rookery were examined for third-stage larvae. Larvae were found only in *Girella nigricans*. *G. nigricans* collected from an uncontaminated area were fed sea lion excrement containing first-stage larvae and the larval development was followed over a 36 day period. The first moult occurred in the intestinal mucosa and sub-mucosa 12–15 days post-infection; the second occurred in the intestinal serosa and mesenteric adipose tissue 25–36 days post-infection. Infected fish were fed to a young uninfected California sea lion. First-stage larvae appeared in its feces 21 days later.

This is the first mode of transmission of a member of the Pseudaliidae in marine mammals to be elucidated, and the first metastrongyloid life cycle wherein a vertebrate intermediate host has been found.


Each spring along the Southern California coast, as well as in amusement parks and research facilities throughout the world, large numbers of wild and captive California sea lions (*Zalophus californianus* Lesson, 1828) die from lungworm infection. This high mortality rate in valuable show and research animals has prompted this study.


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## Materials and Methods

Adult worms and first-stage larvae for identification and morphological studies were recovered from 10 infected California sea lions examined over a nine-month period (22 March 1968 to 12 November 1968). Lungs and trachea were removed intact. Lungs were minced and soaked in flasks with sea water. Adult worms settled to the bottom of the flask and were collected. First-stage larvae, abun-

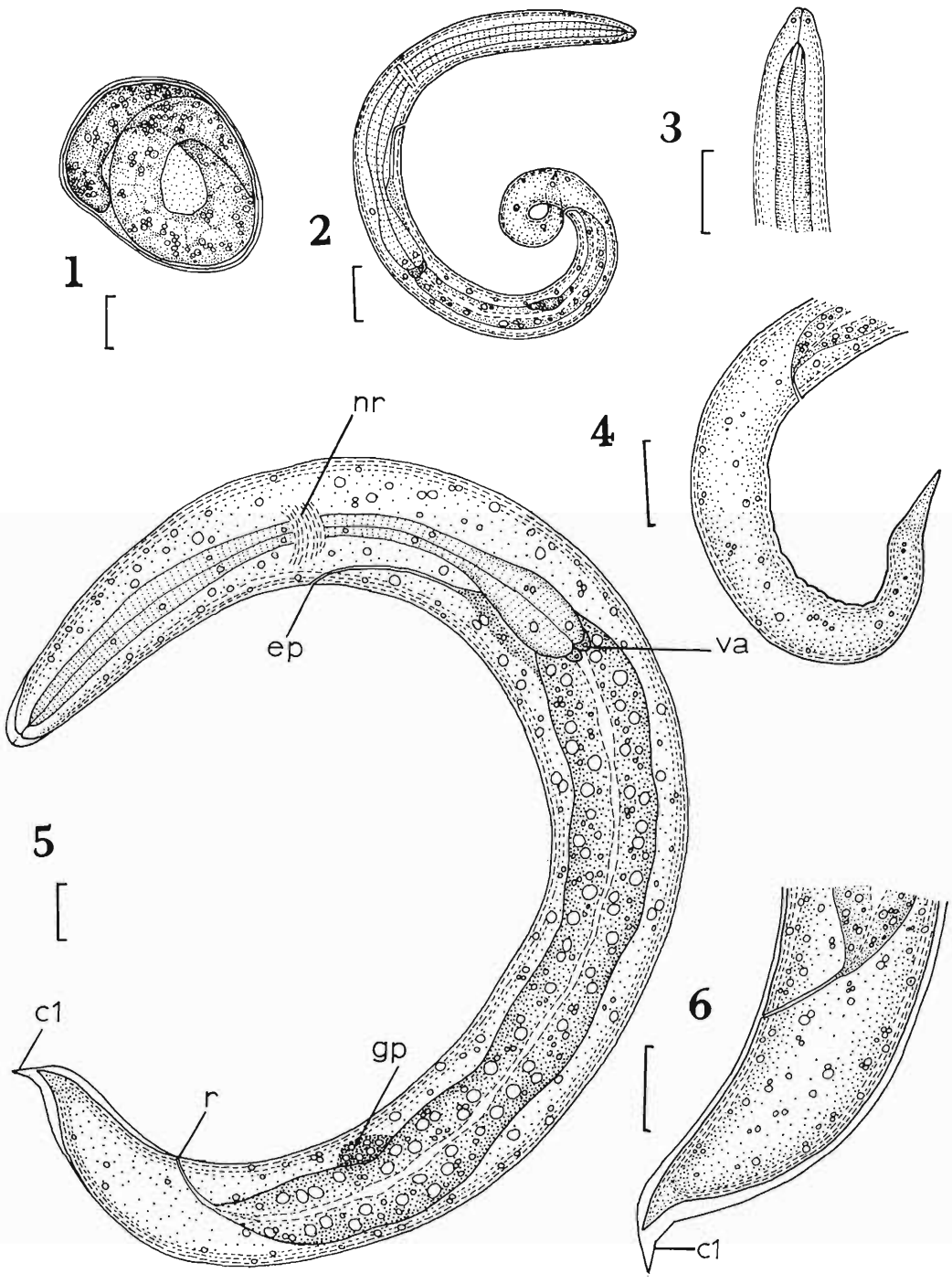

**Table 1. Measurements of larval stages of *P. decorus*.**

| Stage | 1 | | 2 | 3 | |
|---|---|---|---|---|---|
| Number examined | 10 | | 1 | 10 | |
| Length | 240–279 | (260) | 341 | 329–384 | (352) |
| Width | 13–18 | (15) | 25 | 18–22 | (20) |
| Esophagus length | 116–123 | (119) | 123 | 108–125 | (117) |
| Nerve ring—head | 64–72 | (67) | 64 | 54–61 | (56) |
| Excretory pore—head | 68–75 | (73) | 69 | 66–72 | (69) |
| Genital primordium—head | 169–190 | (182) | 238 | 245–315 | (270) |

dant in the mucus of the bronchi and trachea, were also collected.

Two trips were made to the sea lion rookery on San Nicolas Island, California to collect various possible intermediate hosts of *P. decorus* for examination. Molluscs, copepods and fishes were collected from clean pools and pools contaminated with feces.

Molluscs from contaminated pools were identified, minced and Baermannized in acidulated pepsin solution at 37 C (Cable, 1958). The Baerman fluid was examined for larvae. Molluscs from uncontaminated pools were identified, isolated in battery jars with filtered sea water, and kept in a cold water bath (16 C). Mucus from bronchial scrapings and fresh fecal material, both containing first-stage *P. decorus* larvae, were added to each jar. Snails were exposed to larvae for 24 hr, and were examined for infection 30 days later following pepsin digestion.

Copepods from contaminated pools were squashed under coverslips and examined for larvae under the compound microscope. Copepods from uncontaminated pools were placed in culture dishes and exposed to feces and mucus containing first-stage larvae. Exposed copepods were similarly examined for larvae 24, 48, and 72 hr later.

*Girella nigricans*, the "opaleye," a member of the Girellidae or "nibbler" family, were collected from contaminated pools. This fish is an omnivorous scavenger that feeds actively on fecal material. One experimentally infected fish was examined every 24 hr for the first six days and one every three days thereafter. The digestive tract was removed and placed in sea water. Small pieces of the intestinal wall just behind the pyloric ceca were pressed between slides and examined under stereoscopic and compound microscopes for *P. decorus* larvae. When larvae were found, the first 15 mm of intestine was cut into three segments, of which one was fixed in Bouin's fixative for sectioning, one was digested in pepsin and one teased apart in sea water.

Larvae were immobilized by mild heat, and covered with a vaseline-ringed coverslip for study. Permanent whole mounts were made in glycerine or glycerine jelly. Tissues for sectioning were fixed in Bouins fluid, embedded in paraffin, cut at 8 $\mu$ and stained with hematoxylin-eosin. Sections were mounted in Piccolyte resin. Measurements are in microns unless otherwise indicated; ranges are followed by averages.

## Results

### Incidence of *P. decorus* in sea lions

Fourteen immature sea lions of undetermined sex, 3 months to 2 years old, were examined over a 9 month period. All were taken in southern California: six at Point Mugu, seven at San Nicolas Island, and one at Seal Beach. Infections, four of which were massive, were found in 10 of 14 (71%) sea lions examined. In the massive infections the lung surface was covered with small yellowish pustules each of which yielded worms when opened.

### Examination of possible intermediate hosts

All sea lions examined were infected with the lung mite *Orthohalarachne diminuata* (Doetschman, 1944) Newell, 1947. No larvae

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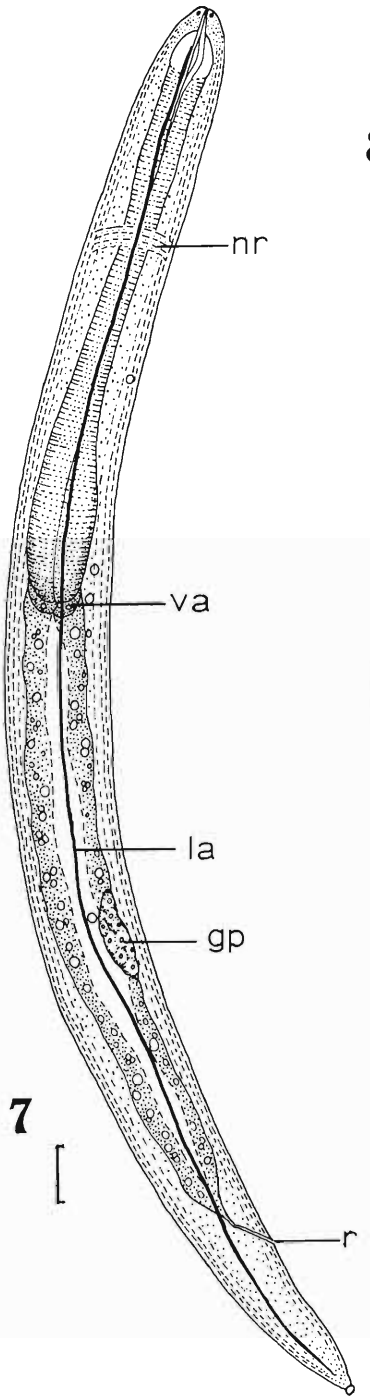
**Figures 1–6.** ***Parafilaroides decorus.*** **1. Egg. 2–4. First-stage larva. 2. Lateral aspect. 3. Anterior extremity, dorsal aspect. 4. Posterior region, showing spike-like tail. 5–6. Second-stage larva. 5. Lateral aspect. 6. Caudal extremity.**

**Scales: Bars = 12 $\mu$ (Figs. 1–4); 10 $\mu$ (Figs. 5–11); 20 $\mu$ (Figs. 12–17).**

**Abbreviations: $C_1$, cuticle of first stage. $C_2$, cuticle of second stage; ep, excretory pore; gp, genital primordium; la, lateral alae; nr, nerve ring; r, anus; va, esophagointestinal valve.**

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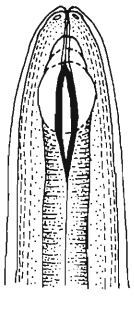

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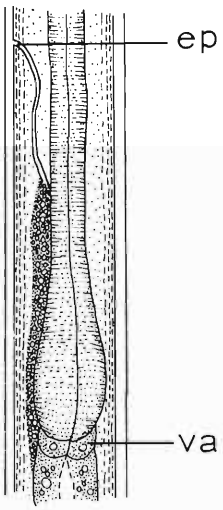


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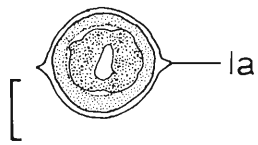


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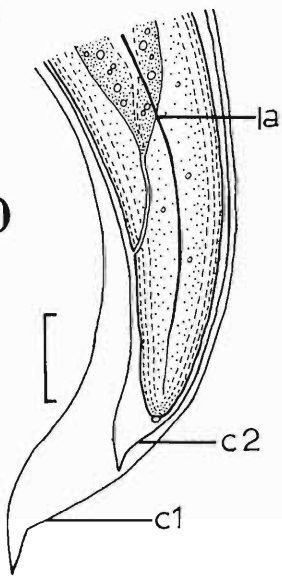


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were found in 50 mites examined although they occur together with first-stage larvae in the mucus of the bronchioles and trachea.

Two hundred and fifty specimens of each of the following mollusc-species from contaminated pools were examined: *Littorina planaxis* (periwinkle), L. *scutulata* (periwinkle, *Tegula funebralis* (turbin snail), *T. brunnea* (turbin snail), *Acamaea* sp. (limpet), *Mytilus edulis* (bay mussel), *M. californianus* (California sea-mussel), *Haliotis cracherodii* (black abalone). Examination revealed no infections. Ten specimens of each species, exposed experimentally to first-stage larvae from infected sea lions were likewise negative.

Larvae were not found in 55 *Trigriopus californianus* (splash pool copepod) collected from contaminated pools. First-stage larvae of *P. decorus* ingested by clean copepods collected from uncontaminated pools died within 48 hr after ingestion.

All of 12 *G. nigricans* from contaminated pools contained numerous larval nematodes, surrounded by a dark red pigment, and located under the intestinal serosa and in the mesenteric fat. They had the characteristics typical of infective larvae of other metastrogyloids, e.g. *Perostrongylus pridhami* (Anderson, 1962), *Trilobostrongylus bioccai* Anderson, 1963. They became active in 15–20 seconds when exposed to artificial digestion at 37 C.

## Experimental infection of *Girella nigricans*

Sixteen *G. nigricans* were collected from uncontaminated pools at San Clemente, California, where sea lions are not common. Ten others previously examined from these pools contained no larvae similar to those found in the specimens from San Nicolas Island. The test fish were fed fecal material from a sea lion previously determined by fecal examination to be heavily infected; all became infected.

Twenty-four hours after infection, first-stage larvae were still in the lumen of the intestine, usually near the mucosa (Fig. 12). By 3 days, larvae had penetrated deep into the mucosa and submucosa (Fig. 13). Five to 12 days after infection they were found immediately under the longitudinal muscle layer of the intestine (Fig. 14). The first moult apparently took place 12–15 days after infection. By 18 days, second-stage larvae were found in the longitudinal muscle layer and beneath the circular muscles (Fig. 15); 12–21 days after infection, some had been walled off by host response and appeared dead (Fig. 16); after 21 days, surviving larvae were found either in the serosa or in the mesenteric adipose tissue. The second moult took place 24–36 days after infection; only one third-stage larva was found at 24 days, while approximately half of the larvae found on day 30 had completed the second moult. By 36 days only third-stage larvae were found, and all were in either the serosal lining or the mesenteric fat (Fig. 17).

To investigate the possibility that fish may serve as transport hosts, 20 pieces of intestine, each estimated to contain approximately 200 third-stage larvae were fed to each of 10 previously unexposed *G. nigricans* and *Fundulus parvipinnis* Girard. The fish were examined after 14 days, but no larvae were found.

## Experimental transmission to sea lion

Fecal samples from captured sea lions waiting shipment at *Sea Lions International*, Santa Barbara, California were examined for *P. decorus* over a seven day period. Two uninfected yearlings were selected and transferred to the Marine Bioscience Facility at Point Mugu, California, where they were examined daily for *P. decorus* larvae for an additional 14 days. None was found. One animal was fed experimentally infected *G. nigricans*, while the control received noninfected synthetic food.

The control animal died from undetermined causes on day 12. A thorough necropsy revealed no larval or adult *P. decorus*. No changes in activity were noted in either animal following the feedings. The experimentally infected sea lion began to pass larvae 21 days after infection.

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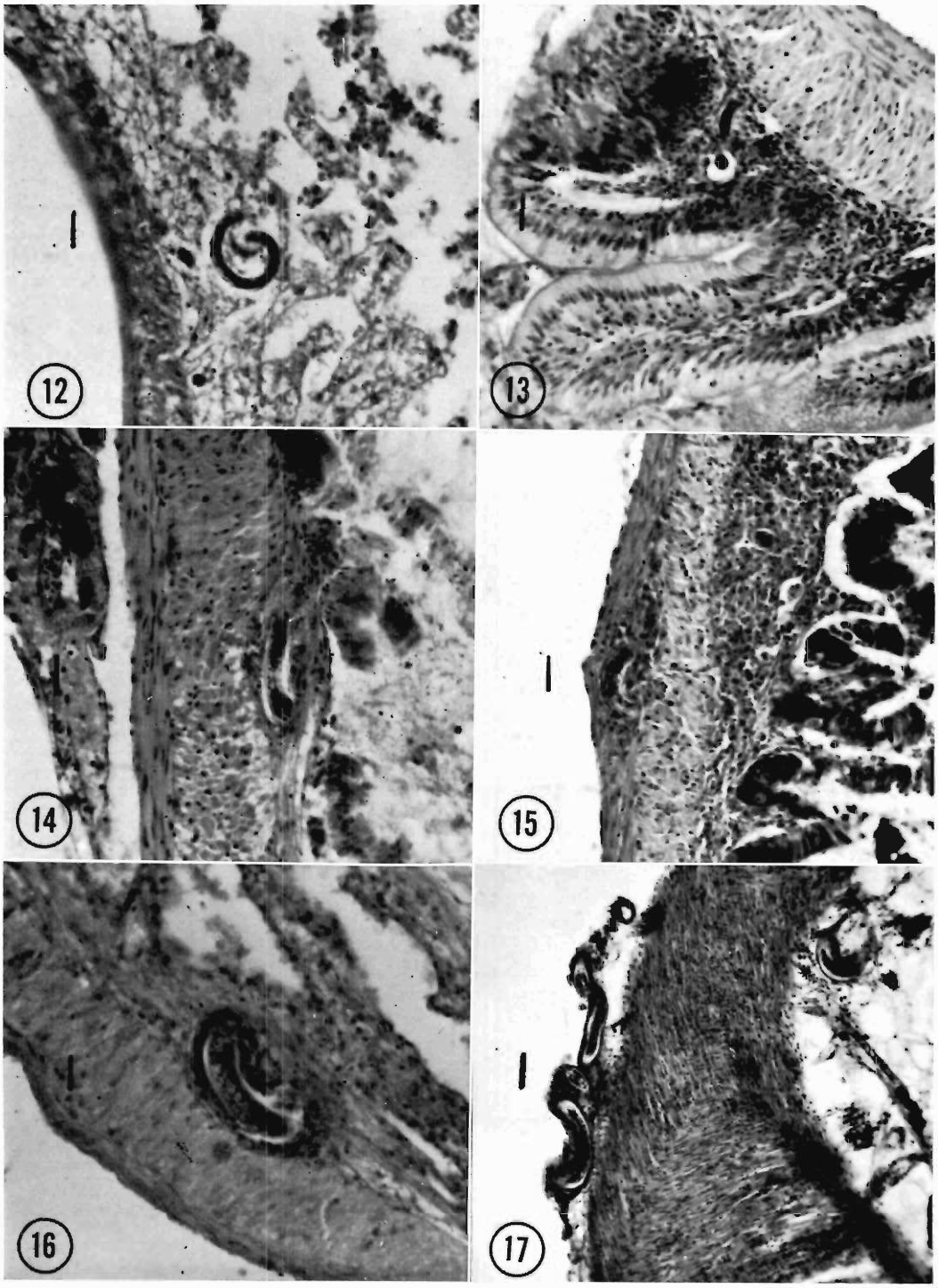
**Figures 7–11.** ***Parafilaroides decorus*, third-stage larva. 7. Lateral aspect. 8. Cephalic region, dorsal aspect. 9. Posterior of esophagus, excretory pore, duct and excretory sinus. 10. Caudal region, lateral aspect. 11. Cross section.**



### Studies of the egg and larvae (Table 1)

EGG (Fig. 1). Twenty eggs removed from two female worms measured 49–68 (57) long by 39–49 (42) wide. The egg capsule is similar to that described by Anderson (1962) for *Perostrongylus pridhami* and *Filaroides martis* in that it consists of two delicate layers, an outer chorion and an inner vitelline membrane.

FIRST-STAGE LARVA (Figs. 2–4). The first-stage larva has a sharp spike-like tail surrounded by loose cuticle. The buccal cavity is a narrow tubular channel connecting the oral opening to the anterior end of the esophagus. A well-developed esophagointestinal valve is present.

SECOND-STAGE LARVA (Figs. 5–6). The second-stage larva is longer and thicker and its body is more granular than the first-stage larva. The genital primordium is larger; the tail pointed but not spikelike; the excretory duct more clearly defined than in the preceding stage; and the first-stage cuticle is retained after the moult.

THIRD-STAGE LARVA (Figs. 7–11). The third-stage larva is slender and serpentine with lateral alae which extend nearly the entire length of the body, beginning approximately 9 $\mu$ from the cephalic extremity and extending to within 5 $\mu$ of the caudal extremity. The tail is blunt and has a terminal knob; both first-and second-stage larval cuticles are usually retained; the stoma is narrow, leading into a dilated buccal-area followed by the thickened walls of the anterior esophagus. The excretory duct leads to a fusiform excretory sinus at the level of the esophageal glands. The esophagus, intestine, and genital primordium are similar in morphology to those of the first two larval stages.

### Discussion

Transmission of *P. decorus* to sea lions at the San Nicolas Island rookery takes place by their ingestion of infected opaleye from contaminated pools. A paratenic host is apparently not required, as sea lions feed directly on the opaleye. A rookery situation is probably ideal for transmission. In these areas there are a high concentration of sea lions, resultant heavy contamination of pools with feces, and an abundance of coprophagous fish to serve as intermediate hosts. Under these conditions sea lions can acquire massive infections of this lungworm.

Animals for exhibition are usually caught at or near rookery areas and bring the infection into captivity with them. Those with heavy infections usually succumb when stressed during rigorous training and show schedules while being maintained on a set diet.

This is the first metastrongyloid life cycle wherein a vertebrate has been demonstrated to act as the first intermediate host. This finding suggests that other metastrongyloids of marine mammals may have a similar life cycle.

### Acknowledgments

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**Figures 12–17.** ***Parafilaroides decorus.*** **Photomicrographs showing location of larvae in *Girella*. 12–14. First-stage larva. 12. In intestinal lumen 24 hours post-infection. 13. In intestinal mucosa 72 hr post-infection. 14. In intestinal circular muscle layer 5 days post-infection. 15–16. Second-stage larva. 15. In longitudinal muscle layer 18 days post-infection. 16. In process of encapsulation in mucosa 21 days post-infection. 17. Third-stage larvae in serosa and mesenteric fat 36 days post-infection.**

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## RESEARCH NOTE

# In vitro and in vivo Excystation of *Clinostomum marginatum* (Trematoda) Metacercariae

Incidental to studies on the development of metacercariae of *Clinostomum marginatum* from frogs in the chick and on the chorioallantois (Fried and Foley, 1970, J. Parasit. 56: 332–335), observations on in vitro and in vivo excystation were made and are reported herein.

In in vitro excystation studies 10 cysts were placed in petri dishes containing either 15 ml of 1% pepsin 1-10,000 (Nutritional Biochemicals Co., Cleveland, Ohio) in Ringer's (Paul, 1960, Cell and Tissue Culture, Williams, Baltimore) adjusted to pH 2.3 with HCl, 0.5% pepsin-Ringer's-HCl-pH 2.3, 0.1% pepsin-Ringer's-HCl-pH 2.3, Ringer's-HCl-pH 2.3, 0.1% pepsin-Ringer's, or Ringer's and maintained at 40 C for up to 2 hr. Table 1 summarizes the data and reveals that 100% excystation occurred within 30 min in either 1% or 0.5% pepsin-Ringer's-HCl-pH 2.3.

In in vivo excystation studies 5 cysts were fed in 1 to 2 ml of Ringer's to each of five day-old unfed chicks which were necropsied 10 to 60 min later. Table 2 summarizes the data and reveals that excysted metacercariae were present in the proventriculus and the esophagus within 10 min and in the mouth cavity within 20. Excysted metacercariae were not recovered in the gizzard, trachea, or duodenum and those in the esophagus were migrating anteriorly along the mucosal surface. Although some excystation may have resulted from cyst damage in the mouth cavity, most metacercariae probably excysted in the proventriculus where acid-pepsin is present.

**Table 1. Summary of in vitro excystation of *C. marginatum* in pepsin-Ringer's-HCl or Ringer's-HCl at 40 C (ten cysts/solution).**

| Time in min. | 1% pepsin pH 2.3 | 0.5% pepsin pH 2.3 | 0.1% pepsin pH 2.3 | Ringer's pH 2.3 |
|---|---|---|---|---|
| 10 | 1 | 0 | 1 | 2 |
| 20 | 5 | 4 | 0 | 0 |
| 30 | 4 | 6 | 5 | 0 |
| 40 | – | – | 0 | 0 |
| 50 | – | – | 0 | 0 |
| 60 | – | – | 1 | 0 |
| 120 | – | – | 1 | 6 |
| Total | 10 | 10 | 8 | 8 |

Zero excysted in 0.1% pepsin in Ringer's (unadjusted).
Zero excysted in Ringer's (unadjusted).

**Table 2. Summary of observations on excysted metacercariae in chicks each fed 5 cysts of *C. marginatum*.**

| Host | No. of min. post-exposure | Mouth cavity | Esophagus | Crop | Proventriculus |
|---|---|---|---|---|---|
| A | 10 | 0 | 1 | 1 | 2 |
| B | 20 | 1 | 1 | 0 | 2 |
| C | 30 | 1 | 2 | 0 | 2 |
| D | 45 | 0 | 0 | 2 | 0 |
| E | 60 | 2 | 0 | 0 | 2 |

Hemenway (1948, Iowa Acad. Sci. 55: 375–381) reported excystation in *Clinostomum* sp. metacercariae at 37 C in acidified pepsin for 1 hr followed by neutral trypsin for 10 to 15 min. In the present study rapid and efficient excystation in vitro in acidified pepsin and the absence of excysted metacercariae posterior to the chick's proventriculus indicate that neutral trypsin is not necessary for excystation in *C. marginatum*.

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