

THE LIFE HISTORY OF THE GASTROPOD
THYONICOLA AMERICANA TIKASINGH,
ENDOPARASITIC IN A SEASONALLY EVISCERATING
HOLOTHURIAN HOST

Maria Byrne

Department of Biology, University of Victoria, Victoria, B.C. Canada V8W 2Y2
Present address: Harbor Branch Foundation, Route 1, Box 196, Fort Pierce, Florida 33450, USA

ABSTRACT

The endoparasitic gastropod *Thyonicola americana* Tikasingh attaches to the intestine of the dendrochirote holothurian *Eupentacta quinquesemita* (Selenka). Evisceration by *E. quinquesemita* is a seasonal event occurring in the autumn and as a result of their attachment to the viscera, *T. americana* is annually expelled from its host. The effect of evisceration on the life cycle of *T. americana* was investigated. Reproduction of the parasite is continuous, but the number of specimens at an advanced reproductive state was highest in summer and decreased coincident with seasonal evisceration. The parasites were not found attached to regenerating or newly regenerated viscera and there seemed to be a 'seasonality' in maturation imposed on *T. americana* by host seasonal evisceration. The optimum period for infection by *T. americana* is in the spring and the minimum length of the parasite's life cycle is approximately six months. Parasites expelled during evisceration perish, and so the majority of *T. americana* do not complete their life cycle. This may be compensated by the high fecundity of successful parasites and their success appears to be amplified by the high density of hosts for infection. The life cycle of *T. americana* is compared with that of entoconchid species parasitic in aspidochirote holothurians.

INTRODUCTION

During a study of evisceration in the dendrochirote holothurian *Eupentacta quinquesemita* (Selenka), an endoparasitic gastropod *Thyonicola americana* Tikasingh was found attached to the viscera expelled from many of the holothurians. *T. americana* is a member of the Family Entoconchidae, a group of shell-less, vermiform prosobranchs, many of which are endoparasitic in holothurian echinoderms (Koehler & Vaney 1903, Ivanov 1949, Tikasingh & Pratt 1961, Lützen 1979). Seasonal evisceration by *E. quinquesemita* was observed in the field and appears to be an annual event, occurring in the autumn (Byrne 1985). The extent of annual evisceration differed in two populations examined and did not involve 100% of the holothurians. As a result of their attachment to the host's intestine, *T. ameri-*

cana are expelled from the host during evisceration. The life history of *T. americana* was examined to see how it is affected by seasonal expulsion from its host and to investigate a potential relationship between reproduction of *T. americana* and evisceration.

Thyonicola americana was first described by Tikasingh (1961) and Lützen (1979) examined aspects of its life history. This species was originally considered to be hermaphroditic (Tikasingh 1961), but Lützen (1979) showed that *T. americana* is cryptically gonochoric with dwarf males inside the females' central cavity. Metamorphosis of female parasites appears to take place while they are penetrating the host's gut (Lützen 1979). That of the male occurs on the male receptacle, a structure found in the female's central cavity. After metamorphosis the males lose all their tissues except for the testis rudiment (Lützen 1979).

Entoconchid parasites of aspidochirote holothurians are thought to depend on release from the host through evisceration to complete their life cycle (Tikasingh 1961, Lützen 1979). The life history of these parasites has been worked out in detail for *Enteroxenos oestergreni* which infects the aspidochirote *Stichopus tremulus* (Lützen 1979). *S. tremulus* is reported to eviscerate annually (Jespersen & Lützen 1971) and the progeny of *E. oestergreni* appear to be released in conjunction with evisceration (Lützen 1979). *Enteroxenos parastichopoli* endoparasitic in the aspidochirote *Stichopus californicus* is also thought to depend on seasonal evisceration to complete its life cycle (Tikasingh 1961, Lützen 1979). A recent study reported that *S. californicus* exhibits seasonal visceral atrophy rather than autotomy (Fankboner *et al.* 1981) and so an alternate means of propagation for *E. parastichopoli* is discussed.

I thank Professor A.R. Fontaine for advice and encouragement. F.A. Pereira da Costa assisted with diagrams. I also thank my diving buddies, especially C. von Carolsfeld. The work was supported by a University of Victoria Fellowship and this report is Smithsonian Marine Station contribution no. 136 and contribution no. 444 of the Harbor Branch Foundation.

METHODS

The occurrence of *Thyonicola americana* was monitored in two populations of *Eupentacta quinquesemita* from November 1981 through February 1983. The two study sites were, Ten Mile Point (TMP) and the Royal Victoria Yacht Club (RVYC), both near Victoria, B.C. (Byrne 1985). Due to the great difference in density of *E. quinquesemita* at the two sites, the monthly sample size collected from TMP was larger, 50-100 holothurians were collected monthly from TMP and 20-60 from the RVYC. Bimonthly samples were taken from September through October 1982 coinciding with seasonal evisceration of *E. quinquesemita*. The holothurians were examined by dissection and the viscera were examined for the presence of *T. americana*.

The reproductive state of *T. americana* at TMP was monitored visually, especially for the presence of parasites with advanced ovarian development, and for ovigerous specimens. The ovaries were visible through the epithelium and ovigerous females were distinguished by the presence of egg capsules which filled their central cavity. The *T. americana* were categorised as immature, with developing ovaries or ovigerous, and for each host the reproductive state of the most mature parasites was noted. For fecundity measurements, the number of egg capsules within four ovigerous specimens was counted and ten capsules from each female were opened and the number of embryos and larvae counted.

RESULTS

Occurrence of Thyonicola americana and Eupentacta quinquesemita at the study areas

The mean population densities of *Eupentacta quinquesemita* at TMP and at the RVYC were 782/m² and 140/m², respectively (Byrne 1985). At TMP, 41% (N=924) of the *E. quinquesemita* with well-developed viscera (see below) had *Thyonicola americana* attached to their intestine, whereas only 0.9% (N=442) of the RVYC holothurians were infected by the parasite.

T. americana were found attached by a narrow stalk to the host's large intestine near the cloaca where the gut pigment changes from brown to yellow (Fig. 1a, b). Within the connecting stalk, is a ciliated tubule, connecting the central cavity of the parasite to the host's intestinal lumen (Lützen 1979). Following seasonal evisceration, *E. quinquesemita* undergoes a 2-4 week period of regeneration (Byrne 1985), and *T. americana* were never found attached to regenerating or newly regenerated viscera. Infection by *T. americana* appears to be limited to holothurians with well-developed viscera. The parasites were occasionally found attached to more anterior regions such as the descending small intestine near the aquapharyngeal bulb, especially in *E. quinquesemita* infected by numerous *T. americana*.

Life history

The life history of *T. americana* is outlined (Fig. 2). The larvae possess a shell and crawl with their pedal mass. They lack a velum and are entirely benthic. Entoconchid larval metamorphosis involves loss of their shell as they penetrate through the host's gut (Lützen 1979). *T. americana* first appear as small round protrusions from the posterior intestine of *E. quinquesemita* (Figs 1a, 2). Then they elongate into the perivisceral coelomic space (Figs 1a, b, 2). As the parasites mature, their elongate bodies begin to grow in coils and their ovaries are an orange-pink colour visible through the epithelium (Figs 1b, 2, 3a-c). The ovigerous parasites are also

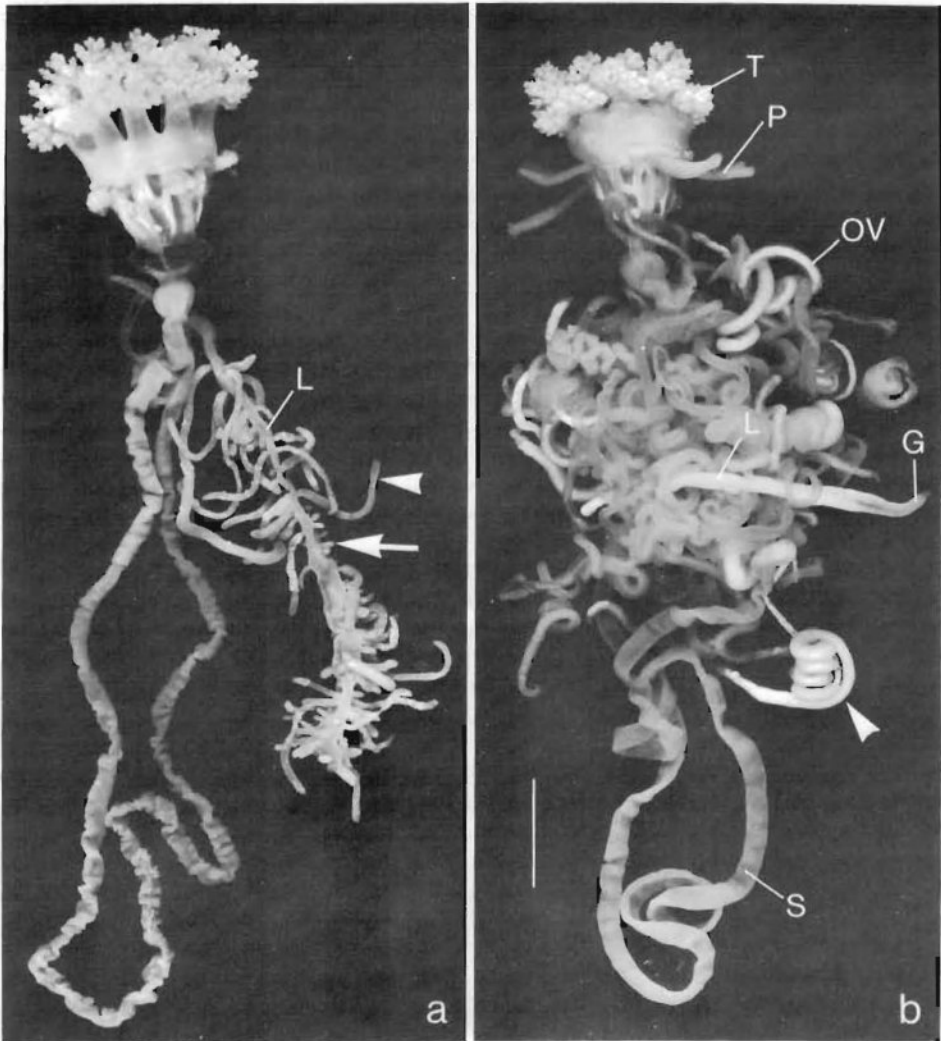
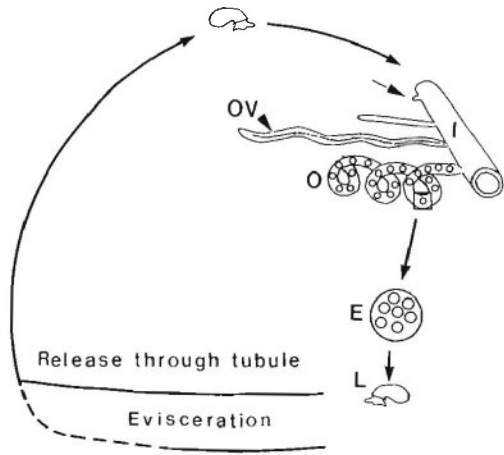


Fig. 1. *Thyonicola americana* Tikasingh attached to the autotomized viscera of *Eupentacta quinque-semita* (Selenka). a, the parasites first appear as small round protrusions (arrow) along the large intestine (L) and elongate into the perivisceral space (arrowhead). b, the parasites grow in coils (arrowhead) and the ovaries (OV) can be seen through the epithelium. The host's large intestine (L) has a yellow pigment compared with the brown colour of the small intestine (S). G, autotomized end of the host's gut; P, autotomized pharyngeal retractor muscle; T, tentacles. Scale = 1.0 cm.

coiled and their central cavities are filled with egg capsules containing developing embryos and larvae (Fig. 3a-d). Ovigerous *T. americana* when straightened are usually 100-200 mm long with a diameter of 1-2 mm and are usually two to three times the length of the host (50-100 mm). Some specimens were found in the coelom no longer attached to the host's intestine.

Fig. 2. Diagrammatic life history of *Thyonicola americana*. The parasite first appears as a small round protrusion (arrow) from the host intestine (1). E, egg capsule; L, shelled larva; O, ovigerous parasite; OV, ovary.



Ovigerous parasites contain 400-600 egg capsules ($\bar{X}_4 = 465$; $SD = 87$) containing 75-150 larvae ($\bar{X}_4 = 134$; $SD = 64$) to make a total of approximately 65 000 young ($\bar{X}_4 = 65\ 000$; $SD = 34\ 000$) per individual parasite. Egg capsules are produced sequentially until the ovaries are completely spent and the entire central cavity is filled with capsules (Fig. 3b, c). This appears to result in sequential embryonic development and larval release. Larval release was not observed, but it is presumably through the ciliated tubule and into the intestinal lumen. Once in the intestinal lumen the larvae probably escape through the cloaca.

Reproduction

Reproduction of *T. americana* appears to be independent of season. Parasites at all stages of maturity were found throughout the study and so introduction of competent larvae to holothurian hosts may be continuous. Specimens with advanced ovarian development and ovigerous females were particularly abundant in summer and just prior to the annual evisceration of *E. quinquesemita* (Fig. 4).

As a result of their attachment to the intestine, *T. americana* were expelled during evisceration. Only 0.9% of the eviscerated holothurians dissected ($N = 442$) had a single parasite free in the coelom that had not been expelled. In autumn 1982, 76% of the *E. quinquesemita* at TMP eviscerated (Byrne 1985). Evisceration occurred from late September through November and this substantially reduced the number of *T. americana* collected during the autumn and winter (Fig. 4). Eviscerated adult parasites eventually perish, degenerating along with the discarded viscera. The body wall of ovigerous females was sometimes punctured during evisceration resulting in a rapid liberation of egg capsules from the central cavity (Fig. 3c). The few ovigerous parasites found in the autumn and winter were associated with holothurians that had retained their digestive tracts through the autumn.

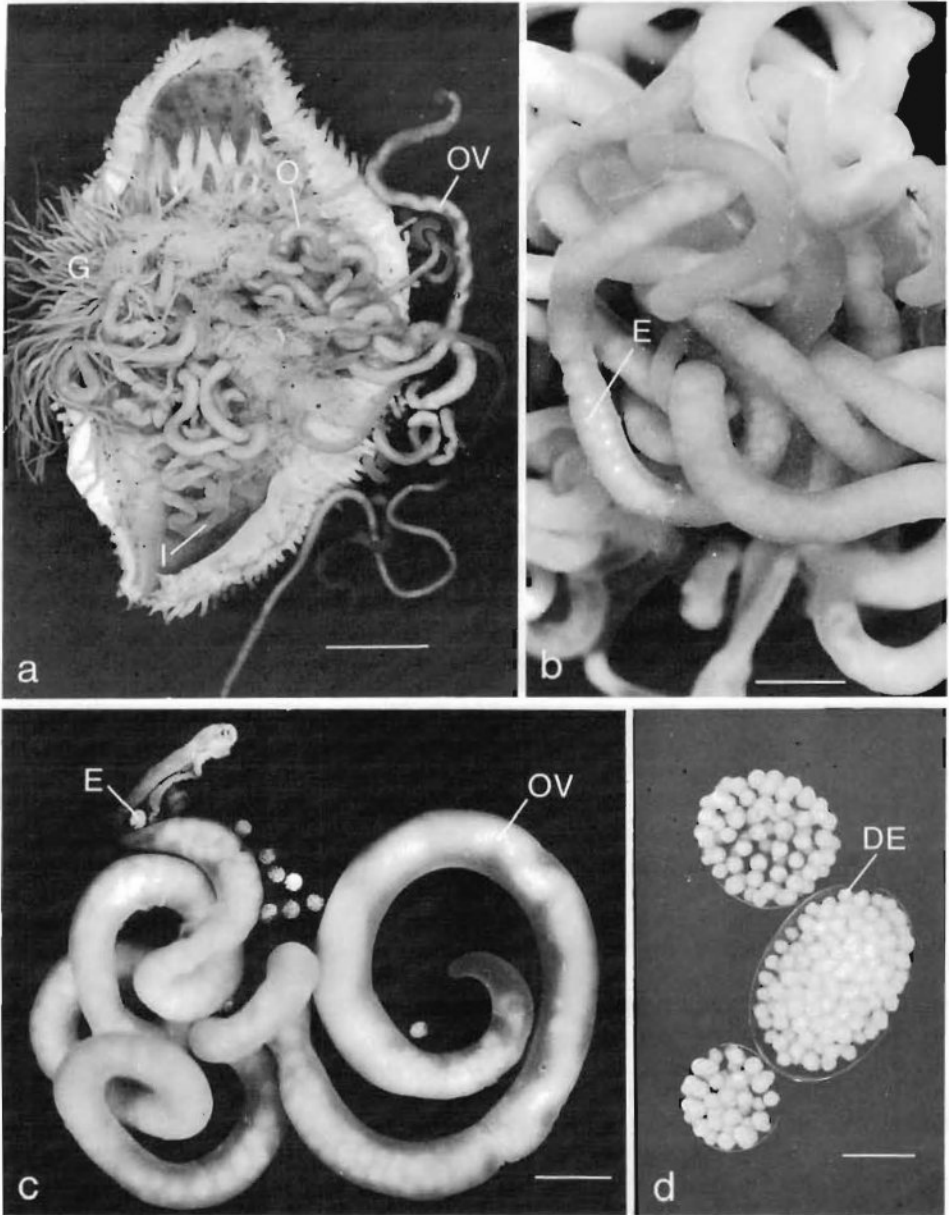


Fig. 3. a, dissected *Eupentacta quinquesemita*. The perivisceral coelom is obliterated by *Thyonicola americana*, five of which are at an advanced reproductive state. G, host's gonad tubules; I, host's intestine; O, ovigerous parasite; OV, ovary. Scale = 1.0 cm. b, ovigerous *T. americana* with egg capsules (E) filling the central cavity. Scale = 2.0 mm. c, ovigerous *T. americana*, some of the egg capsules (E) have been liberated due to rupture of the adult body wall. The ovary (OV) is not spent. Scale = 1.0 mm. d, egg capsules full of developing embryos (DE). The capsule shape and the number of embryos per capsule may vary within a single parasite. Scale = 0.35 mm.

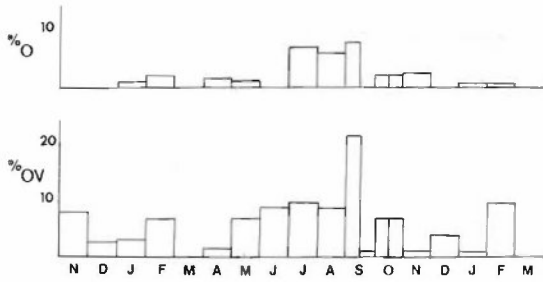


Fig. 4. Frequency histograms showing the percentages of TMP *Eupentacta quinquesemita* with ovigerous (O) *Thyonicola americana* and with parasites with ovaries visible through the epithelium (OV), November 1981-February 1983.

Effect on host

Specimens of *E. quinquesemita* had as many as seventy *T. americana* at various stages of maturity attached to the gut (Figs 1a, b, 3a). Most of the *T. americana* were immature and hosts seldom contained more than one ovigerous specimen. The parasites usually did not fill the host coelom and for the most part did not appear to affect *E. quinquesemita*. Some holothurians contained mature parasites in such numbers, that the coelomic space was completely obliterated (Fig. 3a). The specimen in Fig. 3a had five parasites at an advanced reproductive state that appeared to completely fill the coelom. *T. americana* was observed to interfere with the use of the holothurian's tentacles for suspension feeding. In one case, parasites were coiled around the host's pharyngeal retractor muscles to the point where the tentacles could not be protracted to feed. Heavily parasitized hosts could be detected by their apparent inability to keep their tentacles fully retracted and the orange-pink ovaries of *T. americana* were sometimes visible through the holothurian's body wall.

DISCUSSION

Thyonicola americana infected 49% of the TMP holothurians with well-developed digestive tracts and was virtually absent from the RVYC. This may reflect the poor dispersal powers of *T. americana* larvae and also suggests that a high host density, as found at TMP, may be required for populations of *T. americana* to become established. As for *Enteroxenos oestergreni* larvae (Lützen 1979), the larvae of *T. americana* may have a short free life. The high density of *Eupentacta quinquesemita* probably facilitates successful infection by *T. americana* by reducing the time required for larvae to be taken up by a host.

T. americana were never found attached to regenerating or newly regenerated viscera. This suggests that there may be a minimum size and/or physiological state of the large intestine of *E. quinquesemita* for successful infection by these parasites. *T. americana* was predominantly found attached to the portion of the intestine

posterior to where the gut pigment changes from brown to yellow. The brown pigment of the anterior intestine of *E. quinquesemita* may be associated with waste product accumulations (Byrne 1983), perhaps making these gut regions unattractive to metamorphosing parasite larvae. Attachment to the posterior intestine near the cloaca would also facilitate release of larvae from the host.

Reproduction of *T. americana* appears to occur year-round and infection of the host is potentially continuous. Parasites with developing ovaries and ovigerous specimens were most abundant prior to the seasonal evisceration of *E. quinquesemita*. The decline in the number of ovigerous *T. americana* in the autumn and winter is presumably a result of host evisceration and may also be due to release of progeny through the ciliated tubule in late summer. The number of ovigerous *Enteroxenos oestergreni* also decreased in association with evisceration of its host (Lützen 1979). The abundance of *T. americana* at an advanced reproductive state in summer may indicate a 'seasonality' in maturation imposed on the parasite by annual evisceration. Due to seasonal evisceration, there may be an optimum period for infection of the host in spring when the gut has re-attained a state appropriate for infection. Following this period, the female parasite has to receive a male larva and release progeny by late summer in order to complete its life cycle. Thus, the minimum length of the life cycle of *T. americana* is approximately six months, whereas that of *E. oestergreni* is approximately one year (Lützen 1979). All adult *T. americana* expelled during evisceration degenerate along with the discarded viscera. Consequently, many of the parasites do not complete their life cycle. Embryonic development of *T. americana* is thought to occur entirely within the host, with larval release through the ciliated tubule (Lützen 1979). It is not known whether the *T. americana* embryos expelled during evisceration can complete their development within the egg capsule outside the host. *In vitro* culture of excised egg capsules would be required to see if embryonic development can continue independent of the host.

Visceral autotomy appears to be an annual event in *Eupentacta quinquesemita* (Byrne 1985). Evisceration occurred at both study areas irrespective of the presence or absence of *T. americana*. *T. americana* is not dependent on evisceration for propagation, on the contrary, evisceration appears to have a disruptive effect on its life history. For the most part, *T. americana* does not appear to affect the host. However, specimens of *E. quinquesemita* with a heavy parasite infection may be more prone to eviscerate than less infected hosts (Byrne 1985). As for seasonal evisceration, autotomy induced by *T. americana* would appear to have a detrimental effect on the parasites. In extreme cases, *T. americana* may interfere with the host's gonadal development by competing with the gonads for coelomic space. *Stichopus tremulus* also eviscerates seasonally irrespective of the presence or absence of entoconchids (Lützen 1979). The incidence of evisceration differed in the two holothurian populations, in autumn 1982, 26% of the *E. quinquesemita* from TMP and 50% of those from the RVYC did not eviscerate (Byrne 1985). Ovige-

rous *T. americana* found during the winter were always associated with holothurians that had retained their viscera. These ovigerous parasites may be a source of larvae for infection of holothurians in the spring following visceral regeneration. If *T. americana* larvae have a short free life, then the larvae released by ovigerous parasites retained through the autumn may be crucial for the maintenance of the parasite population.

How *T. americana* larvae enter their host is not known. They cannot swim and so are probably not introduced into the intestine of *E. quinquesemita* by tentacle capture during suspension feeding. The larvae are benthic, so entry through the host's cloaca during cloacal pumping is more likely. Many parasites and commensals of holothurians gain entrance through the cloaca (Hyman 1955). Evisceration induces a seasonal cessation in feeding by *E. quinquesemita* for at least 2-4 weeks (Byrne 1985). The respiratory trees are not expelled and cloacal pumping appears to continue. Therefore, parasite larvae may enter the host through the cloaca while the gut is regenerating. Tikasingh (1962) suggested that infection of aspidochirote holothurians occurs through the mouth during deposit-feeding activities and/or through the anus.

T. americana lacks a gut and the host's coelomic fluid is presumably the major source of nourishment. The ciliated tubule, although maintaining a connection to the intestinal lumen, appears to function for larval release (Lützen 1979) and not for nutrition. Entoconchids parasitic in aspidochirote holothurians lose this tubule connection during development.

It has been suggested that *Enteroxenos oestergreni* and *E. parastichopoli*, entoconchid parasites of the aspidochirote holothurians *Stichopus tremulus* and *S. californicus* respectively, depend on host evisceration to complete their life cycle (Tikasingh 1962, Lützen 1979). In these species the ciliated tubule connection closes during development and ovigerous parasites detach from the gut, lying free in the host's coelom. To complete their life cycle, ovigerous *E. oestergreni* expelled during evisceration degenerate and their progeny complete development within the egg capsule outside the host (Lützen 1979). The capsule degenerates releasing larvae which then reinfect *S. tremulus* by an unknown mechanism. Sporozoan and turbellarian parasites found in the gut of *S. tremulus* also appear to depend on seasonal evisceration to complete their life cycle (Jespersen & Lützen 1971).

Annual gut atrophy occurs in *S. californicus* and it appears that seasonal evisceration may not occur in this species (Fankboner *et al.* 1981). As a result, *E. parastichopoli* may not depend on annual release from its host to complete its life cycle. A recent report on the life cycle of *Anoplodium hymanae*, a turbellarian symbiont found in the coelom of *S. californicus* (Shinn, in press) provides evidence for an alternate propagative method for *E. parastichopoli*. *S. californicus* has ducts connecting the coelom to the posterior end of the rectum and it is through these ducts that the egg capsules of *A. hymanae* appear to exit from the host (Shinn, in press). Perhaps *E. parastichopoli* also utilizes these ducts for larval release. *S. californicus*

will eviscerate if provoked (Swan 1961) and so some *E. parastichopoli* may complete their life cycle through evisceration. Following larval release, the adult parasites may degenerate in the host's coelom or be expelled if evisceration occurs.

Despite the apparent lack of seasonal evisceration in *S. californicus*, the attachment positions of entoconchid parasites to the gut of aspidochirote holothurians and in addition, the detachment of ovigerous parasites, appear to be correlated with the sites of visceral autotomy. The larvae of *E. parastichopoli* and *E. oestergreni* usually attach to the host's oesophagus (Tikasingh 1961, Lützen 1979), a portion of the aspidochirote digestive tract not expelled during evisceration. As a result, immature parasites are usually retained during evisceration, whereas ovigerous parasites are expelled. Therefore, although evisceration may not be crucial to their life cycle, when it does occur it does not have a disruptive effect. This is in contrast to the case for entoconchids parasitic in dendrochirote holothurians that eviscerate. As for *T. americana*, these parasites remain attached to the posterior intestine throughout their life cycle with larval release through the ciliated tubule. Dendrochirote evisceration results in autotomy of the entire digestive tract and this disrupts the life cycle of the attached parasites.

The effect of the high proportion of *T. americana* which do not complete their life cycle due to seasonal evisceration may be compensated by the high fecundity of those that do. At TMP the success of *T. americana* progeny is probably amplified by the high density of *E. quinquesemita* available for infection. One might expect a seasonal reproductive cycle in *T. americana* closely associated with annual evisceration of its host, but continuous reproduction may be more appropriate because the extent of annual evisceration within and between populations of *E. quinquesemita* appears to be variable from year to year (Byrne 1985).

REFERENCES

- Byrne, M., 1983. Evisceration and autotomy in the holothurian *Eupentacta quinquesemita* (Selenka). – Ph. D. Thesis, University of Victoria.
- Byrne, M., 1985. Evisceration behaviour and the seasonal incidence of evisceration in the holothurian *Eupentacta quinquesemita* (Selenka). – *Ophelia* 24: 75-90.
- Fankboner, P.V., G.L. Cameron & T. Smith, 1981. Annual loss of the gut by the sea cucumber *Parastichopus californicus* (Stimpson). – (Abstract). – W. S. N. Annual Meeting, Santa Barbara, p. 18.
- Hyman, L.H., 1955. The Invertebrates: Echinodermata, pp. 238-244. McGraw-Hill, New York.
- Ivanov, A.V., 1949. The structure and development of the endoparasitic mollusc *Parenteroxenos dogieli*. II. Organization of the larva and postlarval metamorphosis. – *Isv. Akad. Nauk, SSSR, Ser. Biol.* 2: 109-134.
- Jespersen, A. & J. Lützen, 1971. On the ecology of the aspidochirote sea cucumber *Stichopus tremulus* (Gunnerus). – *Norw. J. Zool.* 19: 117-132.
- Koehler, P. & C. Vaney, 1903. *Entosiphon deimatis* nouveau mollusque parasite d'une holothurie abyssale. – *Revue suisse Zool.* 11: 23-41.
- Lützen, J., 1979. Studies on the life history of *Enteroxenos* Bonnevie, a gastropod endoparasitic in aspidochirote holothurians. – *Ophelia* 18: 1-51.

- Shinn, G. L., Reproduction of *Anoplodium hymanae*, a turbellarian flatworm (Neorhabdocoela, Umagillidae) inhabiting the coelom of sea cucumbers; production of egg capsules, and escape of infected stages *without* evisceration of the host. – Biol. Bull. (in press).
- Swan, E. F., 1961. Seasonal evisceration in the sea cucumber, *Parastichopus californicus* (Stimpson). – Science, N. Y. 133: 1078-1079.
- Tanaka, Y., 1958. Feeding and digestive processes of *Stichopus japonicus*. – Bull. Fac. Fish. Hokkaido Univ. 9: 14-26.
- Tikasingh, E. S., 1961. A new genus and two new species of endoparasitic gastropods from Puget Sound, Washington. – J. Parasit. 47: 268-272.
- Tikasingh, E. S., 1962. The microanatomy and histology of the parasitic gastropod, *Comenteroxenos parastichopoli* Tikasingh. – Trans. Am. microsc. Soc. 81: 320-327.
- Tikasingh, E. S. & I. Pratt, 1961. The classification of endoparasitic gastropods. – Syst. Zool. 10: 65-69.

1

2

3