HOST DISTRIBUTION AND DEVELOPMENT OF *PSEUDODELPHIS OLIGOCOTTI* (DRACUNCULOIDEA: NEMATODA), A PARASITE OF EELGRASS BED FISHES

by

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B.Sc., McGill University, Montreal, 1991

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES
(Department of Zoology)

We accept this thesis as conforming to the required standard.

THE UNIVERSITY OF BRITISH COLUMBIA

August 1999

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Date OCTOBER 12, 1999

DE-6 (2/88)

ABSTRACT

Transmission is a key point in parasitic life cycles and should be accurate for specific hosts if parasites are particular in their host requirements. Occasionally breadth of host range is correlated with mode of transmission: many parasites are discriminating in their transmission and have narrow host ranges, while some with indiscriminate transmission have broad host ranges, reflecting relaxed host requirements or a similarity between hosts. This thesis investigates the mode of transmission and host range of parasite *Pseudodelphis oligocotti* (Nematoda: Dracunculoidea) because it poses a problem: *P. oligocotti* infects nine fish species but only completes development in two - *Apodichthys flavidus* and *Gobiesox meandricus*. Presumably, the seven other fishes serving as unusual hosts are inappropriate for parasite development due to some physiological reason, yet the two successful host species seem very different. I propose that *P. oligocotti*'s mode of transmission accounts for this pattern. To determine the life cycle and host range of *P. oligocotti*, I surveyed 24 coastal fish species from nine British Columbia localities for prevalence and intensity of infection, stages of parasite development, and their distribution in host tissues. *Pseudodelphis oligocotti* infects nine species of coastal marine fishes. It infects *A. flavidus* at the highest rate and intensity: prevalence of infection was as high as 80%, and mean intensity was as high as 19 worms per infected host at certain localities. Subadult and adult *P. oligocotti* occurred in the body cavity of all nine fish hosts. Larvigerous *P. oligocotti* were only recovered from *A. flavidus* and one specimen of *G. meandricus*, where they occurred in the hepatic sinus. Infective first-stage larvae were recovered from gills and associated tissues of *A. flavidus*. Histology and scanning electron microscopy confirmed that larvae breach the gills to gain access to the external environment. I experimentally infected potential intermediate host *Tigriopus californicus* with larvae from larvigerous *P. oligocotti*, recording mortality rates due

to infection and larval development. Up to 57.1% of the copepods became infected with an average 1.26 worms per infected host. Larvae developed to the third-stage (infective to fish) in 14 to 15 days at 15 – 24.4°C. Copepod mortality did not increase as a result of infection. Intermediate hosts containing third-stage larvae were fed to uninfected *A. flavidus*. Infections were established in two of the 10 specimens. Results demonstrate that *P. oligocotti* is transmitted between fishes by ingestion of infected free-living copepods. Fishes that *P. oligocotti* infects share habitat with *A. flavidus* and many ingest free-living copepods. To examine whether ecological association between hosts predicts how far *P. oligocotti* develops in those hosts — an indication of host suitability — I characterized fishes by their diets, microhabitats, and taxonomy from literature sources. Hosts that are more ecologically similar to *A. flavidus* support more advanced stages of *P. oligocotti* than those that are phylogenetically related. This suggests that opportunity for infection determines parasite host range.

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ACKNOWLEDGEMENTS

Many thanks are due to my supervisor Martin Adamson and my supervisory committee Dolph Schluter, Carl Walters, Don McPhail, and Don Ludwig, for their efforts towards making me a better scientist and communicator. Although the process has been sometimes painful (but mostly fun), I'm thankful especially to Martin for bringing me closer to my goal: I always wanted to be a parasitologist when I grew up. I'm thankful to the other curious minds I got to joust with: Tracey Appleton for her inspiration and early work on the system, Esther Reid and her insatiable questions, Stephen Connor for his unbridled enthusiasm seasoned with a hint of sarcasm, Amanda Brown "keeping me honest", Allyson Miscampbell for her selfless acts of sacrifice, and Tammy Laberge for all the supportive exchanges. Mark MacLean's editorial comments of the final draft are especially appreciated. I am also indebted to Mike Kent for the crash course in histopathology and his editorial help on parts of the thesis pertaining to histopathology. Fishing for data, often in the middle of winter in the dead of night, would have been impossible without the helping hands of many game scientists, fellow graduate students, friends, supporters and assistants: Durrell Kapan, Martin Adamson, Tracey Appleton, Claire Thompson, Esther Reid, David Brisco, Jennifer Ondrack, Tanh Dhong, Martin Rebele, Dan Yuellet, J.J. Lee, Tammy Lee, Ross Shaw, Stephen Connor, Amanda Brown, Graham Blair, Allyson Miscampbell, Tammy Laberge, Glen MacDonald, Danny Kent and John Fisher of the Vancouver Public Aquarium, Maggie Booth, John Rinon, Dave Warman, Victor Shieh, Laurence Déspres, Kirsten Gummeshun, Moira Lemon (CWS), Rob Butler (CWS) and his daughter Holly, David Suzuki, Vancouver Island fishing team Michael Bennett, Marilyn Bennett, and Doris (Gram) Bennett, Wendy Kaplan, Steve Heard, Mounir Nader, Nick Saavas, Jen Sunday, Bryan Yu, Mark Rigby, Sam Chen, Christine Adamson, Tim Goater and the parasitology class at Malaspina, Greg Sandland, and any poor soul I've

forgotten who got wet for my thesis project. Your efforts were indispensable. My thanks go to Patti Beer at the Vancouver Public Aquarium, for taking good care of my fishes. I would like to thank Tim Goater for providing additional samples from Piper's Lagoon, Danny Kent for samples he collected on my behalf at Point Roberts, and Terry Sullivan for providing additional samples from Sidney Spit. I'm deeply grateful to Jeff Marliave for my first detailed introduction to the intertidal zone, and for his enthusiasm for fish and all things scientific that he selflessly imparted along with heaps of information whenever I required either. I'm especially thankful to Al Lewis for being such a tremendous resource of information on copepods and otherwise (for the meagre price of my supervisor's scalp). I thank Esther Reid in her special role as friend and fellow biologist: her tireless searches for "hopeful monsters" revealed our first glimpse of larvae in the gill filaments. I want to thank my neighbour Jim Cockburn the tax-payer (a closet worm-enthusiast) for his good humor and suspension of disbelief (and for all the penguins).

I have saved my loved ones to thank for last, because they've had such a hand in making me who I am, and have borne the brunt of my thesis stress. Without their unconditional love and support this document would not exist. My deepest thanks to Mom and Dad who never let me forget that I could do anything; Abby for drawing me out of my writing funk so that I could smell the flowers; Grampy Jay for always giving me a hard time while wearing a big smile; Gram, my champion, who always makes me feel wonderful; my Aunt Bonnie, the first biologist I ever knew, and my role model – I always wanted to be just like her. I would like to recognize the enormous contributions of my loving friends, in particular Samantha Hicks, whose unconditional support and ready laughter enhanced the pleasure of my life, and whose early demise at the peak of her life taught me the importance of living life to its fullest, just as she did. Finally, I owe a million thanks to my partner, Durrell Kapan, for each of the many long hours

he dedicated to fishing, technical support, editing, emotional bolstering, my amusement, the map, and finally for his unshakable confidence in me despite my own opinion on the matter.

I gratefully recognize and thank NSERC for 2 years of graduate funding in addition to project support from an NSERC operating grant to Martin Adamson.

CHAPTER 1: GENERAL INTRODUCTION

Introduction

One of the key points in a parasite's life cycle that determines its reproductive success is the transmission of its progeny to the next host. This may occur by way of an intermediate host, vector, or free-living stages. Assuming that parasites have particular host requirements and that all hosts are not equal, natural selection should act to make transmission as accurate as possible. However, for those parasites that depend on intermediate hosts and their ingestion by final hosts for transmission, there is little opportunity to choose the final host. Transmission and host ecology play important roles in creating a parasite's host range (Adamson and Caira 1994). Parasites' ability to develop in a range of hosts determines potential ways in which parasites may speciate: by cospeciation with hosts, or by capturing unusual hosts (Chabaud 1959, Holmes and Price 1980).

Any organism in (on) which a parasite lives for all or part of its life cycle is a *host.* Implicit in the use of this term is that the parasite as a species meets certain developmental requirements in a host and/or is successfully transmitted by that host. However, for practical reasons, the fate of a parasite cannot always be ascertained, and *host* often refers to any organism infected by a parasite, regardless of that parasite's fate. Some parasites require multiple hosts to complete one reproductive cycle. The host in which sexual reproduction occurs is called the *definitive* or *final host*, while hosts in which younger or non-sexually reproducing stages occur are referred to as *intermediate hosts* if they are required for the parasite's continuing development. *Vectors* are a special subset of hosts: they actively transport parasites between (other species of) hosts (Dogiel et al. 1964). In this study, distinction is drawn between final hosts that the parasite

merely infects without developing to maturity, and those that it infects and develops to maturity in regularly: I refer to them as *unusual (final) host* and *principal (final) host* respectively. When a parasite completes its development in an unusual host while retaining its normal morphology, Chabaud (1959) refers to it as *parasite transfer*. If that parasite then becomes isolated in the newly acquired unusual host and subsequently speciates, *host capture* is said to have taken place (Chabaud 1959).

Method of transmission and host ecological relationship with other potential hosts are occasionally correlated with parasite host range. The number of hosts and their taxa that makes up a parasite's host range is used to indicate a parasite's host specificity. *Host specificity* is defined as the "degree to which a parasite is able to mature in more than one host species" (Roberts and Janovy Jr. 1996). All parasites show host specificity, but the degree varies. Many parasites are highly host specific: Monogenea, whose transmission between fish hosts is restricted (direct contact between hosts is often necessary), are renowned for their high degree of host specificity (Rhode 1979). There are at least two exceptions, both Hawaiian monogeneans. One species infects seven families of fish hosts, while the other infects 11 families of fish hosts. In both cases, host species live sympatrically in diverse coral reef systems (Rhode 1979). Host specificity of monogeneans may result from strict requirements to match host physiology or simply restricted opportunities for parasite exposure to multiple hosts. Certain pinworms of Anurans have broad host ranges when there are many sympatric hosts available (Adamson and Caira 1994, data from Adamson 1981). However, these pinworms are in fact specific to certain host types (tadpoles) that do not group together by conventional taxonomy but may nonetheless be alike physiologically and are definitely ecologically associated.

This thesis examines the previously unknown life cycle and host range of an endoparasitic nematode of marine fishes, *Pseudodelphis oligocotti* (Dracunculoidea: Guyanemidae), which although first described from tidepool sculpin, *Oligocottus maculosus*, was never observed to develop into mature stages in this host (Adamson and Roth 1990). It is not unusual for a parasite to frequently infect hosts that are essentially dead ends for the parasite. Of 20 metazoan salmonid parasites investigated by Leong and Holmes (1981), 10 of these infected hosts in other fish families in addition to Salmonidae, but only 2 parasite species reached maturity in these other fish hosts. Another study reports that sockeye salmon are frequently infected by 3 main species of parasitic helminths only one of which can mature in sockeye: the other 2 parasites come from other fish hosts in the lake (Bennett, Adamson, and Margolis 1998).

Clearly within a parasite's host range as we perceive it, hosts vary in their suitability. Holmes (1976) observes using data collected by Leong (1975) that a parasite's host range commonly includes multiple hosts of variable suitability in which development may or may not proceed, with one ideal host that is most commonly infected in nature.

In Chapter 2 I characterize *P. oligocotti*'s host range by examining its abundance, frequency of infection, and development in various hosts. I identify its primary host as that in which *P. oligocotti* completes its development. In hosts that it infects but does not complete development in (such as type host *Oligocottus maculosus*) I record where along its developmental sequence *P. oligocotti* eventually fails. Since *P. oligocotti*'s degree of developmental success is variable and reflects variation in host suitability, in Chapter 5 I explore the question: what qualities do more suitable hosts share?

Pseudodelphis oligocotti's host distribution may reflect its method of transmission. Typical of dracunculoids, which occupy sites in their hosts other than the

intestine, *Pseudodelphis oligocotti* lives in the blood of definitive fish hosts. Members of the Dracunculoidea use very different transmission strategies, involving vectors or free-living intermediate hosts: certain species are transmitted between fish hosts by blood-sucking arthropods (Tikhomirova 1975), while species like the renowned Guinea worm (*Dracunculus medinensis*) deposit larvae directly into the water where free-living copepods ingest them, to in turn be ingested by the next vertebrate host (Moorthy 1938). In Chapter 3, I demonstrate that *P. oligocotti* is transmitted between fish hosts by a free-living harpacticoid copepod, a common food item for many small nearshore fishes inhabiting eelgrass (*Zostera marinus*) beds and rocky intertidal zones where *P. oligocotti* is found (Miller et al. 1980). Larvae extracted from larvigerous female nematodes living in the hepatic sinus of penpoint gunnel, *Apodichthys flavidus*, were fed to lab-cultured splashpool harpacticoid copepods *Tigriopus californicus*. Ingestion of parasitic nematode larvae by a copepod is described for the first time. *Pseudodelphis oligocotti*'s morphogenesis to the fish-infective third-stage in *T. californicus* is formally described, and is indicative of *T. californicus*' suitability as an intermediate host.

By protecting parasites from adverse environmental conditions and packaging them in an attractive (to final hosts) manner, intermediate hosts extend a parasite's distribution in space and time relative to that of free-living or directly transmitted nematodes (Chabaud 1957, Inglis 1965, in Anderson 1984). This may have been an important adaptation on the part of the parasite for transmission in the highly dispersive aquatic environment (Inglis 1971). In Chapter 3, intermediate host *T. californicus* mortality due to infection by *P. oligocotti* is investigated because premature death of the intermediate host limits a parasite's availability to infect final hosts.

Dracunculoids occupy extra-intestinal sites (such as blood, skin, *et cetera*) in the vertebrate definitive host (Adamson 1986), sites from which they must gain

access to the external environment when the time comes to transmit. Blood-dwelling dracunculoids access the external environment either by active migration of the larvigerous females to peripheral arteries, or by the intervention of haematophagous vectors. *Pseudodelphis oligocotti* does neither. In Chapter 4, I demonstrate the unique nature among zooparasitic nematodes of *P. oligocotti*'s method of transmission from the vertebrate host. By observing *P. oligocotti*'s distribution within the host by stage of development, the tissue sites occupied by larvigerous female parasites and first-stage copepod-infective larvae indicate that it is the larvae themselves which breach the gills and gain access to the external environment. It has already been suggested that we look for this mode of host egress among certain blood-dwelling nematodes whose life cycles are as yet unknown (Appy et al. 1985).

In summary, in this thesis I present the life cycle of a parasite with a broad host distribution. Upon determining *P. oligocotti*'s life cycle, I have discovered that it transmits to fish final hosts by ingestion of free-living copepods, which acquire the parasites by ingesting larvae that actively breach the fish host's gills. This goes far in explaining the diversity of fish species infected by *P. oligocotti*, many of which eat copepods. However, further life cycle observations indicate that many of these hosts are unsuitable. In the final chapter I demonstrate that the most suitable of these alternate hosts are the most similar in diet and habitat but not necessarily related in form, phylogeny, or presumably physiology. This essentially correlates a host's rate of exposure to a parasite with that host's suitability in terms of parasite development. The ability to develop in hosts that are ecologically associated but not necessarily phylogenetically related is essential for an important mode of parasite speciation, that by host capture (Chabaud 1959).

CHAPTER 2: *PSEUDODELPHIS OLIGOCOTTI* DISTRIBUTION IN COASTAL MARINE FISHES

Introduction

Pseudodelphis oligocotti (Nematoda: Dracunculoidea) occurs in at least 9 species of coastal marine fishes. Although *P. oligocotti* was first described from tidepool sculpin (*Oligocottus maculosus*) captured at Popham Island, Stanley Park, Vancouver, and Bamfield Marine Station, Bamfield, British Columbia, fully mature stages (larvigerous females) of the parasite were not found in the 344 fish examined (Adamson and Roth 1990). Under the suspicion that *O. maculosus* is not in fact the principal host for *P. oligocotti*, I surveyed fishes at various localities around the southern British Columbia coast to determine *P. oligocotti*'s host distribution and identify its primary host(s). In this chapter I examine *P. oligocotti*'s abundance in different hosts at different localities. I chart its development in various hosts and by doing so identify *P. oligocotti*'s primary host and determine where it fails in other hosts.

Although parasites vary in their host range (Rhode 1979), which may depend on a number of historical and ecological factors (Adamson and Caira 1994), most parasites are host specific to some degree according to the number of taxa or types of hosts they infect (Holmes and Price 1980, Adamson and Caira 1994). Even when a parasite's host range includes multiple hosts, often there is a particular host in which it grows more quickly, grows larger, produces more progeny, and is present more frequently and in greater abundance. In other hosts, parasites may occur less frequently, and growth and development may be retarded, even halted at an early stage (Dogiel et al. 1964; data from Leong 1975, cited in Holmes 1976). Because hosts vary in their suitability, one might expect *P. oligocotti* to vary in its abundance, growth and development in different host

species. The host (or hosts) in which it regularly completes development is the principal host contributing to *P. oligocotti*'s propagation in nature. The frequency with which *P. oligocotti* infects various hosts and its abundance in those hosts reflects the principal host's ecological and/or physiological relationship with other fishes. We might expect this relationship to vary geographically, since different habitats may be more or less supportive of *P. oligocotti*. Numerous sites are surveyed for *P. oligocotti* and characterized by fish host community, wave exposure, and types of substrate to determine parasite geographic range and suitable habitat.

The definitive host fish for *P. oligocotti* transmits parasite larvae into an aquatic environment, and we expect these larvae to go through an intermediate host before they are infective to another fish (as do all other members of the superfamily Dracunculoidea, see Anderson 1992 for review). Not all parasites make it back into an appropriate fish host. I describe the fate of parasites in unusual hosts wherein they do not complete development. These unusual hosts vary in some respect, since there is variation in the point at which a parasite's development is halted (see Chapter 5). A parasite is said to *transfer* to an unusual host when its development reaches completion in that host (Chabaud 1959).

The point along a parasite's developmental sequence where it is interrupted is variable in *P. oligocotti* depending on the host species, and may indicate variation in host qualities. The closer a parasite gets to completing its development in a host, the fewer the chance modifications necessary before successful transfer to that host occurs. In host environments that are most unsuitable, the parasite never establishes, dying immediately after ingestion. Hosts that are more suitable may provide appropriate chemical cues for the parasite to proceed further along the sequence of generalized developmental steps: exsheathment, penetration, migration, growth, or finally, transmission (MacInnis 1976).

The goal of this chapter is to determine *P. oligocotti*'s primary definitive host(s). This requires examining the parasite's development, abundance and frequency of infection in a range of hosts at different localities. *P. oligocotti*'s development in various hosts will be characterized by whether development is completed, and if not, where along the developmental sequence the parasite fails.

Methods

Field Collections

Fish were captured from coastal British Columbia mainly by one of two methods: dragging a pole seine (length 2m, height 1.5m, diagonal mesh size 11 mm) through subtidal eelgrass (*Zostera marinus*) beds, and overturning rocks in rocky intertidal flats adjacent to eelgrass beds. Seining sessions consisted of 10 hauls, ranging in length from 10-90 seconds. Samples therefore covered areas ranging from approximately 16-136 m^2 (assuming constant seining rate of 0.8m/s). *Hippoglossoides elassodon* (flathead sole) from Barkley Sound were collected by another method, using a 2H bottom trawl towed at 82 to 167 meter depths by the W.E. Ricker (Department of Fisheries and Oceans vessel) in conjunction with a federal survey of west coast Vancouver Island in May 1998. All collection sites occurred throughout the coastal mainland of British Columbia to west coast Vancouver Island, British Columbia (Table 2.1). Many of these sites were sampled only once to determine the parasite's geographic range. However, I sampled primary collection sites Lumberman's Arch/Figurehead Point, Point Roberts and Roberts Bank more frequently. Lumberman's Arch and Figurehead point were sampled on spring tides from: spring to mid-winter in 1991, 1992, and 1995; throughout the year in 1993 and 1994; sporadically in spring, summer, and fall 1996-1997; and spring 1998. Point Roberts and Roberts Bank were sampled on spring tides, twice in the spring of 1993, throughout the year in 1994, and sporadically from 1995 to 1997.

Once captured, fish were usually placed in a salt water holding tank and processed within 5 days of capture. Fish were identified to species, measured in total length and sexed (usually upon dissection except in the case of the sexually dimorphic *Syngnathus leptorhynchus*), in addition to being dissected for parasites.

Dissections

Dissections generated data on *Pseudodelphis oligocotti*'s prevalence (percentage of individuals infected, Margolis et al. 1982), intensity (mean number of worms per infected individual, Margolis et al. 1982) and development for each fish species and locality. Fish intended for dissections were held in a temporary salt water holding tank at the Department of Zoology. Dissections were completed usually within 72 hours – infection from other fish during this time is highly unlikely since the transmission cycle of other dracunculoids takes on the order of months and requires an intermediate host (see Anderson 1992 for review). Before dissecting them, I anaesthetized fish in tricaine methanesulfonate (MS222) at a concentration of approximately 0.0065g/l-seawater/cm total length of fish. After motion had ceased (approximately 4 min) fish were placed on a dissecting tray and measured for total length (head to tip of tail). I removed the head with a scalpel and set it aside for examination of gills and collection of otoliths. I made an incision at the base of the tail and took a blood sample using a standard hematocrit tube. With a dissecting microscope, I examined the blood sample for circulating stages of *P. oligocotti*. I then made a ventral incision from anus to the juncture of pectoral fins, and pinned the fish open for examination of body cavity. I removed worms and placed them in 0.67% sodium chloride (NaCl) solution for sorting by stage and sex. When dead worms were found, some of them encapsulated within host tissue, they were enumerated and stored in a solution of 70% ethanol, 5% glycerine, to which a few crystals of phenol per litre had been added. Gonads were removed and placed on slides for examination for worms. The intestine was saved in 70% ethanol, 5% glycerine with a few crystals

of phenol per litre added, for future examination of stomach contents. I then examined the liver, mainly the hepatic sinus, and pericardium for nematodes. I removed any nematodes, liver (after initial examination) and heart, and examined the common cardinal vein which branches dorsally from the pericardium, collecting any nematodes found therein. I set the liver and heart aside for histological examination. I then returned to the head, removing the gills first by making a ventral incision to expose the buccal cavity, then severed the anterior and posterior connections right and left side in turn to extract the full set of four gill arches together. The right set was placed on a slide where the gill arches were separated as gently as possible and laid anterior side up. I placed another slide on top and examined each gill arch for nematode larvae trapped in the meniscus formed between the two slides. The left set of gill arches was placed in 10% buffered formalin for histological or scanning electron microscope (SEM) examination at a later date.

Nematodes typically undergo five developmental stages from larva to adulthood: the third to fifth (adult) stages occur in the final, in this case fish, host; the first stage is transmitted from gravid adults in the final host to intermediate hosts (Anderson 1992). First-stage may also be found in the final host, on their way out. Second-stage larvae develop in the copepod intermediate host. With the aid of a microscope, I staged nematodes as first stage, third or fourth stage (as one category), fifth stage, gravid females with developing embryos, or gravid females with first-stage larvae (larvigerous). First-stage larvae were distinctive with a dorsal cephalic swelling and little cellular differentiation. Combined third- or fourth-stage was assigned if gonads were undeveloped, or, if developed, nematodes did not possess a patent (open and functioning) vulva (in the case of a female) or a coiled tail (in the case of a male). Fifth, adult stage was assigned if either the vulva was patent (in the case of a female) or the tail was coiled (in the case of a male) (Adamson and Roth 1990). Larger females were examined to

determine whether the uterus contained either developing embryos or first-stage larvae. Female nematodes whose uterus contained first-stage larvae I usually set aside for experimental infections of copepods (Chapter 3). All other nematodes were fixed in a heated solution of 70% ethanol, 5% glycerine, to which a few crystals of phenol per litre had been added, labeled by fish host, tissue site in the host, sex, and stage, and stored for future examination.

Results

I collected 1386 marine fish belonging to 24 species (Table 2.2, Table 2.3) from 11 British Columbia localities between 1991 and 1998. Nematodes were recovered from the body cavity, ovaries, heart, pericardium, hepatic sinus and gills of fish hosts, and identified as *Pseudodelphis oligocotti* based on the reduced buccal capsule, long, divided oesophagus, shape of the uterus in females, and the males' simple conical tale (Adamson and Roth 1990). *P. oligocotti* was present in 9 fish species (426 individuals, Table 2.3) but was most prevalent over all sites (excluding species only caught and examined once) in the pholid *Pholis laeta* (54% infected, Table 2.3). Pholids *Pholis ornata* and *Apodichthys flavidus*, and *Gobiesox meandricus* (Gobiesocidae) were also infected at a high rate over all sites (again, excluding those fish caught and examined only once). *Apodichthys flavidus* was the only fish to consistently produce mature parasites (worms with infective first-stage larvae *in utero*). One specimen out of 15 *Gobiesox meandricus* also contained larvigerous females (Table 2.3). Pholid *Apodichthys flavidus* showed the highest rate of infection at any one site (excluding species caught only once) – 80% at Figurehead Point (Table 2.4). Figurehead Point is a rocky intertidal zone adjacent to the eelgrass beds of Lumberman's Arch and together they constitute the main study site. At main study site Lumberman's Arch/Figurehead Point, in addition to high infection rates in the three pholids (*Pholis laeta, P. ornata* and *Apodichthys flavidus*), *Pseudodelphis oligocotti* was prevalent in *Syngnathus leptorhynchus* (24%

infected) and *Gobiesox meandricus* (46% infected, Table 2.5). *Leptocottus armatus* was infected at a rate of 15%. At this site *P. oligocotti* was also recovered from *Gasterosteus aculeatus*, *Artedius lateralis*, and *Oligocottus maculosus,* but sample sizes for these species are too small to generate reliable prevalences (Table 2.5).

Prevalence of *Pseudodelphis oligocotti* in all fishes varied between sites (Table 2.4). *P. oligocotti* occurred in fishes from 6 of the 9 sites surveyed (where Lumberman's Arch and Figurehead Point are considered a single site, see above, and excluding Popham Island, which was primarily surveyed by another study, Adamson and Roth 1990). Refer to Table 2.1 for a list of sites surveyed and Table 2.4 for infected sites. *Apodichthys flavidus* was one of the infected species at five of these six infected sites (Figure 2.1). Although *A. flavidus* was found at all sites infected with *P. oligocotti*, at one of these sites (Roberts Bank) *P. oligocotti* only occurred in other fish species (Table 2.4). Three sites did not support *P. oligocotti:* Porpoise Bay, Barkley Sound, and Sidney Spit. Of the three uninfected sites, specimens of *A. flavidus* were only recovered from Sidney Spit (Figure 2.1: sites with uninfected *A. flavidus* are marked with an empty pie; sites where no *A. flavidus* were caught are marked with an asterix). The five sites supporting infected *A. flavidus* have sandy beaches with eelgrass beds adjacent to extensive cobble (extending from supralittoral to subtidal). Wave exposure is moderate. Roberts Bank, where other fish species beside *A. flavidus* are infected, is a shallow man-made embayment sheltered from wave action, with an extensive eelgrass bed on a silty substrate. The closest rocky intertidal zone may be at Point Roberts. Sidney Spit, where fish including *A. flavidus* were uninfected, is also a shallow unexposed embayment with a silty bottom and extensive eelgrass beds. At uninfected site Porpoise Bay, where no *A. flavidus* was collected, eelgrass beds were deeper than other sites, and there was no adjacent rocky intertidal zone. Sites sampled in and around uninfected site Barkley Sound were distant from rocky shorelines at depths 82 to 167 meters and covered a variety of substrates.

Infected site Piper's Lagoon was exposed to some wave action and supported eelgrass beds on a sandy substrate. Infected site Tofino Harbour Crab Dock was more sheltered but also supported eelgrass beds on a sandy substrate.

As reported above, at 5 of the 6 sites where fish were infected with *P. oligocotti*, *A. flavidus* was also infected (Table 2.4, Figure 2.1). At one infected site, Point Roberts, however, *A. flavidus* were infected at a very low rate (9%) while another pholid species (*Pholis ornata*) was infected at a prevalence of 25%. Roberts Bank supported *A. flavidus* that were infection-free (68 *A. flavidus* sampled), but other hosts were infected with *P. oligocotti* at low prevalences (*Syngnathus leptorhynchus* at 2%, *Pholis ornata* at 14%, and *Pholis laeta* at 20%).

Stanley Park's Lumberman's Arch and the adjacent Figurehead Point were the primary, most frequently surveyed sites. At these sites, intensity was highest in *A. flavidus*, with a mean of 19 worms per infected fish (Table 2.5). This is 3 times greater than worm burdens in the next most intensely infected hosts, Syngnathus *leptorhynchus* (mean intensity of 5.5 worms), and the single specimen of the type host *Oligocottus maculosus* (6 worms) that I caught. *Pholis* spp. bore the next highest worm burdens, at 3.9 and 4.7 worms per infected fish (Table 2.5).

Third-stage larvae through to fifth-stage adults of *P. oligocotti* were recovered from the body cavity of all infected hosts. However, mature forms of *P. oligocotti* (females gravid with first-stage larvae) were recovered from only 2 of the 9 infected fish species: penpoint gunnel (*A. flavidus*) and northern clingfish (*Gobiesox meandricus*) (Table 2.3). From the former host individuals sampled at main study sites Lumberman's Arch and Figurehead Point, 14.4% of all *P. oligocotti* were females containing infective first-stage larvae (Table 2.5). Although 1 of the 7 *P. oligocotti* recovered from 7 infected *G. meandricus* was larvigerous, the host specimen was recovered from Piper's Lagoon, not Lumberman's Arch/Figurehead Point, and is hence not included in Table 2.5. Female

nematodes gravid with larvae were recovered from the hepatic sinus, the pericardial sac, and the common cardinal vein of *A. flavidus*. The larvigerous female *P. oligocotti* collected from *G. meandricus* was found in the hepatic sinus. Other organs of this host specimen were not examined for first-stage larvae. In *A. flavidus*, first-stage larvae were recovered from between the gill filaments. The hepatic sinus is the final tissue site occupied by *P. oligocotti*. From here larvigerous *P. oligocotti* release first-stage larvae into the host's blood stream: larvae are carried to the gills, which they breach to gain access to the external environment (Chapter 4).

Parasites in an advanced stage of development (female nematodes with developing embryos that were not yet larvae *in utero*) were supported by *Pholis laeta* (crescent gunnel), *Oligocottus maculosus* (tidepool sculpin), and *Syngnathus leptorhynchus* (bay pipefish) (Table 2.5). In *Pholis laeta* and *Syngnathus leptorhynchus*, female worms containing developing embryos occurred in the body cavity, sometimes pericardium, and occasionally ovary but never in the hepatic sinus. In *Oligocottus maculosus*, females with developing embryos were recovered only from the body cavity (Adamson and Roth 1990).

The remaining 4 infected host species, *Pholis ornata*, the stickleback *Gasterosteus aculeatus*, and sculpins *Leptocottus armatus* and *Artedius lateralis*, never supported *P. oligocotti* individuals with developing embryos or first-stage larvae *in utero* - development did not advance beyond the fifth-stage adult. Because most infected individuals of species such as *Apodichthys flavidus* contain multiple stages of *P. oligocotti* at once (Chapter 4), it is likely that mature stages would have been recovered from infected individuals if they could develop in that species. Nonetheless, the extremely low sample sizes of *Gasterosteus aculeatus* and *Artedius lateralis* (each represented by one infected individual), gives me less confidence in concluding that these two species never produce mature stages. In all 4 species

parasites were found exclusively in the body cavity except for the occasional worm recovered in the pericardium of *Pholis ornata*. One specimen of *Pholis ornata* that was held in captivity at the Vancouver Aquarium with infected *A. flavidus* for 34 months did contain larvigerous females in the hepatic sinus.

The abundance of larval stages (third or fourth) of *Pseudodelphis oligocotti* in unusual hosts *S. leptorhynchus* and *P. ornata* peaks two to three months after peaks in abundance of larvigerous females in *A. flavidus* (Figure 2.2). Third or fourth stage larval abundance peaks in *A. flavidus* at the same time of year as larval abundance in unusual hosts. Based on developmental experiments in Chapter 3, I expect a minimum delay of 17 to 18 days after the transmission of first-stage larvae from fish definitive host before third-stage larvae are infective to the next fish host via copepod intermediate hosts. Once in the fish host, larvae may spend 61 days in the third and fourth larval stages (Chapter 4).

Discussion

Infection of nine coastal marine fish species by *P. oligocotti* suggests that it has a broad host range. Other dracunculoid nematodes infect up to 4 final host species (Anderson 1992). However, in light of *P. oligocotti*'s development in addition to its high prevalence and intensity in *A. flavidus*, this is the principal host that contributes to *P. oligocotti*'s transmission. Other hosts vary in percent infected, intensity of infection and degree of development of *P. oligocotti*. Five other species from this study, in addition to *Oligocottus maculosus* from which *P. oligocotti* was first described (Adamson and Roth 1990), are regularly infected (*Gobiesox meandricus*, *Pholis laeta*, *Syngnathus leptorhynchus*, and *P. ornata*), but only one (*G. meandricus*) ever contained larvigerous *Pseudodelphis oligocotti* in the hepatic sinus. *Oligocottus maculosus* (as reported by Adamson and Roth 1990), *Pholis laeta* and *Syngnathus leptorhynchus* all support an advanced stage of *Pseudodelphis oligocotti*: gravid females with developing embryos. *Pholis ornata* are frequently infected but

P. oligocotti was never observed to advance beyond the subgravid adult (L5) in wild-caught fish. However, in one specimen held captive for 34 months with infected *A. flavidus*, a larvigerous *P. oligocotti* female was found in the hepatic sinus. It is not unusual for a parasite's host range (which includes hosts suitable for development) in nature to differ from its host range in a laboratory setting (Solter and Maddox 1998). The captive specimen of *Pholis ornata* may have acquired its advanced infection because it was held in close quarters with infected *A. flavidus* and therefore was exposed to more opportunities for infection, via copepod intermediate hosts. Vancouver Aquarium staff stocked tanks in which these fishes were held with local sand and coral — potential intermediate copepod hosts may have been introduced into the water. Direct transmission (directly from fish to fish, bypassing an intermediate host) is unlikely and without precedence in the dracunculoids. Neither copepods nor parasites can move between tanks because all seawater is passed through a 2-foot thick, #16 mesh sand gravity filter before recirculating (John Rawle, Director of Seawater Systems, Vancouver Public Aquarium, personal communication).

Pseudodelphis oligocotti was described from type host *Oligocottus maculosus* (Adamson and Roth 1990). Adamson and Roth (1990) report a low mean intensity and prevalence (1.7 and 22%, respectively) in *O. maculosus* compared to that in *A. flavidus*. Nor did the researchers ever recover female worms containing first-stage larvae from 76 infected fish out of 344 examined (Adamson and Roth 1990). Specimens of *Pseudodelphis* sp. found in this study agree morphologically with the description of *P. oligocotti*. Given the broad host distribution I have reported as well as the high number of host species that do not produce infective stages, it is not surprising that *P. oligocotti* has been described from a type host that may be inappropriate from the point of view of the parasite.

Pseudodelphis oligocotti's occurrence in so many species of fish hosts may be attributable to its method of transmission. Many Dracunculoids transmit by way of copepod intermediate hosts (Anderson 1992). *Pseudodelphis oligocotti* transmits between fish hosts in a free-living harpacticoid copepod (Chapter 3). Harpacticoid copepods are part of the diet of many fishes that live in the eelgrass beds and/or nearby rocky tidal zones (Miller et al. 1980). Coastal waters are used as feeding and nursery grounds for the young of many marine fish species, and breeding/spawning grounds for the adults of many species (Jones 1962), making coastal communities particularly diverse. Habitats that support many sympatric host species that are ecologically similar have been correlated with broad host ranges in parasites (Adamson and Caira 1994). Pinworms (Nematoda: Oxyurida) generally have a limited range of transmission (see Anderson 1992 for review). Pinworm host specificity often reflects the degree of ecological isolation of their hosts: the pinworm *Gyrinicola batrachiensis* infects 7 species of tadpoles from three different anuran families, where tadpoles congregate together in shallow water and feed indiscriminately, often on pinworm eggs (Adamson 1980, Adamson 1981). Where most fish monogeneans are restricted to a single species, genus, or family of host, again because of restricted transmission, two species of Hawaiian monogeneans from coral reef systems infect fishes from 7 to 11 different families (Rhode 1979).

Pseudodelphis oligocotti's abundance is almost as variable among locations as it is between host species at a single location (Figure 2.1, Table 2.4). However, all locations examined in this study that support *P. oligocotti* had *A. flavidus*, and this host was always infected with one exception: Roberts Bank. The three localities that did not support *P. oligocotti* either had no *A. flavidus* (Porpoise Bay and Barkley Sound), or were silt/mud bottomed (Sidney Spit). *Apodichthys flavidus* may be absent from Porpoise Bay because there were no nearby rocky intertidal areas in which *A. flavidus* likes to breed. *Apodichthys flavidus* may be absent from Barkley

Sound collections because sampling occurred at depths greater than *A. flavidus* occurs: *A. flavidus* does occupy shallower regions of Barkley Sound (Hart 1973, J. D. McPhail, University of British Columbia, personal communication). Adamson and Roth (1990) report *Pseudodelphis oligocotti* in *Oligocottus maculosus* specimens recovered from sites that included Bamfield Marine Station, Barkley Sound. Similar to Sidney Spit, Roberts Bank also has a silt/mud bottom, but although its *A. flavidus* were uninfected, other fishes were infected at a low rate. These fishes may have acquired their infection from other nearby sites, such as Point Roberts. Localities with silty or muddy bottoms, without nearby rocky intertidal zones, may be poor habitat for *P. oligocotti* because they are poor habitats for *A. flavidus*, which breeds under cobble, and possibly the intermediate host(s). For example, the splashpool copepod *Tigriopus californicus,* an intermediate host for *P. oligocotti* under laboratory conditions (Chapter 3), inhabits tide pools rather than tidal flats. That certain intertidal populations of fishes do not have *P. oligocotti* may simply be a result of random chance rather than differences in habitat. Little exchange occurs between members of different intertidal populations, because of the restricted movements of intertidal fish larvae, the stage at which fishes would normally disperse (Marliave 1986, Horn and Gibson 1988).

Pseudodelphis oligocotti establishes and develops to adulthood in nine out of 24 fish species surveyed, but shows variation in extent of development depending on the host. In all fishes where it is found, *P. oligocotti* migrates to the body cavity and develops to the fifth adult stage. However, in four of these species (*Pholis ornata*, *Gasterosteus aculeatus*, *Leptocottus armatus*, and *Artedius lateralis*) no later stages were ever observed in nature. Only in one species, *Pholis ornata*, did these mature forms migrate beyond the body cavity, occasionally appearing in the pericardium, which is adjacent to the hepatic sinus of the liver but still isolated from the blood stream. Only once, under artificial conditions, did a fully mature

parasite appear in the hepatic sinus. In the normal host, the hepatic sinus is the final tissue site. *P. oligocotti* transmits larvae to the external environment by releasing them into the blood upstream of the gills, which larvae then breach (Chapter 4). Thus although cues may be present in *Pholis ornata* to induce parasite migration towards the final tissue site, development does not proceed under natural conditions. In three fish species (*Syngnathus leptorhynchus*, *Pholis laeta*, and *Oligocottus maculosus*), development proceeds to the formation of embryos in the uterus of female worms (data on *O. maculosus* comes from Adamson and Roth 1990). Presumably parasites mate in the body cavity. However, in *Oligocottus maculosus*, migration never ensues, ovigerous females appearing in the body cavity alone (Adamson and Roth 1990). Hence development proceeds to a certain extent even without the preceding event of migration. In *Pholis laeta* ovigerous females appear in the pericardium; cues are presumably present to induce migration towards the final tissue site before transmission. Development proceeds further than in the first group of fish hosts, but does not proceed to completion. In *Syngnathus leptorhynchus*, subgravid adult (L5) stages occur in the pericardium and hepatic sinus in addition to the body cavity, indicating that *P. oligocotti* sometimes migrates to the final tissue site. Female worms with developing embryos *in utero* have been found in the host's body cavity, ovary and pericardium, indicating that development proceeds to a certain extent regardless of tissue site.

It is not clear exactly why *P. oligocotti* fails to develop to a stage capable of transmission in many of the hosts it infects, some of which are infected frequently. Failure to develop usually indicates that tissue sites lack the appropriate cue(s). Presumably appropriate tissue sites possess essential nutrients for or act as inducers of parasite development (MacInnis 1976, Read and Skorping 1995). Parasites may use any of a multitude of cues to find sites and develop within the host. The liver fluke *Fasciola hepatica* has six different

behavioural phases in the vertebrate host, each in a different host tissue where specific chemical cues induce specific responses (Sukhdeo 1990). Tissue specificity in parasites can be stricter than host specificity, and may even underlie most of the host-specific relationships between hosts and parasites (Adamson and Caira 1994). *Fasciola hepatica* infects herbivores: its emergence in the host intestine is stimulated by bile salts formed by herbivores but not by carnivores (Sukhdeo and Mettrick 1986).

Table 2.1 Locations surveyed and number of times each was surveyed for *Pseudodelphis oligocotti* and its hosts.

Sampling Site	Location	Latitude	Longitude	Number of visits 1991-98
Lumberman's Arch[1]	Stanley Park, Vancouver	49°15'00"N	123°07'00"W	67
Figurehead Point[1]	Stanley Park, Vancouver	49°15'00"N	123°07'00"W	8
Point Roberts	Washington, U.S.A.	48°59'08"N	123°04'36"W	11
Roberts Bank	Delta	49°05'00"N	123°11'00"W	10
Sidney Spit	Southern Gulf Islands	48°39'00"N	123°20'00"W	2
Tofino Harbour Crab Dock	Tofino, westcoast Vancouver Island	49°08'99"N	125°53'51"W	1
New Brighton Beach	East Vancouver	49°15'00"N	123°07'00"W	2
Popham Island[2]	Howe Sound	49°21'40"N	123°29'00"W	>1
Piper's Lagoon	Nanaimo	49°10'00"N	123°56'00"W	3
Porpoise Bay	Sunshine Coast	49°30'00"N	123°46'00"W	2
Barkley Sound	westcoast Vancouver Isl.	48°27'58" - 49°15'44"N[3]	125°08'64" - 127°05'71"W[3]	1

[1] Main study sites: adjacent but different in habitat

[2] Sampled only once in this study, but surveyed extensively in another study by Adamson and Roth 1990

[3] Multiple sites sampled by boat and bottom trawl in conjunction with DFO study.

Table 2.2 List of fish species caught and examined for *Pseudodelphis oligocotti*. Fish taxonomy follows Hart (1973).

Family	Scientific Name	Common Name	Code[1]
Batrachoidiformes			
Batrachoididae	*Porichthys notatus*	plainfin midshipman	PN
Gobiesociformes			
Gobiesocidae	*Gobiesox meandricus*	northern clingfish	GM
Gasterosteiformes			
Aulorhynchidae	*Aulorhynchus flavidus*	tubesnout	AuFl
Syngnathidae	*Syngnathus leptorhynchus*	bay pipefish	S
Gasterosteidae	*Gasterosteus aculeatus*	three-spined stickleback	GA
Perciformes			
Embiotocidae	*Cymatogaster aggregata*	shiner perch	CA
Stichaeidae	*Anoplarchis purpurescens*	high cockscomb	AP
Stichaeidae	*Xiphister mucosus*	rock prickleback	XM
Stichaeidae	*Lumpenus sagitta*	Pacific snake prickleback	LS
Pholidae	*Apodichthys flavidus*	gunnel, penpoint	A
Pholidae	*Pholis laeta*	gunnel, crescent	PL
Pholidae	*P. ornata*	gunnel, saddleback	PO
Scorpaeniformes			
Hexagrammidae	*Hexagrammus stelleri*	whitespotted greenling	HS
Hexagrammidae	*H. decagrammus*	kelp greenling	HG
Cottidae	*Leptocottus armatus*	Pacific staghorn sculpin	LA
Cottidae	*Artedius fenestralis*	padded sculpin	ArFe
Cottidae	*A. lateralis*	smooth-headed sculpin	AL
Cottidae	*Oligocottus maculosus*	tidepool sculpin	OM
Cottidae	*Enophrys bison*	buffalo sculpin	EB
Cottidae	*Blepsias cirrhosus*	silver spotted sculpin	BC
Pleuronectiformes			
Pleuronectidae	*Platichthys stellatus*	starry flounder	PS
Pleuronectidae	*Hippoglossoides elassodon*	flathead sole	FS
Salmoniformes			
Salmonidae	*Oncorhyncus nerka*	sockeye salmon	ON
Rajiformes			
Rajidae	*Raja binoculata*	big skate	RB

[1] Used in Chapter 5 to refer to species.

Table 2.3 Number of each fish species examined for *Pseudodelphis oligocotti*, number infected, prevalence (percent infected) and numbers that contained mature forms of the parasite. Blanks in the last 2 columns indicate that no individuals contained these forms.

	No. examined[1]	No. infected[1]	Prevalence (% infected)	No. containing females with developing embryos	No. containing larvigerous females
Porichthys notatus	1	0	0		
Gobiesox meandricus	15	7	47		1[2]
Aulorhynchus flavidus	1	0	0		
Syngnathus leptorhynchus	653	145	22	10	
Gasterosteus aculeatus	1	1	100		
Cymatogaster aggregata	2	0	0		
Anoplarchis purpurescens	15	0	0		
Xiphister mucosus	1	0	0		
Lumpenus sagitta	2	0	0		
Apodichthys flavidus	402 (435)	182	45	67	95
Pholis laeta	105 (108)	57	54	1	
P. ornata	63 (66)	28	44		1[3]
Hexagrammus stelleri	1	0	0		
H. decagrammus	4	0	0		
Leptocottus armatus	45	4	9		
Artedius fenestralis	9	0	0		
A. lateralis	3	1	33		
Oligocottus maculosus[4]	345	77	22	>0[4]	
Enophrys bison	8	0	0		
Blepsias cirrhosus	1	0	0		
Platichthys stellatus	1	0	0		
Hippoglossoides elassodon	11	0	0		
Oncorhynchus nerka	1	0	0		
Raja binoculata	1	0	0		

[1] Includes only fish examined shortly after they were caught. Numbers in brackets include those held in captivity or otherwise manipulated.

[2] Fish recovered from Piper's Lagoon.

[3] Fish held in captivity for 34 months, see text for explanation.

[4] Includes 344 individuals from Adamson and Roth (1990) study: 76 were infected; an unknown number contained female worms with developing embryos.

Table 2.4 Proportion of fish infected with *Pseudodelphis oligocotti* for each infected species at each locality positive for *P. oligocotti*. Sample size for each species caught by site follows in parentheses. Sites occur throughout coastal British Columbia.

	Lumberman's Arch[1]	Figure-head Pt[1]	New Brighton	Point Roberts	Roberts Bank	Piper's Lagoon	Tofino Harbour	Popham Island[2]
Gobiesox meandricus	1 (2)	0.36 (11)	0 (1)			1 (1)		
Syngnathus leptorhynchus	0.24 (599)			0 (6)	0.02 (46)	0 (1)		
Gasterosteus aculeatus	1 (1)							
Apodichthys flavidus	0.75 (213)	0.80 (5)	0.40 (5)	0.09 (78)	0 (68)	0.58 (12)	0.60 (5)	
Pholis laeta	0.65 (74)	0.78 (9)	0 (2)	0.13 (8)	0.20(5)			
P. ornata	0.77 (30)			0.25 (16)	0.14 (7)			
Leptocottus armatus	0.15 (27)			0 (3)	0 (8)			
Artedius lateralis	0.5 (2)			0 (1)				
Oligocottus maculosus	1 (1)							0.22 (344)

[1] Sites lie adjacent to each other and together are considered main study site. Fish communities differ because LA consists of eelgrass beds & FP of rocky inter- to subtidal zones.

[2] Data from Adamson and Roth (1990). Includes occasional specimens from Stanley Park, Vancouver, and Bamfield Marine Station, Bamfield, British Columbia.

Table 2.5 Summary of infection of fishes at main study sites (Lumberman's Arch and Figurehead Point): percent infected with *Pseudodelphis oligocotti*, mean number of parasites per infected fish, variance to mean intensity ratio, and percentage of worms that reach maturity.

	Number examined[1]	Percent infected	Mean intensity	Variance: Mean intensity	Percent of worms with embryos[2]	Percent of worms with larvae[3]
Gobiesox meandricus	13	46	3.0	3.3	0	0
Aulorhynchus flavidus	1	0				
Syngnathus leptorhynchus	599	24	5.5	7.9	1.3	0
Gasterosteus aculeatus	1	100	1.0		0	0
Apodichthys flavidus	218	75	19.0	15.3	8.2	14.4
Pholis laeta	83	66	4.7	10.9	1.1	0
P. ornata	30	77	3.9	3.6	0	0
Leptocottus armatus	27	15	1.8	0.5	0	0
Artedius lateralis	2	50	1.0		0	0
Oligocottus maculosus	1	100	6.0		0	0

[1] Note "Number examined" differs from Table 2.3 because sites differ: this table focuses only on the main study sites.

[2] Female worms containing developing embryos *in utero*

[3] Female worms containing first-stage larvae *in utero*

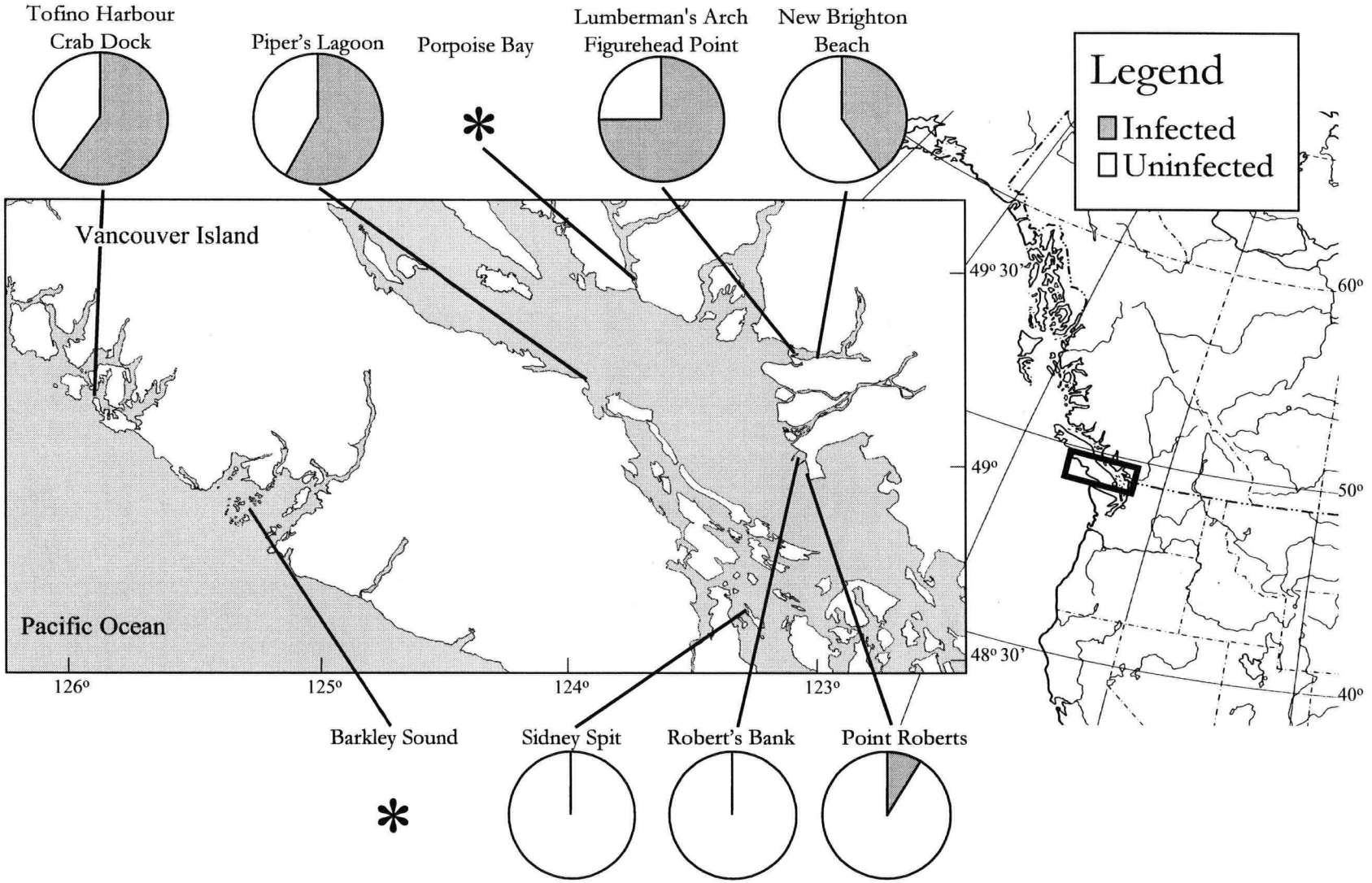

Figure 2.1 Map of British Columbia localities surveyed for *Pseudodelphis oligocotti*. Proportion of infected *Apodichthys flavidus* is indicated by shaded portion of pies. Empty pies mean that *A. flavidus* was uninfected with *P. oligocotti*. Localities marked with an asterix indicate that sites were sampled but no *A. flavidus* were captured. *P. oligocotti* did not occur at these sites in any fish surveyed.

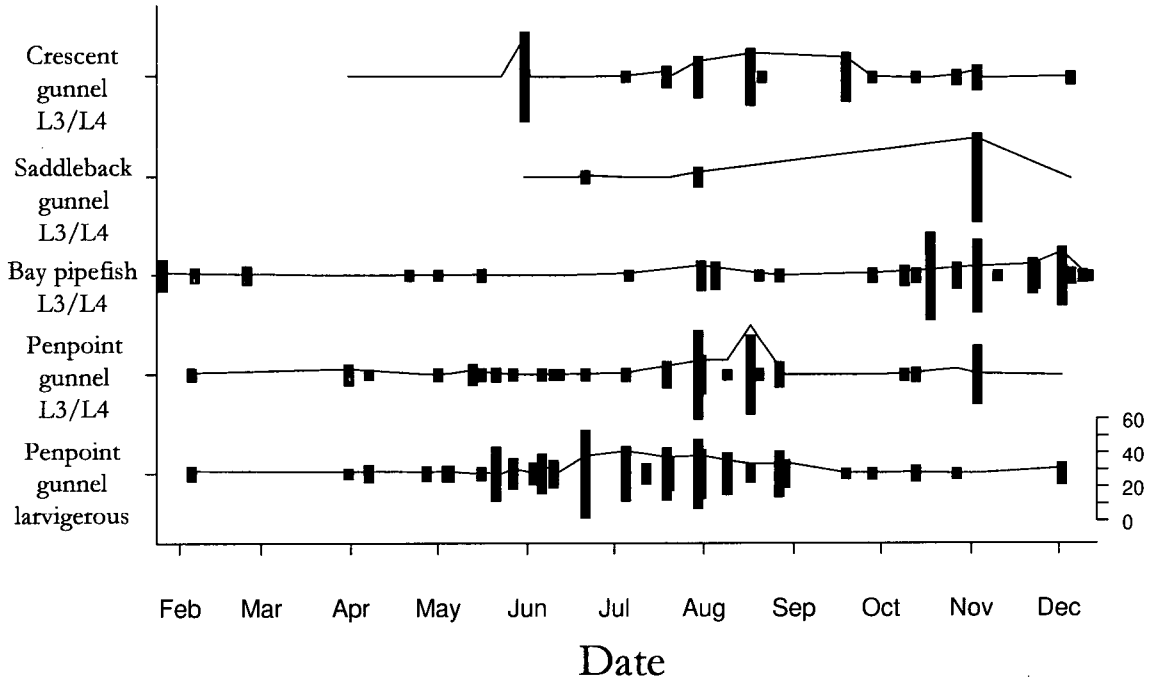

Figure 2.2 Seasonal relationship between total number of larvigerous *Pseudodelphis oligocotti* recovered from *Apodichthys flavidus* caught at particular time of year and total number of larval stages of *P. oligocotti* (L3 or L4) in *A. flavidus*, bay pipefish (*Syngnathus leptorhynchus*), saddleback gunnel (*Pholis ornata*), and crescent gunnel (*Pholis laeta*) caught at a particular time of year. Vertical bars represent number of worms (scale bar in far right corner applies to all categories). Lines represent robust (3 iterations) estimates of each point using the nearest 0.1625 points of the data set to generate the estimate (lowess function, Mathsoft 1998).

CHAPTER 3: DEVELOPMENT OF *PSEUDODELPHIS OLIGOCOTTI* (NEMATODA: DRACUNCULOIDEA) IN THE INTERMEDIATE HOST, A HARPACTICOID COPEPOD

Introduction

Dracunculoid *Pseudodelphis oligocotti* is a member of the family Guyanemidae. Other members of the Guyanemidae infect freshwater fishes (Petter 1974, Petter 1987, Petter and Dlouhy 1985, Costa et al. 1991), and none of their life cycles has been described. This study is the first effort to establish the manner of use and identity of the intermediate host for a member of this family. Life cycles have been investigated for closely related families, but these have been in freshwater systems (Anderson 1992). Thus, this species offered an opportunity to undertake the first study of transmission of a marine dracunculoid.

Dracunculoids typically transmit by way of free-living copepods or arthropod vectors according to their site of infection in the final host. Some dracunculoids that live in the blood of the final host (members of the genera *Skrjabillanus* and *Molnaria*) deposit their larvae into the blood stream. Larvae are then ingested by a haematophagous arthropod vector and eventually reintroduced into another host (Tikhomirova 1971, Tikhomirova 1975, Tikhomirova 1980, see Adamson 1986 and Anderson 1992 for reviews). Another dracunculoid that lives in the blood (*Philometra obturans*) actively breaches gill arteries: gravid females deposit larvae directly into the water, where larvae are ingested by free-living copepods (Molnar 1976, Molnar 1980, Moravec 1978, Moravec and Dykova 1978). Many dracunculoids, such as the infamous Guinea worm *Dracunculus medinensis*, occupy subcutaneous sites in the final host: gravid females break through the skin to release their larvae into the water. These larvae are also ingested by free-living copepods (Moorthy 1938, Fedchenko 1871, see Anderson 1992 for review).

Pseudodelphis oligocotti lives in the blood of its fish final host, *Apodichthys flavidus*. Gravid parasites presumably release larvae into the blood upstream from the gills (Chapter 4). In this chapter I examine whether *Tigriopus californicus* can serve as intermediate host for *P. oligocotti*. This requires a description of larval morphogenesis in the intermediate host.

Within the intermediate host, parasites are protected from adverse environmental conditions and are packaged more attractively to the final host. Thus intermediate hosts extend the life of parasitic larvae in space and time relative to that of free-living or directly transmitted nematodes (Chabaud 1957, Inglis 1965, in Anderson 1984). Premature mortality of the intermediate host also curtails the parasite's life. However, few studies have looked closely at mortality rates in the intermediate host due to infection. Some authors report on days post-infection at which 50% of the infected copepods have survived (Ko and Adams 1969). In addition to examining *P. oligocotti*'s ability to infect a free-living intermediate host, and its morphogenesis in that host, I also examine intermediate host mortality due to infection by *P. oligocotti*.

Methods

Experimentally infected copepods were derived from two sources: lab-reared marine copepods, *Tigriopus californicus*, and multiple species of wild-caught marine copepods other than *T. californicus* from Lumberman's Arch, British Columbia. I used marine copepod *T. californicus* in this study because it is widespread in the intertidal zone and hence not an unlikely candidate for the transmission of *Pseudodelphis oligocotti* to *A. flavidus* in nature. Furthermore, cultures of *Tigriopus californicus* were readily available: it is a robust species, tolerant to a range of salinities and temperatures (Lewis et al. 1998).

Lab rearing

Alan D. Lewis (Earth and Ocean Sciences, University of British Columbia) supplied source *T. californicus* from his lab cultures started in the late 1960's with organisms from Barkley Sound, British Columbia. I maintained *T. californicus* colonies in 1-litre Erlenmeyer flasks, feeding them a pinch of Wardley's basic tropical fish flakes every few months. This built up algae and bacteria in the water – the real sustenance of the colony. I left temperature to vary with that of the room (range 19.4 - 24.4°C). Windows provided natural light for better algal growth. Seawater used in all copepod rearing and experimentation was collected from Georgia Strait, British Columbia, filtered through 54 micron mesh, heated on maximum power for 10 minutes by microwave in 1 liter volumes for sterilization, and allowed to cool to room temperature. Brand of microwave used was Sharp model no. R-2A75C, which ran at 120V and 600W. The seawater was heated to 50°C by this procedure. I did not boil the seawater, as this can cause the precipitation of non-living organic matter. I assumed that microwaves killed living material. Colonies kept in sterilized filtered seawater never suffered massive die-offs, as did earlier colonies kept in seawater derived from commercial mixes.

Field collections

I collected copepods at Lumberman's Arch, Vancouver, British Columbia and the adjacent Figurehead Point (49°15'N, 123°07'W), from habitats where *A. flavidus* occurs and may ingest them. To target planktonic copepods, I swept a fine mesh (64 microns, 11 inch diameter opening) plankton trawl several times at mid-depth through 0.6m deep water at low tide. I uprooted and scraped eelgrass samples of their epiphytic invertebrates. I collected tidepool water samples in 50-ml jars from rocky Figurehead Point. Collections were made seven times throughout May, June, and July of 1994, and once in May 1997. I obtained 16

different copepod species from these three sources. Wild-caught copepod species were stored in jars of sterilized filtered natural seawater in a cold room at 11°C.

Transmission experiments

Using a 253-micron Nitex® nylon mesh strainer, I isolated equal numbers of lab-reared adult copepods randomly, without regard to gender, into finger bowls (8.3-cm diameter, 3-cm depth). Numbers of copepods per dish ranged from approximately 10 to 50 but was held constant within a trial. A trial is an experiment that uses the same fish host as a source of worms. For each trial, 1 to 5 replicate experimental dishes were treated with equal doses of infective larvae: 1 dish was not treated with infective larvae, as a control. Controls were used in trials of August 10, 1995, May 1, 1996, April 17 and 28, 1997 only. To elicit appetite, in later trials I waited 3 to 5 days after isolating copepods in sterile seawater before attempting to infect them. Table 3.1 summarizes transmission experiments.

I obtained adult female *Pseudodelphis oligocotti* gravid with first-stage larvae from the hepatic sinus of *A. flavidus* originating from Lumberman's Arch or Figurehead Point. I placed gravid female nematodes in 0.67% sodium chloride (NaCl) and burst them with fine probes. The released larvae were pipetted into the copepod-bearing finger bowls. I used from one to three larvigerous female nematodes, all from the same fish host, as sources in a given trial. Number of source females represents the dose of worms applied in a given trial (Table 3.1), assuming number of females is correlated with total number of larvae. I divided larvae equally between 1 to 5 experimental replicate dishes of copepods. Copepods from untreated control dishes were manipulated in a similar manner to the experimentally infected copepods (see below). Using a dissecting

microscope, I observed larval behaviour and copepod feeding behaviour upon release of first-stage larvae into the feeding chamber.

Inspection of copepods for infection and nematode development *in vivo*

The duration of copepod exposure to infective larvae varied between trials and depended on the activity level of larvae that remained uneaten. I generally allowed from two to four days to pass before removing larvae and transferring infected copepods to a fresh dish. To check copepods for infection, I pipetted them individually onto a slide along with a drop of seawater medium. I placed two strips of moistened No. 10 Whatman® filter paper along either side of the drop to cushion a cover slip. In this way the copepod was held in position but not harmed. I examined copepods under 40X power with a compound microscope for approximately 30 seconds. If the copepod was infected I placed it in a fresh dish of seawater labeled for intensity of infection. I discarded uninfected copepods. Controls were similarly placed on slides, compressed by cover slips and illuminated under 40X microscopy for approximately 30 seconds then returned to a fresh control dish. Temperature at which I stored copepods varied between trials from uncontrolled room temperature (19.4 - 24.4°C) to the constant 15°C of an environment chamber.

The process of mounting copepods on slides and examining them sometimes resulted in a fatal accident. For this reason, copepods were examined for parasitic nematode development only every second day, as per the initial inspections described above. Although the slide's cover slip pinned down the copepods, their nematode parasites continued to writhe around quite actively. Nonetheless, with each inspection I estimated relative size of the nematode with respect to its host (proportion of length and width), nematode activity level scored from zero to 1 (0 indicating death, 1 being most active), and signs of

molting (best seen as loose cuticle at head or tail). Every nematode molt marks its transition to the next stage of development. Nematode larvae initially fed to copepods were at the first stage of development, L1.

Development in intermediate host

Occasionally I killed an infected copepod to examine its nematode(s) for developmental studies. I fixed nematodes from dead infected copepods in a solution of 70% ethanol, 5% glycerine, to which a few crystals of phenol per litre had been added, before mounting them on slides for examination. Because of small sample sizes, I was limited in the number of worms available for detailed morphological examinations and measurements. Thus the number at each stage of development varies. I measured worms by first drawing them with a *camera lucida* (drawing tube) attached to a compound microscope. I then measured dimensions of the drawing with a length of unwaxed Johnson & Johnson's dental floss laid along the worm's length at the center of the drawing, and marked off organs of interest (anterior end, nerve ring, oesophagus, intestine, genital primordium, anus, posterior end). I converted measurements to microns with the aid of a drawing of the micrometer for scale. Because larvae from this family of nematodes have never been described before, I had to stage larvae based on the morphological differences I observed and the days on which I observed morphological types relative to days I observed molts, using first-stage larvae *ex utero* as a reference point.

Intermediate host mortality

Mortality rate was assessed in 3 trials, based on August 10, 1995, May 1, 1996, and April 28, 1997 experimental infections (Table 3.1). The first and last trials were conducted at room temperature, while the second trial was conducted at 15°C. Infected copepods used for mortality measurements were the results of transmission experiments. Since experimental infection rates were usually much

lower than 50% (Table 3.1), numbers of copepods per dish, replicate dishes and level of treatment (intensity of infection) were limited. In trial 1 I isolated 11 and 12 singly infected copepods randomly into two separate dishes. I manipulated 50 uninfected copepods, held in a control dish, in an identical manner, so as to measure mortality in experimental dish(es) due to infection alone. I monitored these three dishes for mortality of copepods, recording the proportion dead in each dish every day for up to 16 days. In trial 2 I isolated into 4 separate dishes according to level of infection: 28 singly infected copepods, 12 copepods infected with 2 larvae each, 2 copepods infected with 3 larvae each, and 1 copepod infected with 4 larvae. For up to 12 days, I checked these and a control dish of 34 uninfected copepods daily for proportion dead. Finally, in trial 3 I isolated 11 singly infected copepods, 1 copepod infected with 2 larvae, 1 copepod infected with 3 larvae, and 18 uninfected copepods into 4 separate dishes according to level of infection. I assessed daily proportion of dead copepods per dish as a function of days post-infection and worm burden. I used cubic splines to interpolate the data, which smoothed the data with locally weighted regressions based on approximately 0.5 to 3% of the data at a time (λ = 0.005 to 0.029 depending on the sample size with a particular infection load, at 3 degrees of freedom) (Mathsoft 1998).

Results

None of the 235 individuals from 16 different species of wild-caught copepods were infected with nematodes from the wild. I was unsuccessful in experimentally infecting wild-caught species of copepods: in only 2 of 8 attempts did wild-caught copepods survive beyond the first day of exposure. In one of the two attempts for which wild-caught copepods did survive, embryos from the source nematode were still immature, unfurling from their coiled positions sluggishly or not at all. In the other attempt, copepods simply did not appear to

engage the larvae – I did not observe any instances of active ingestion of larvae by wild-caught copepods.

Attempts to infect lab cultured *Tigriopus californicus* were frequently successful. Parasite larvae released from larvigerous female nematodes sank quickly to the bottom of the experimental dishes and writhed about tail down. *T. californicus* appeared to encounter larvae with their brush-like tarsi. Feeding took longer than 30 seconds; copepods maneuvered larvae into their mouths and ingested them whole but by small increments. Larvae were unharmed and presumably moved into the copepod's haemocoel shortly after being ingested. I successfully infected 147 *T. californicus* in 12 of 16 trials. The average number of larvae in infected copepods was 1.26 (minimum 1, maximum 4) but most contained a single larva (Figure 3.1).

Transmission rates to intermediate hosts

Infection success (% of copepod hosts infected per copepod hosts exposed) varied between 0 and 57.1% (Table 3.1). Trials were aborted if all hosts died within the first day of exposure (I cannot tell whether a dead host had had a chance to eat before death and therefore been exposed). Infections were unsuccessful if female nematodes did not contain enough first-stage larvae *in utero*. Female nematodes often contained a mix of developing embryos and first-stage larvae. The former unfurled slowly and behaved sluggishly if prematurely released. If more than 50% of a female nematode's embryos were still developing and had not reached first-stage, the experiment was aborted. Variation in success of infection in *Tigriopus californicus* was, if anything, negatively related to the dose of adult worms (where mean of percentage infected in each replicate dishes within a trial is used to represent percent infected for that trial, and "n", the number of trials using *T. californicus*, is 15: Spearman's $\rho = -0.52$, $p = 0.05$ on Freeman Tukey transformed percentages of infected) (Zar 1996).

Exposure time had no effect on success of infection. I varied exposure from 2 to 7 days, but at room temperature only an average of 16% (n = 4) of the uningested larvae were still moving 2 days post release. At 15°C larvae survived 4 days post release.

Intermediate Host Mortality due to Infection Load

The activity of *P. oligocotti* larvae inside the copepod host was high. Relative to the host, larvae grew in width from 0.25 to 1 times the width of the host's lower intestine, and in length from 0.3 to 1.2 times the host's body length. Nonetheless, mortality rates were no higher in infected copepods than in uninfected copepods, regardless of worm burden. Results are based on 2 trials at room temperature and 1 trial at 15°C. Mortality rates were similar amongst all three trials regardless of temperature. For each trial, each dish's daily proportion dead was averaged across all days. First and third trials, both conducted at uncontrolled room temperature, were pooled (variance ratio test $F_{3,4} = 0.19, p = 0.33$). Mean daily proportion dead for each dish was not significantly different between the two experimental temperatures (Wilcoxon rank sum test on 7 replicates at room temperature and 5 replicates at 15°C; $p = 0.25$). I plotted daily proportion of copepods dead in a finger bowl by day since exposure and level of infection, coding temperature with different symbols, and assessed the data for pattern using a cubic spline. Daily proportion of uninfecteds dead was not noticeably different from daily proportion of infecteds dead, except that mortality rates of copepods with 1 or 2 nematode larvae increased towards the end of the experiment (Figure 3.2). Copepods rarely harboured more than 2 worms; sample sizes are inadequate to conclude whether or not worm burdens of 3 and 4 are more harmful than 1 or 2. The single copepod infected with 4 nematode larvae survived until the end of the experiment.

Nematode Development in the Intermediate Host

Development of larvae in the copepod intermediate host occurs in the host's haemocoel. Larvae are expected to molt twice, passing from first to second-stage (L1 – L2) and then second to third-stage (L2 – L3). Measurements are given in microns: mean followed by range in parentheses.

First-stage Larvae, Ex Utero *and in Copepods*

The following description in based on 5 first-stage larvae collected from female worms or fish gills, 2 from copepods held for 2 days post exposure at room temperature, and 2 from copepods held at 15°C for 6 and 7 days. The latter two groups' measurements fell within the range of a given measurement for larvae *ex utero* or from gills hence I consider them together. First stage larvae 588 (450-709) long. Width at nerve ring 27 (18-47), at anus 23 (18-35). Cuticle forms tooth-like swelling in dorsal buccal area (Figure 3.3A). Nerve ring 83 (65-114) from anterior end, or 14.8% (12.7-16.5) along body length. Excretory pore posterior to nerve ring, 95 (76-111) from anterior extremity or 17.8% (16.7-19.3) along body length. Excretory gland ends anterior to oesophageal-intestinal junction. Oesophagus undivided, 181 (121-224) long and 11 (6-28) wide at anterior extremity. Larvae from copepods are characterized by marked oesophageal-intestinal valve. Intestine 187 (129-216) long; its lumen may be narrow or wide. Genital primordium not always discernible (identified in 3 of 9), ventral at 175 (159-185) from anterior extremity. Genital primordium 29.3 - 34.6% along body length from anterior extremity, and 22 (8-32) long. Rectum 57 (41-86) long. Ratio of intestine and rectum to oesophagus 1.41 (1.04-1.93). Tail 122 (95-144) long and very attenuated, narrowing well before the end (Figure 3.3B).

L1-L2 molt

First to second-stage molting larvae were recovered days 4 – 11 at 15°C (6 individuals) and day 5 at room temperature (1 individual). Length 514 (403-631). Width at nerve ring 28 (22-43), at anus 21 (18-31). Molting cephalic denticle evident. Excretory pore posterior to nerve ring with excretory gland ending anterior to oesophageal-intestinal junction. Oesophagus 156 (102-185) long, 9 (8-11) wide at anterior end, not noticeably differentiated into muscular and glandular portions except in one specimen: oesophagus muscular and glandular portions 85 and 87 long respectively. Oesophageal-intestinal valve well developed. Intestine 215 (121-322) long, cells distinct, lumen variable in width. Genital primordium evident in 4 of 7 specimens: 25 (14-49) long, located ventrally, bimodal for longitudinal position: 38.5% (31.4-47.3) along body length in 3 specimens, 56.5% along body length in 1 specimen, well posterior to oesophageal-intestinal junction. Bimodality could represent different sexes. Rectum 57 (24-81) long, lumen often expanded with molting epithelium. Ratio of intestine and rectum to oesophagus 1.75 (1.36-2.01). Tail 78 (62-112) long. First-stage's elongated tail is replaced with a stubby one; in 6 specimens molted L1 tail extends to 119 (110-134) (Figure 3.3C).

Second-stage larvae

I recovered second-stage larvae from 2 copepods that had harboured larval nematodes for 8 days at room temperature. Both specimens were damaged and incomplete. One measurable specimen at least 778 long without tail (missing). Head simple, without cephalic swelling, buccal opening subtriangular (Figure 3.3D). Oesophagus 11 wide at anterior end. Other specimen (anterior portion only) 19 wide at the nerve ring. Excretory pore 62 and nerve ring 63 from anterior end. Excretory gland ends near junction between muscular and glandular oesophagus. Muscular and glandular portions of oesophagus both 119 long each. Oesophagus 8 wide at anterior end (Figure 3.3E).

Third-stage larvae

Larvae recovered on developmental day 14 at room temperature (1 complete specimen, 2 damaged specimens) and 15 to 16 at 15°C (3 specimens) were third-stage. Body filiform, tail conical. Head with two pairs of cephalic papillae and a single apical papilla. Lateral aspect of oral opening flanked by cuticle in the form of an inverted "v" (Figure 3.3F). Length 1146 (918-1359). Width at nerve ring 27 (23-33), at anus 17 (15-21). Nerve ring 96 (84-111) from the anterior, or 8.5% (7.1-10) along body length. Excretory pore posterior to nerve ring, at 134 (119-160) from anterior, or 11.8% (10.8-13.4) along body length. Excretory gland ends between muscular-glandular oesophageal junction and oesophageal-intestinal junction. Oesophagus 8 (7-10 wide), well differentiated into muscular and glandular portions 226 (176-295) and 352 (290-462) long respectively. Oesophageal-intestinal valve well developed. Intestine 395 (312-454) long. Genital primordium 649 (563-743) from anterior extremity, well posterior to oesophageal-intestinal valve, at 58.7% (54.8-61.3) along body length in 3 of 4 specimens, adjacent to oesophageal-intestinal valve, at 51.7% along body length in 1 specimen. Rectum 62 (59-64) long. Ratio of intestine and rectum to oesophagus less than previous stages, at 0.839 (0.805-0.927). Tail 89 (78-108) long (Figure 3.3G).

Effect of temperature on development

There was much variation in rate of development between individuals; first-stage and molting worms were recovered anywhere from 4 to 11 days at 15°C. At least some larvae reach third-stage by day 14 at room temperature and by day 15 at 15°C. Temperature had no effect on length of resultant L3 infective larvae (2-sample t-test on mean lengths at uncontrolled room temperature and 15°C, $t_4 = 0.39, p = 0.71$). To further compare rate of larval development at 15°C and room temperature, I looked for peak frequencies of developmental days on which

molts were observed (loose cuticle indicating a molt). At 15°C the first peak, presumably the first molt, occurred on the 7th day. No second peak in molting was observed, although molting was observed steadily until the end of the experiment (Figure 3.4). At room temperature (19.4 - 24.4°C) the first peak was observed on the 5th day and another on the 12th day (Figure 3.4). Therefore an increase of approximately 5°C advances the first molt by two days but may be less important as the second molt approaches.

Discussion

Intermediate host in nature

Tigriopus californicus successfully serves as intermediate host for *Pseudodelphis oligocotti* in the laboratory. I do not know whether *T. californicus* serves as an intermediate host of *P. oligocotti* in nature. None of the wild-caught copepod species were naturally infected with nematode larvae, but this is not surprising considering the ephemeral nature of this stage of development and the vastness of the habitat to survey. *Tigriopus californicus* is therefore our best lead, but its usefulness as an intermediate host for *P. oligocotti* in nature would depend on the potential for contact between *T. californicus* and *P. oligocotti* first-stage larvae, and between infected *T. californicus* and *A. flavidus*. *Tigriopus californicus* occurs in rocky supralittoral splashpools from Alaska to Baja California. *Apodichthys flavidus* are epibenthic and range in distribution from shallow subtidal to tidepools above the low-tide horizon (Yoshiyama 1981, Yatsu 1981, Hay et al. 1989). The potential for contact appears low. However, *T. californicus* may make excursions into penpoint habitat: the marine environment regularly inundates some splashpools (Metaxas and Scheibling 1993). Laminar flow of splashpool-derived water has been observed at nearshore depths within a couple of centimeters above the substrate (Jeff Marliave, personal observation). *Apodichthys flavidus* may also make excursions into *T. californicus*' habitat: splashpools are known to host the

occasional fish (Lewis et al. 1998) — fish predation impacts *Tigriopus californicus* abundance in Washington tidepools (Dethier 1980).

Whether or not *A. flavidus* encounters *Tigriopus californicus* often, *A. flavidus* obviously encounters *Pseudodelphis oligocotti* at a sufficient rate to sustain *P. oligocotti*'s life cycle in nature. *Pseudodelphis oligocotti* is a probably a generalist at the intermediate host level. It is common for a parasite's host range in a laboratory setting to differ from its host range in nature since in nature a parasite may not encounter the full set of hosts it could possibly use (Solter and Maddox 1998). Non-specificity at the intermediate host level (even when a parasite may be specific at the definitive, or final, host level) has been observed among many parasitic nematodes. *Avioserpens taiwana* (Dracunculoidea: Dracunculidae) infects several genera at both the intermediate and final host level (Wang et al. 1983). *Avioserpens mosgovoyi* infects multiple species of freshwater copepod intermediate hosts and 2 genera of waterbird final hosts (Supryaga 1965, Supryaga 1971 in Moravec 1994). *Philometra obturans* (Dracunculoidea: Philometra) develops in several genera of copepods, 2 genera of fish paratenic hosts but only one final host (Moravec 1978). *Anguillicola crassa* infects a copepod and an ostracod intermediate host (Petter et al. 1990). In fact, Anderson (1992) proposed that generalist requirements at the intermediate host level was necessary for the transfer of terrestrial parasitic nematodes from terrestrial to aquatic invertebrates and subsequently to other aquatic hosts.

This is the first detailed account of copepods ingesting parasitic nematode larvae in a marine system (Janet W. Reid, National Museum of Natural History, Smithsonian, personal communication). Bashirullah and Ahmed (1976) observed active ingestion of *Camallanus adamsi* larvae on the part of freshwater copepods; other authors acknowledge infection of copepods by ingestion but do not describe it (see Moravec 1994 and Anderson 1992 for life cycle reviews). First-

stage *P. oligocotti* larvae sink after release, consistent with most other members of the Dracunculoidea, which cannot maintain themselves in the water column (Anderson 1992, Clark 1994). Larvae are then ingested whole by *T. californicus*. *Tigriopus californicus* is a generalist reported to ingest a variety of things (detritus, fecal pellets, diatoms, bacteria, organic flocs, protists, small crustaceans) (Lewis et al. 1998) all of which are smaller than first-stage *P. oligocotti* larvae. Elements of *T. californicus*' oral cone (such as the labrum, labium and mandibles) are armed with silica-based teeth and/or spinous projections and setae for the grinding or positioning of food (Lewis et al. 1998). These structures may however lack the musculature to break up something as large as a nematode. Grinders act instead like friction pads to move nematodes along. The gnathobases (parts of the mandible) move in opposition and, in addition to bearing teeth for breaking or holding, bear spinous process that are used to move food or remove, tooth-brush style, food particles from larger objects. Gnathobases thus act to move food continuously into the oesophagus (Lewis et al. 1998).

The average intensity of laboratory copepod infection by *Pseudodelphis oligocotti* does not appear to be as high as that of other members of the Dracunculoidea, nor does it reach the same peak intensities. *Tigriopus californicus* is a small copepod (1 – 1.4 mm in length, Powlik et al. 1997, Al Lewis, personal communication): a single larva appears to fill the host's haemocoel. Copepods infected with *Philometra obturans* generally contained 2 –3 worms but could range up to 12, higher intensities occurring in larger species of intermediate host copepods (Moravec 1978). Larger species Moravec studied were 1 to 2 mm in length (females, *Eucyclops* spp.) and 2 to 4 mm (females, *Macrocyclops fuscus*) in length (Wilson 1932). *Philonema oncorhynchi* mean intensity in *Cyclops bicuspidatus* is 2 but ranges up to 18 (Ko and Adams 1969). *Cyclops strenuus* (only species for which measurements were available and are presumably indicative of the genus) ranges in length from 0.95 to 1.35 mm (females) (Wilson 1932). Average intensity for

Camallanus adamsi is 4 larvae per copepod but ranges up to 7 (Bashirullah and Ahmed 1976). For one of the copepod species Bashirullah and Ahmed infected, *Mesocyclops leukarti*, measurements for the genus range from 1 to 1.5 mm (Wilson 1932). In addition, number of larvae in a given size of exposure chamber may influence mean intensity of infection. Infections by *Philometra ovata* varied in intensity depending on size of the exposure chamber: more intense infections resulted when copepods were held in closer quarters with infected larvae (Moravec 1980). However, by holding constant the number of source *Pseudodelphis oligocotti* and volume of the exposure chamber, it is unlikely that density of infective larvae varied enough to detect its influence.

Rates of *T. californicus* infection in the laboratory depended primarily on maturity of female worms, and were higher after copepods had been prestarved. In other studies, rates of successful infection of copepod intermediate hosts by *Philometra obturans* were influenced by number of infective larvae in the exposure chamber (Moravec 1978). This did not appear to be the case herein (Table 3.1), although the range of variation examined was low. *Philomena oncorhynchi* infected copepods at a higher rate (80-90%) when exposure time was extended from 24 to 48 hours (Ko and Adams 1969). However, exposure time had no affect on transmission rates in this study. This is not surprising, since 83.3% of free-swimming larvae were dead by day 2 of exposure (Table 3.1). Beyond 2 days transmission rate must reach a plateau.

Maturity of adult female nematodes greatly influenced percentage of successful copepod infections by larvae *ex utero*. Ovoviviparous *Pseudodelphis oligocotti* females contain a range of developmental stages from eggs to first-stage larvae. As females mature, a greater proportion of developing embryos have matured to first-stage larvae. Perhaps there is a gradient of developmental readiness amongst larvae *in utero* that is both a function of female age and longitudinal position

along the uterus from ovary to vulva. Even if one manipulates the number of fully mature female source worms, larval numbers would be diluted proportionally with uninfective larvae and, although increasing absolute numbers, would not affect the rate at which copepods encounter infective larvae.

Proportion of infected *Tigriopus californicus* dead is not appreciably higher over the course of developmental days than proportion of uninfecteds dead, except perhaps at the end of an experiment when proportion dead increases to approximately 10% dead in singly and doubly infected copepods (Figure 3.2). Moravec (1978) observed greater mortality in infected copepods than in uninfecteds. In his study, mortality rates were greatest at the beginning of the experiment. Heavily infected copepods (greater than 2 worms) have been observed to remain on the bottom more than those with lighter infections (Moravec 1978, Moorthy 1938). Infected copepods lying on the bottom may suffer from insufficient aeration (Moorthy 1938). In this study, finger bowls provided the optimal shallow environment for copepod growth. Thus mortality is likely due to direct rather than indirect behavioural effects of infection. Worms in copepods moved around vigorously, often jostling the host's eyespot or compressing its intestine. I suspect them to be more harmful as they grow larger, although the data presented in this study are insufficient to draw this conclusion.

There was considerable variation in rate of development among individuals, as indicated by the occurrence of both first-stage, and molting worms from day 4 to day 11 at 15°C. *Philomena oncorhynchi* shows considerable variation in rate of development between individuals (Ko and Adams 1969). Nonetheless, *Pseudodelphis oligocotti* developed more rapidly than *Philonema oncorhynchi*: at a given temperature (15°C), the former reached third-stage in 15 days whereas the latter reached third-stage in a minimum of 21 days. Of the Dracunculoidea, members'

of the genus *Dracunculus* development times are most similar to that of *Pseudodelphis oligocotti* (Anderson 1992).

In *Pseudodelphis oligocotti* an increase of approximately 5°C advances the first molt by two days but may be less important as the second molt approaches. However, there was considerable variation between individuals. Many dracunculoid life cycles have delayed rates of development with decreasing temperature (see Anderson 1992 for review). An increase of 5°C almost doubles the rate at which molting occurs in *Philonema oncorhynchi* (Ko and Adams 1969). More data are required to quantify the effect of temperature on *P. oligocotti*'s rate of development. If a change of 5°C alters the rate of development by 2 days, *P. oligocotti*'s developmental time in nature is probably longer than approximately 15 days. Given that local seawater temperature is between 9 and 11°C, 17 to 18 days may be necessary to develop to the third larval stage assuming that the effect of temperature on development is linear. The higher experimental temperatures used in this study may have made it difficult to recover second-stage larvae. Very few second-stage specimens were found relative to the number of first and third-stages. Perhaps the second stage is very short: the accelerating affect of higher temperatures would make this stage even less noticeable in copepod surveys.

Pseudodelphis oligocotti larvae are the first to be described in the family Guyanemidae. First-stage larvae have a cephalic dorsal swelling characteristic of dracunculoids. Larvae are most similar in form to those of *Philonema agubernaculum* and *P. oncorhynchi* (Dracunculoidea: Philometridae). The first larval stage of all three species has a dorsal cephalic swelling, an elongated tail, and a simple undivided oesophagus. I did not find any complete specimens of *P. oligocotti* second-stage larvae, but the third larval stage of all three species has a divided oesophagus with a long multicellular glandular portion. Excretory pore and gland are in similar positions. Body lengths of larval stages are also similar

(Ko and Adams 1969, Vik 1964 for descriptions). Other members of the Philometridae do not resemble *P. oligocotti* as much. First-stage larvae of *Philometra abdominalis, Ph. obturans, Ph. ovata* all have conical tails and blunt heads, but the former two each have a dorsal cephalic swelling similar to *P. oligocotti* whereas the latter does not. *Philometra* spp. larvae have a few large oesophageal glands with giant nuclei at the base of the oesophagus, which *P. oligocotti* does not. Larvae of *Philometra* spp. are similar to *P. oligocotti* in length (Moravec 1977, Moravec 1978, and Moravec 1980). The first larval stage of another philometrid, *Ichthyofilaria canadensis,* is more different from *P. oligocotti* than *Philonema* spp. are: it is sheathed, with a blunt head and tail, and half the size (Appy et al. 1985). *Philonema* spp. larvae resemble those of *P. oligocotti* more than do larvae of dracunculoids in the families Dracunculidae and Anguillicolidae. Larvae of *Avioserpens* spp. have conical tails similar to those of *P. oligocotti*, but also have an extremely long oesophagus whose posterior portion consists of three long oesophageal glands with prominent nuclei. Lengths at third-stage are half the size of *P. oligocotti* (Moravec and Scholz 1990, Moravec 1994). First larval stage of dracunculoid *Dracunculus medinensis* is similar in size, has as a dorsal cephalic swelling and an oesophagus of similar proportions to *P. oligocotti*, but unlike *P. oligocotti* it has a ridged cuticle and very attenuated tail (Moorthy 1938). Anguillicolid *Anguillicola crassa* larvae are much more stout than those of *P. oligocotti* (Petter et al. 1990).

Table 3.1 Summary of experimental infections of *Tigriopus californicus* with first-stage larvae of *Pseudodelphis oligocotti* from *Apodichthys flavidus* originating from Lumberman's Arch/Figurehead Point, British Columbia.

Infection Date	Adult Worm Dose[1]	Days Exposed[2]	Copepod Type	Percent Infected[3]	Total # copepods
February 5, 1993	0.5	4	*Tigriopus californicus*	27.4 (11.1-43.8, n = 2)	41
May 6, 1993	1	4	*T. californicus*	14.3	8
May 21, 1993	3	4	*T. californicus*	0	7
August 17, 1993	1	1	*T. californicus*	45	11
August 18, 1993	1	2	*T. californicus*	30.8	13
May 27, 1994	2	3	*T. californicus*	8	25
June 6, 1994	1.33	4	*T. californicus*	2.5 (0-5, n = 2)	61
June 21, 1994	0.33	2-6	*T. californicus*	34.1 (23.8-47.6, n = 3)	72
July 20, 1994	1.5	6-7	*T. californicus*	1.25 (0-2.5, n = 2)	55
August 10, 1995	2.25	1-4	*T. californicus*	11.8 (0-22.4, n = 4)	199
April 19, 1996 [4]	1.6	2-4	*T. californicus*	9.64 (0-16.7, n = 5)	168
May 1, 1996 [4]	0.33	1-4	*T. californicus*	46.8[5] (40-57.1, n = 3)	87
April 17, 1997 [6]	1	2	*T. californicus*	0	21
April 28, 1997	1	2	*T. californicus*	41.9[7]	31
May 27, 1997 [8]	1	2-3	wild-caught spp.	0	49
July 21, 1997	1	2	*T. californicus*	0	47

[1] Female worms pooled and their larvae divided evenly between replicates if applicable

[2] Multiple days of exposure refer to replicates.

[3] Range and sample size refer to replicates (given below where applicable)

[4] Trial held at 15°C instead of room temperature

[5] Copepods withheld food for 5 days.

[6] Trial held at 17.5°C instead of room temperature.

[7] Copepods withheld food for 3 days.

[8] Trial held at 10°C instead of room temperature.

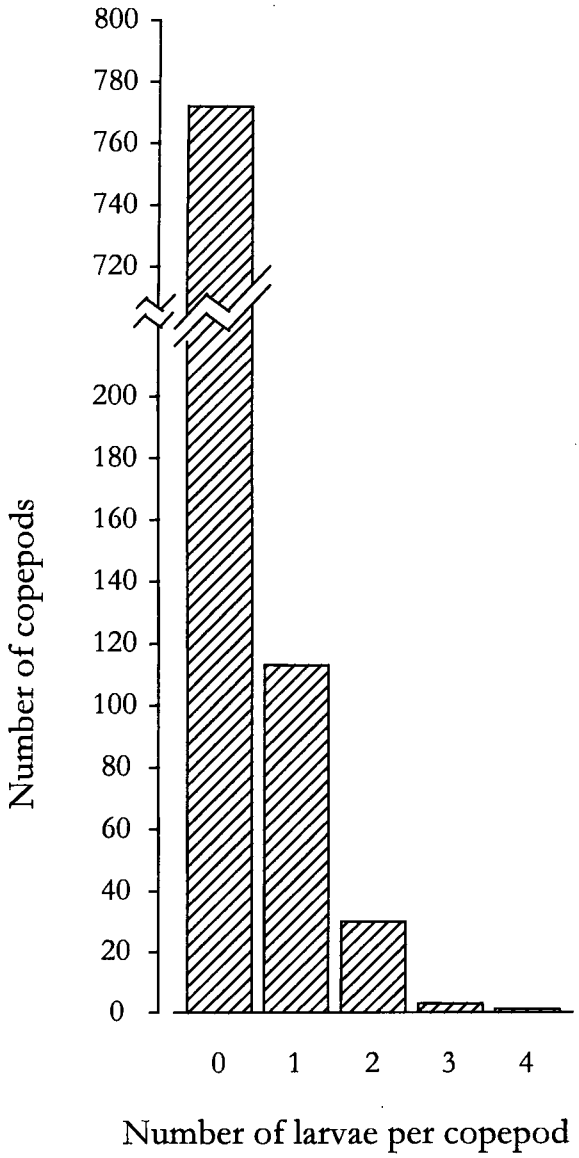

Figure 3.1 Frequency of infections ranging in intensity from zero to 4 larval nematode *Pseudodelphis oligocotti* parasitizing copepod *Tigriopus californicus* as a result of experimental infections.

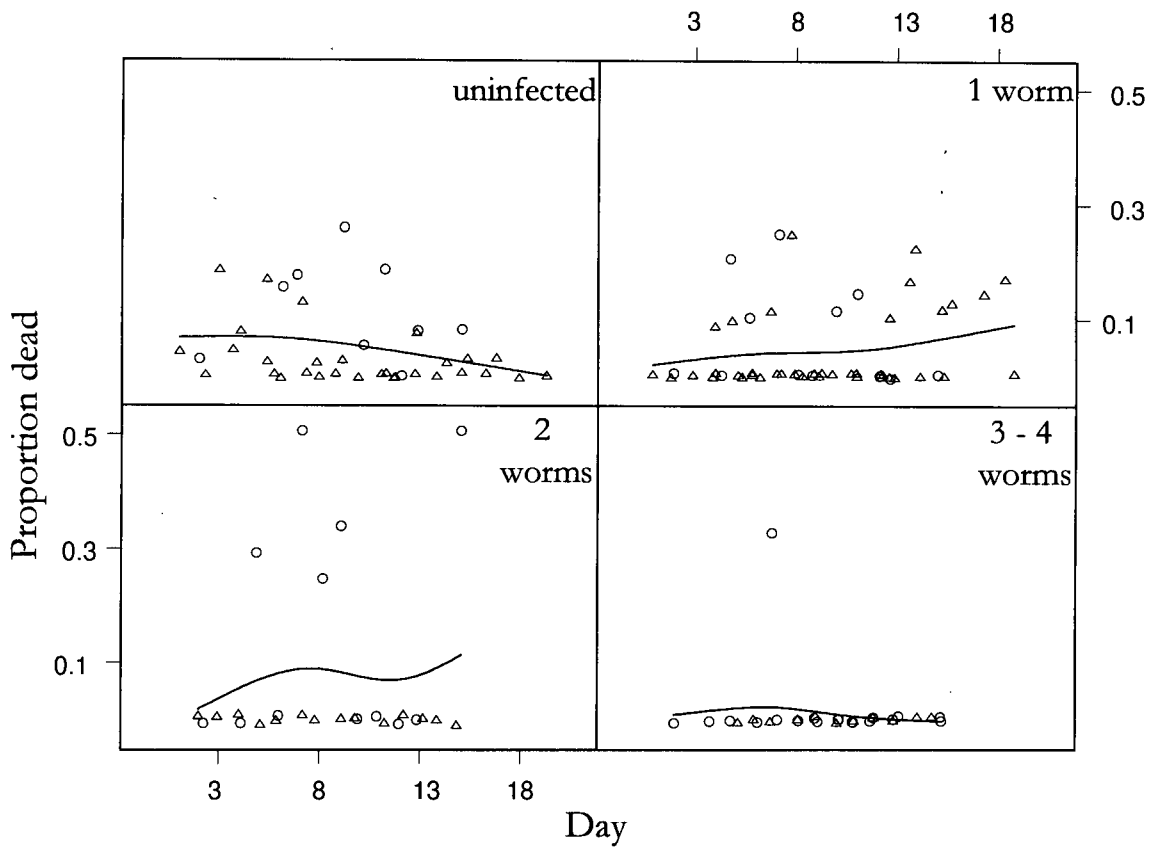

Figure 3.2 Daily instantaneous mortality rate (proportion dead) of copepod *T. californicus* infected with *Pseudodelphis oligocotti* larvae. Symbols code for dishes maintained at two different temperatures: open circles represent those dishes maintained at 15°C, and open triangles represent those dishes maintained at uncontrolled room temperature (19.4 to 24.4°C). Lines are cubic spline models of the data, where estimates are based on 0.5 to 3% of the data at a time depending on intensity category, with 3 degrees of freedom. Days are numbered from the beginning of an experimental infection.

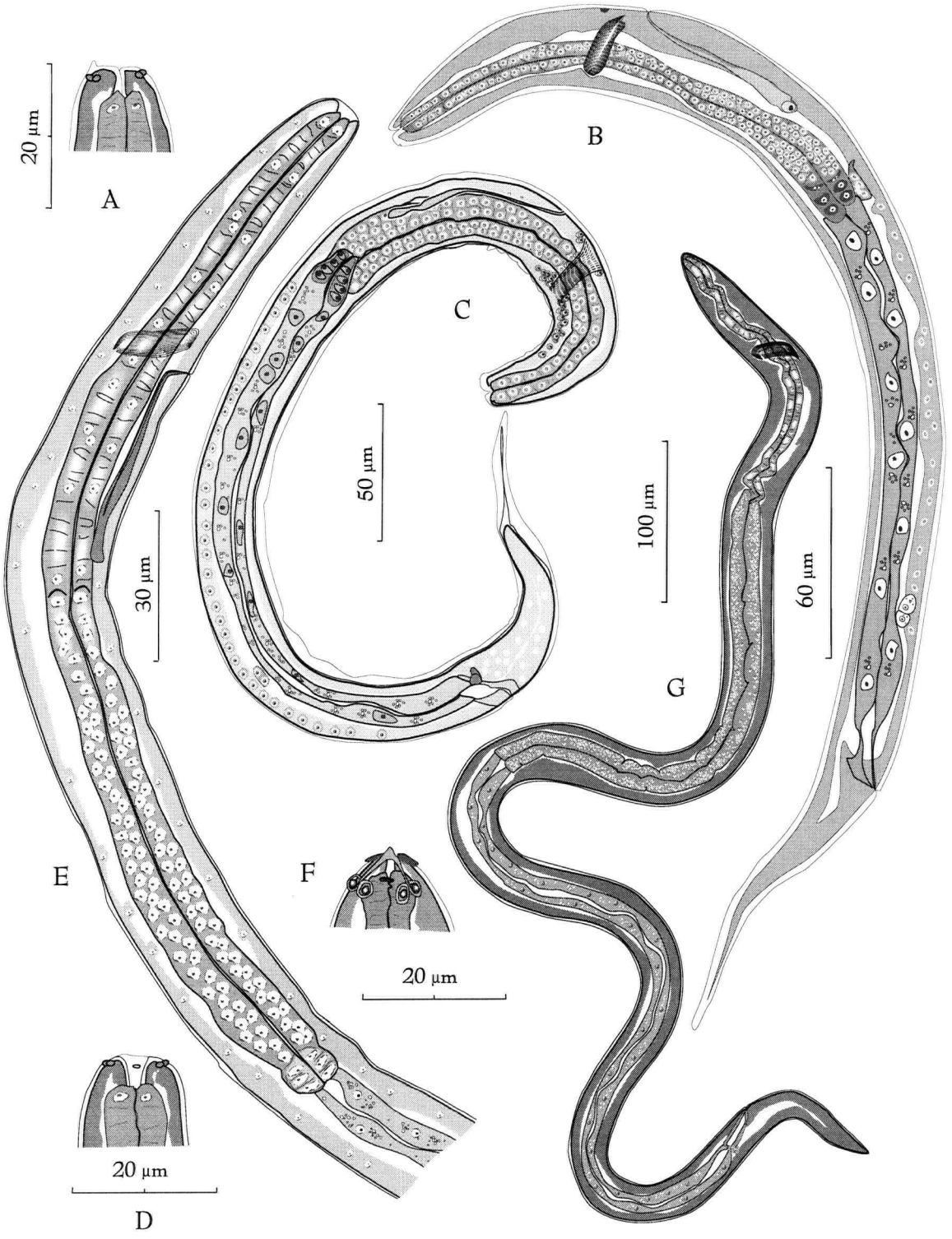

Figure 3.3 First to third stage larvae of *Pseudodelphis oligocotti* recovered from experimentally infected *Tigriopus californicus*. A. First-stage larva from day 7 copepod, cephalic lateral view, B. First-stage larva from day 7 copepod, lateral view, C. First to second-stage molt from day 6, D. Second-stage larva from day 8 copepod, cephalic lateral view, E. Second-stage larva, anterior end from day 8 copepod, F. Third-stage larva from day 14 copepod, cephalic lateral view, G. Third-stage larva from day 14 copepod.

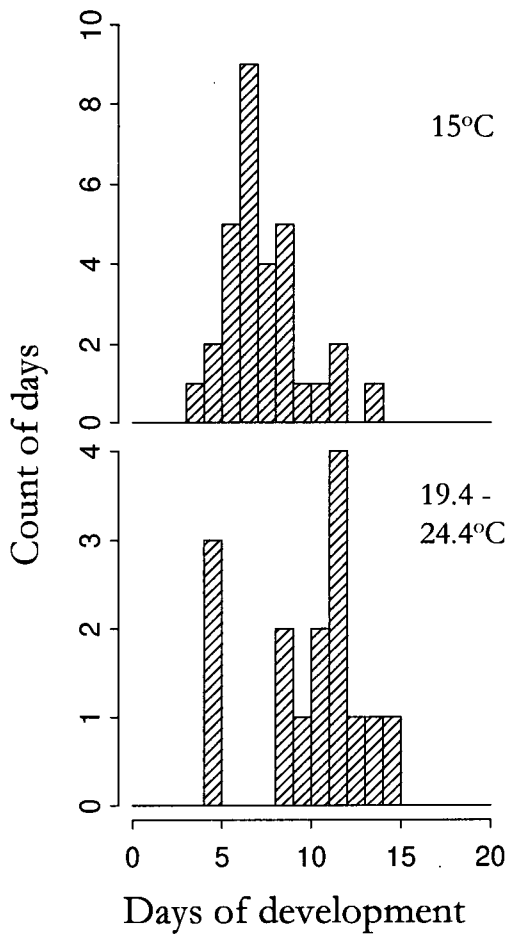

Figure 3.4 Frequency distribution of developmental days on which molts of larval *Pseudodelphis oligocotti* developing in live *Tigriopus californicus* were observed, at two different experimental temperatures. Data does not include sacrificed copepods and their larvae.

CHAPTER 4: DEVELOPMENT OF *PSEUDODELPHIS OLIGOCOTTI* (NEMATODA: DRACUNCULOIDEA) IN THE FINAL HOST, PENPOINT GUNNEL (*APODICHTHYS FLAVIDUS*)

Introduction

A parasite's reproductive success depends on its transmission among hosts, which in turn depends on a parasite's ability to release its transmitting stages into the external environment. Zooparasitic nematodes solve this problem in a variety of ways; those that live in the host's intestine simply pass their eggs or larvae out with the host's feces; many tissue dwellers have elaborate migratory phases to reach the external environment or may be transmitted by vectors (Adamson 1986, Anderson 1992 for review). Like all dracunculoid nematodes, *Pseudodelphis oligocotti* (Guyanemidae) lives in an extra-intestinal site in the final host, *Apodichthys flavidus* (Perciformes: Pholidae) and is presented with the difficulty of getting its larval stages to the external environment.

Pseudodelphis oligocotti lives in the blood stream of its final host. Other dracunculoids that live in the host's blood stream transmit in two different ways: *Molnaria* and *Skrjabillanus* spp. (Dracunculoidea: Anguillicolidae) live in the host's mesenteries and serosa of various organs; first-stage larvae are carried in blood to the skin where ectoparasitic brachiurans ingest larvae with their blood meal (Tikhomirova 1971, Tikhomirova 1980, Tikhomirova 1975, see Adamson 1986 and Anderson 1992 for reviews). Gravid females of *Philometra obturans* dwell in the heart, but migrate from the ventral aorta to the gill arteries to gain access to the external environment; larvae are released directly into the water where they are ingested by free-living copepods (Molnar 1976). *Ichthyofilaria* spp. live on the surface of the liver and in the mesentery; first-stage larvae circulate in the host's blood and body fluids, but how they reach the external environment is unknown

(Appy et al. 1985). Appy et al. (1985) suggest that larvae may be ingested by haematophagous arthropods or exit the gills themselves. In Chapter 3 I demonstrated that a free-living copepod (*Tigriopus californicus*) could serve as an intermediate host. This chapter addresses the problem of how *P. oligocotti* get its larval stages into the external environment where free-living copepods can ingest them.

In this chapter I make detailed observations by stage and tissue site of the course of *Pseudodelphis oligocotti* infection in penpoint gunnel (*Apodichthys flavidus*). I report on attempts to experimentally infect specific parasite-free *A. flavidus* by feeding them copepods infected with *P. oligocotti*. This represents the first experimental infection of a marine host with a dracunculoid. I demonstrate that *P. oligocotti* lives in the body cavity and blood system of its fish host; larvigerous females release larvae into the blood, which carries larvae directly to the gills. Larvae breach the gills to gain access to the water. Larvae do not enter the fish's general circulation, so there is no opportunity for transmission by haematophagous arthropods. Free-living copepods act as intermediate hosts (Chapter 3).

Methods

Field collections

I collected penpoint gunnel, *Apodichthys flavidus* by dragging a pole seine (length 2m, height 1.5m, diagonal mesh size 11 mm) through subtidal eelgrass beds, and by overturning rocks in intertidal zones adjacent to eelgrass beds. I sampled 427 *A. flavidus* over 6.5 years from a variety of coastal sites on the mainland and Vancouver Island, British Columbia (Table 2.1 and Figure 2.1, Chapter 2). I sampled many of these sites only once; others I repeatedly sampled. Lumberman's Arch and Figurehead point were sampled on spring tides from: spring to mid-winter in 1991, 1992, and 1995; throughout the year in 1993 and

1994; sporadically in spring, summer, and fall 1996-1997; and spring 1998. Point Roberts and Roberts Bank were sampled on spring tides, twice in the spring of 1993, throughout the year in 1994, and sporadically from 1995 to 1997. The latter site was unique in that *A. flavidus* caught there were not infected with *Pseudodelphis oligocotti* and Roberts Bank served as a source of uninfected hosts for experimental infections.

Once captured, *A. flavidus* befell one of three fates: 1.) I necropsied some along with the other fish species for data on the adult *Pseudodelphis oligocotti*'s prevalence, intensity, and development (see Chapter 2), 2.) I reserved certain individuals from Lumberman's Arch/Figurehead Point to use as a source of *Pseudodelphis oligocotti* for experimental infections. These were held at the Vancouver Public Aquarium. 3.) I reserved other individuals originating from Roberts Bank until I was ready to experimentally infect them. These were isolated into separate tanks at the Vancouver Public Aquarium. Neither copepods nor parasites can move between tanks because all seawater is passed through a 2-foot thick, #16 mesh sand gravity filter before recirculating (John Rawle, Director of Seawater Systems, Vancouver Public Aquarium, personal communication). Sand grains that make up #16 mesh sand range in diameter from 1.18 mm down to 0.65 mm (50-70% falling in the latter category) (Target Productions Ltd., personal communication). Both sources assure me that, in their experience, nothing as large as a first-stage larva of *P. oligocotti* (average 0.027 mm in diameter) would get through this filter.

Necropsies and staging of worms

Before dissection I measured fish total length and approximated the age of *A. flavidus* from length-frequency distributions for all those caught in a particular month (see Devries and Frie 1996 for standardized methods).

I necropsied *A. flavidus* for *Pseudodelphis oligocotti* according to the dissection protocol given in Chapter 2. With a hematocrit, I took blood samples from base of the fish's tail to look for circulating stages of *P. oligocotti.* I broke the contents of the hematocrit into a petri dish and examined its contents with a dissecting scope. All worms recovered were held in 0.67% sodium chloride (NaCl) until I counted, sexed and staged them. *Pseudodelphis oligocotti* develops through three stages in the fish host, each separated by a molt: third-stage larval (L3), which is the stage acquired from the copepod intermediate host, fourth stage larval (L4), and fifth stage (L5), the adult. In the case of *Pseudodelphis oligocotti*, sexes became discernible late in the fourth stage. Sexing adult nematodes can be done with the naked eye: males have an obvious coiled tail with a prominent spicule. Adult gravid females are grossly enlarged and have ovaries that contain developing embryos or first-stage larvae (larvigerous). Distinguishing subgravid adult females from later juvenile stages, and staging juveniles themselves, requires the use of a microscope. The presence of a patent vulva (open to the exterior and functional) marks an adult female. A vulva that is prepatent, or closed, indicates that the worm is a fourth stage female. Worms with an undifferentiated but extensive gonad were still termed fourth stage but could not be sexed. The occasionally molting fourth to fifth stage worm confirmed how long a fourth stage gonad might be. The smallest worms ever found in penpoint were considered third-stage; gonads consisted of a few cells. I was not always able to differentiate between third and fourth stage nematodes with small, undifferentiated gonads; for the purpose of analysis I combined larval stages of worms into a single category – L3/L4. I never observed a third to fourth stage molting nematode.

After staging and sexing worms, I fixed most in a heated solution of 70% ethanol, 5% glycerine, to which a few crystals of phenol per litre had been

added; many mature female nematodes that were larvigerous I set aside for experimental infections of copepods (Chapter 3).

Tissue location and rate of larval host exit

During host dissections, I made detailed observations as to which tissue sites different stages occupied. Presence of larvigerous females predominantly in a particular tissue will be suggestive of the site of egress. The presence of first-stage larvae *ex utero* in a particular host tissue will confirm it. Since the gills were suspected as the site of egress, for each fish host I examined one set of gills under the light microscope with transmitted and incident light. The other set of gills I preserved in 10% buffered formalin and set aside for scanning electron microscopy (SEM).

Occasionally I reserved some *A. flavidus* as sources of infection. I wanted to know whether they were transmitting first-stage larvae (to ensure that worms were sufficiently mature to infect copepods – see Chapter 3). To ultimately measure the rate at which *A. flavidus* was shedding larvae, I first weighed a 4-liter bucket of sterile filtered seawater (see Chapter 3 for methods of sterilization and filtration), added the fish (rinsed first with seawater), re-weighed fish and bucket, and subtracted the weight of the bucket to derive the fish weight. After a certain amount of time I rinsed the fish and transferred it to another bucket of fresh sterile filtered seawater. I filtered the recently vacated seawater using 54-micron mesh and examined the filtrate microscopically for L1 larvae. I repeated this procedure 2 or 3 times, rinsing the fish between occasions so as to dislodge any larvae from the previous trial.

Histopathology

To confirm the host tissue distribution of various stages of parasite, and to assess host tissue reaction to infection, I preserved a sample of host organs in 10% buffered formalin and set them aside for histological treatment. Livers for

histological examination were selected from hosts that were a range of different sizes: 110 to 285 mm in length. In total, I examined 14 *A. flavidus* livers histologically by slide-mounting cross-sections of wax-embedded samples stained with hematoxylin and eosin.

Experimental infections with *Pseudodelphis oligocotti*

Lab rearing

Fish that were not dissected immediately after collection were held live at the Vancouver Public Aquarium, in separate tanks according to their origin. Lumberman's Arch penpoints served as a source of larvigerous female nematodes for experimental infections. *Apodichthys flavidus* from Roberts Bank was consistently free of infection; before each experimental infection, I dissected a random subsample of the captive population to confirm that they had remained so. Seawater filters at the Vancouver Public Aquarium are fine enough to eliminate intermediate host and nematode larva movement between tanks (John Rawle, Director of Seawater Systems, Vancouver Public Aquarium, personal communication). Aquarium staff fed fish live brine shrimp once a day. One day prior to an experimental infection, staff withheld food from uninfected gunnel so that they would be motivated to eat experimentally infected copepods.

Transmission Experiments

Nematode larvae become infective to fish final hosts at the third stage of development, L3. After I had observed two waves of molting separated by a growth phase in the nematode population infecting copepods (Chapter 3), I fed the surviving copepods to uninfected *A. flavidus* originating from Roberts Bank. In addition to subsampling captive penpoints to confirm that they were infection-free, in later trials I paired each experimentally infected fish with a control fish. This was done to ensure that infections were not being acquired from the Aquarium water itself.

The number of infected *T. californicus* remaining alive after their nematodes developed to the third infective stage dictated the number of *A. flavidus* (penpoint gunnel) infected. I attempted to give each fish a dose of 2 to 3 infected copepods. I used feeding chambers to isolate experimental *A. flavidus* and contain any infected *T. californicus* that were not eaten. Feeding chambers were large shallow dishes, 11" diameter by 3" deep, with darkened bottoms, lids and sides, into each of which I placed a penpoint gunnel 5 - 10 minutes before a feeding attempt so that it could acclimatize. I used two different methods of feeding infected copepods to *A. flavidus*. In one, I loaded an infected copepod into a disposable glass pipette whose tip had been painted red with nail polish. This was dangled in front of the penpoint gunnel. As the fish gulped at the red pipette tip, I injected the copepod into its mouth. Copepods that were not swallowed were recaptured for another attempt. Captive *A. flavidus* raised on brine shrimp are occasionally uninterested in ingesting the diminutive *T. californicus*: amphipods are more similar to brine shrimp in size. If repeated attempts using the above method failed, I employed a second method. I isolated and left to acclimatize *Melita californica* amphipods (captive colonies standard supply at the Vancouver Public Aquarium). As above, I loaded an infected copepod into a glass pipette and released it into the amphipod's path. The amphipod usually ate it within a few minutes. Using a turkey baster, I then released the amphipod into a feeding chamber with *A. flavidus*. Directing it into the penpoint's mouth was unnecessary; fish would ingest the amphipods within a few minutes.

I recorded the number of successful feeding attempts and the worm burden supported by each ingested *T. californicus* copepod so that experimental *A. flavidus* worm burden could be predicted. Experimentally infected *A. flavidus* and their paired controls were retired to individual aquaria and held for a variable length

of time, from 2 to 8 months. I attempted experimental infections of *A. flavidus* four times using a total of 9 experimentally infected individuals.

Results

Frequencies of lengths of *A. flavidus* caught in May form a peak with a mode at 35-38 mm; there is an absence of lengths between 61 mm and 80 mm. Hence for this sampling period, 0 to 61 mm is designated the youngest age group, age 0 (Devries and Frie 1996). The smallest infected *A. flavidus* was 80 mm and caught in May. It was infected with a single adult (L5) nematode. The host's size for a May catch places it in the age 1 group. The next smallest *A. flavidus* that was infected was 84 mm and caught in July. It too was infected with adult (L5) nematodes. It is unclear whether an 84-mm fish caught in July is a fast growing young of the year (age 0) or a slow growing yearling (Figure 4.1). Otoliths were not used to age fish due to lack of independent age calibration (Devries and Frie 1996).

The largest *A. flavidus* caught was 322 mm in total length. It was infected with 15 adult nematodes in the body cavity, 5 adult nematodes in the hepatic sinus of the liver along with 5 larvigerous females also in the hepatic sinus. Large fish always contained gravid or larvigerous worms. Many large *A. flavidus* (but less than 262 mm) also supported third and fourth stage larvae of *P. oligocotti* (Figure 4.2, Figure 4.3). Abundance of subgravid adults (L5) was correlated with host length, although host length only explained 20% of the variation in parasite abundance (Pearson's correlation coefficient on log-transformed variables $r = 0.45$, $r^2 = 0.20$, $p = 0.00$ at 160 degrees of freedom). Abundance of larvigerous *P. oligocotti* was also correlated with host length, although host length only accounted for 34% of the variation in parasite abundance (Pearson's correlation coefficient on log-transformed variables $r = 0.59$, $r^2 = 0.34$, $p = 0.00$ at 160 degrees of freedom).

Smaller *A. flavidus* either did not have or had fewer late-stage (L5, gravid with developing embryos, or larvigerous) nematodes, but had slightly more larval stages (L3/L4) than larger fish (Figure 4.2). The largest individuals did not contain larval stages of *P. oligocotti* (Figure 4.3). Larval stages appeared in hosts between 84 and 262 mm. Adult fifth stage (L5) nematodes occurred in the full range of infected host sizes (80 mm – 322 mm). Gravid female nematodes occurred in *A. flavidus* between 120 and 322 mm long (Figure 4.4).

There was little data collected from mid-November to end of March, but when sampling occurred, most stages of nematode were recovered regardless of season (Figure 4.5). First stage larvae (L1) were not surveyed in gills until later in the study so their distribution with time of year is incomplete. Late larval (L3/L4) stages showed a slight peak at the end of summer. This appeared to be followed by a pulse in adult (L5) stages in late November. Adult stages were also abundant from May to September. The abundance of ovigerous, and larvigerous females was fairly consistent throughout the spring and summer.

Each stage of *Pseudodelphis oligocotti* occurs in a particular site within the host. Third and fourth stage nematodes (larvae) were recovered almost exclusively (99.6% of all L3 or L4 worms) from the body cavity (Table 4.1). Similarly, adult males were largely restricted to the body cavity (99.4% of adult males). Adult females, on the other hand, were distributed between the body cavity (24%) and the hepatic sinus (71%). Of the dead worms recovered (stage unknown), 99% were in the body cavity. Ovigerous and larvigerous females were recovered almost exclusively from the hepatic sinus of the liver (95% and 97% respectively). They were also found overflowing into the atrium of the heart and down the common cardinal vein. The hepatic sinus is connected directly to the heart, which is in turn connected directly to the gills: there are no other intervening tissues. Blood flows directly from heart to gills to be oxygenated

(Lagler et al. 1962). First stage larvae (L1) were found primarily in the gill region (89% of L1's recovered). In 20 of 54 hosts examined that had larvigerous females in the liver, first-stage larvae were recovered from gills; they were seen in wet mounts, found in gill wash, seen in cross-section in histological preparations, and also in SEM of gill tissue. A total of 132 larvae were recovered in this manner. No larvae were ever recovered from blood samples taken from the base of the tail. *Apodichthys flavidus* infected with larvigerous females shed these first-stage larvae at a rate of up to 1 every 4 minutes (Table 4.2).

Histological examinations and field-emission scanning electron microscopy confirmed that first-stage larvae recovered from between gill filaments or in gill wash were originating from the blood vessels of the primary gill lamellae themselves (Figure 4.6 and 4.7). *Apodichthys flavidus* gills are similar in structure to those of other teleosts: pairs of gill primary lamellae (or filaments) branch off from the supporting gill arch. Each primary lamella has a row of secondary lamellae running down front and back, perpendicular to the primary lamella (for a diagram refer to Yasutake and Wales 1983). Diameter of the blood vessels in the primary lamellae is 0.036 mm at tip and 0.108 mm at base. First-stage larvae, which are on average 0.038 mm in width (Table 4.3), presumably travel to the very tip of the primary gill lamella. Gills from hosts infected with gravid female nematodes were infiltrated with more immune cells such as macrophages and lymphocytes than uninfected gills. Blood vessel walls were thickened, particularly around the larvae themselves (Figure 4.8).

Histopathology

Samples selected for histological examination varied in intensity and stage of infection from uninfected livers to those containing up to 29 adult gravid female worms. Specimens of *A. flavidus* examined for histopathological changes ranged in length from 110 to 285 mm.

Uninfected livers (2 specimens, one from an uninfected fish, one from a fish with 9 adult nematodes in the body cavity only – a mild infection) had hepatocytes that appear to be organized somewhat concentrically, presumably around blood vessels. Their nuclei were uniform, monomorphic and monochromatic. The cytoplasm of hepatocytes varied in the amount of glycogen or lipid. Hepatocytes from the mildly infected fish were glycogen-rich, whereas uninfected hepatocytes contained more lipid droplets, indicated by clear bubbles in the cytoplasm (Figure 4.9A, Slauson and Cooper 1990).

Infected livers contained gravid female nematodes, which lay somewhat coiled along the length of the fish hepatic sinus. In heavy infections, the host's hepatic sinus was very distended. Infected livers fell into two classes, those with larvigerous females, and those with less-developed females with developing embryos *in utero*. Livers in the former group demonstrated the most severe pathological changes, but within each group the severity of tissue reaction also increased with worm burden. Livers infected with 2 - 8 ovigerous females (3 specimens) and 2 - 7 larvigerous females (4 specimens) showed the least reaction. The amount of cellular lipid compared to glycogen in hepatocytes still appeared variable. Some but not all specimens showed nuclear pleomorphism of the hepatocytes. There was no inflammation of the hepatic sinus epithelium itself, although there was evidence of mild to moderate multifocal chronic inflammation on the surface of the liver (peritonitis) and around blood vessels (perivascularitis). Macrophages were the predominant inflammatory cell, along with the occasional plasma cell and lymphocyte. Livers infected with 31 and 38 ovigerous females (2 specimens) were moderately to severely inflamed at multiple foci. Inflammation was chronic, i.e., macrophages predominated. Hepatocyte nuclei in these livers were pleomorphic and showed variable staining. In one specimen, worms in the hepatic sinus caused pressure atrophy of the surrounding hepatic tissue. Another liver exhibited diffuse infection by

encapsulated first-stage larvae, presumably spilled over from larvigerous female nematodes in the hepatic sinus. Granulomatous lesions had formed in association with these larvae. Livers infected with 21 - 29 larvigerous females (3 specimens) also exhibited multifocal, chronic inflammation. Hepatocyte nuclei were also pleomorphic and the tissue architecture appeared disorganized. I noted pressure atrophy of the liver tissue surrounding the hepatic sinus. In addition, 2 specimens had granulomatous lesions (Figure 4.9B-D).

Experimental infections

Of the four attempts to infect captive uninfected *A. flavidus*, two resulted in infections. One fish examined 61 days post infection harboured 2 male fourth-stage larvae. Larvae were 5.34 to 5.37 mm in length (Table 4.3). Another experimental gunnel examined 33 weeks (231 days) post infection produced 2 fifth stage worms (adult males), also in the body cavity. These worms ranged from 15.64 to 15.91 mm in length. Nematodes collected from experimentally infected fish were of similar dimensions to those collected in the wild of the same stage (Table 4.3).

Discussion

Observations of *Pseudodelphis oligocotti*'s development from necropsies, as well as successful experimental infections indicate that *A. flavidus* is an appropriate definitive host, even though *P. oligocotti* was originally described from type host tidepool sculpin, *Oligocottus maculosus* (Adamson and Roth 1990). Gravid stages of the parasite were restricted in distribution to the hepatic sinus, which is directly upstream from the gills. First-stage larvae were restricted mainly to the gills. This provides evidence that mature *P. oligocotti* release larvae into the blood stream from whence they gain access to the exterior by breaking out of the gills. There

is no opportunity for intervention by a blood-sucking vector in *P. oligocotti*'s transmission.

Necropsy results yielded a distribution of parasite stages within the host from which I deduced the migration and tissue sites of development of *Pseudodelphis oligocotti*. Ingested third-stage larvae presumably penetrate the intestinal wall and migrate into the host's body cavity. They develop through fourth stage to the adult fifth stage in the body cavity. The males remain in the body cavity where they presumably mate with females. Males and unstaged/unsexed dead worms are rarely found outside the body cavity. Adult females migrate to the hepatic sinus. Since males almost never occur there, females must normally be mated before migrating to this site. Perhaps mating induces migration, although there was one exceptional fish specimen in which a third/fourth stage larva migrated to the hepatic sinus. Among certain dracunculoids vaginal plugs are in place after females are mated (Crichton and Beverley-Burton 1975). This was not observed in *Pseudodelphis oligocotti*. Distribution of gravid females (both larvigerous or with developing embryos) in the hepatic sinus of the liver indicates that this is the final site from which first-stage larvae pass to the exterior. Occasionally, gravid females are found in the atrium of the heart and the common cardinal vein. The gill region is directly downstream from the heart, hepatic sinus and common cardinal vein. First stage larvae were restricted to the gill region (132 first-stage larvae recovered from wet mounts) and were never recovered from the general circulation (all blood samples from the tail were negative). Histological cross-sections and scanning electron microscopy confirmed that first-stage larvae break through the blood vessels of the primary lamellae of the gill to reach the external environment. This is the first record of such a method of transmission. Similar to *P. oligocotti*, some blood-dwelling dracunculoids (*Molnaria* and *Skrjabillanus* spp.) release first-stage larvae into the blood of the host, but in these cases larvae are carried to the skin where ectoparasitic brachiurans ingest them

with a blood meal (Tikhomirova 1971, Tikhomirova 1980, Tikhomirova 1975, see Adamson 1986 and Anderson 1992 for reviews). *Philometra obturans* also lives in the blood but gravid females migrate to the gill arteries to release larvae directly into the water (Molnar 1976). The method by which *Pseudodelphis oligocotti* disseminates its larvae is yet another solution to the problem getting larval stages into the external environment. For some blood-dwelling nematodes the method of transmission is still unknown. *Ichthyofilaria* spp. (Dracunculoidea: Philometridae) live on the surface of the definitive host's liver and in the mesentery; first-stage larvae circulate in the host's blood and body fluids, but we do not know how they reach the external environment (Appy et al. 1985). The method of transmission demonstrated for *P. oligocotti* sets a new precedent for the transmission of blood-dwelling parasites whose life cycle's are yet to be completed.

Wild-caught *A. flavidus* typically become infected by *Pseudodelphis oligocotti* when they are yearlings (age 1); age 0 are young of the year, and age 1 are yearlings in between their first and second year of life (Devries and Frie 1996). The smallest fish infected was an 80-mm yearling caught in May, infected with an adult nematode. In the fall, *A. flavidus* migrate from eelgrass beds to adjacent rocky sub/intertidal zones to spawn, and return to eelgrass beds in the spring. Yearling fish are probably infected over their second winter, when young of the year are just emerging. Young of the year may become infected only later in the season because that is when they become exposed, or because there is a certain minimum size requirement (in this case, presumably 80 mm) for a nematode to establish.

My determination of the first age class of *A. flavidus* (mode at 35 – 38 mm in the spring, and greater that 75 mm in late summer, Figure 4.1) roughly agrees with Hart (1973) who reports that *A. flavidus* are 20 to 40 mm in the spring of their

first year, and 100 to 120 mm by the end of the summer of their first year. I was unable to determine host age with certainty beyond the first age class. Fish length frequencies were examined by month because time of year may affect age composition of samples. *Apodichthys flavidus* has a discrete spawning period (Hart 1973) which makes individuals amenable to length-frequency aging (Devries and Frie 1996). I examined length frequencies of late spring and summer samples, after *A. flavidus* has returned from its spawning area; spring and summer samples included the most complete distribution of age groups. Modeling age from peaks in length frequencies in a sampling period assumes unbiased sampling of fish age groups. This assumption is justified because, for a given time of year, the same sampling apparatus recovered a full range of sizes (according to Hart 1973). Modes in length frequencies were only distinct for the smallest sizes. Beyond this, variance increases with the mean length of the fish, giving rise to overlapping annual length frequency distributions (Devries and Frie 1996, Jones 1962) (Figure 4.1). This phenomenon results from non-uniform growth between ages.

Parasite abundance (subgravid adult (L5) and larvigerous stages only) increased with host size (as an index of fish age) probably through the accumulation and growth of parasites throughout a host's life. *A. flavidus* individuals continually acquire *P. oligocotti* throughout their lives: all parasite stages were present concomitantly in all but the largest, oldest fish, which did not harbour L3/L4 larvae. The restriction of earlier nematode stages to all but the largest hosts may be the result of diet shifts as fish age. Fish often vary their diet with age (Horn and Gibson 1988, Bane and Bane 1971, Yoshiyama 1980). Copepods, intermediate hosts of *Pseudodelphis oligocotti,* may not be considered profitable prey to penpoints greater than 262 mm, resulting in reduced exposure to *P. oligocotti* unless small copepods are accidentally ingested. There is no age-related diet data on *A. flavidus* itself in the literature. However, captive gunnel seemed to show a

preference for the larger amphipods over the copepods. Alternatively, many host populations are believed to acquire immunity to parasites with age, exhibiting lower intensities and rates of infection in older members (age at which host populations acquire immunity depends on transmission rates; see Woolhouse 1998 for review). The absence of gravid nematode stages in young fish may be a result of the time it takes parasites to mature from time of initial infection. Many large gunnel also supported third and fourth stage larvae of *Pseudodelphis oligocotti*, inferring that they acquire new infections throughout their lives (Figure 4.2).

Many parasites are adapted to transmit when susceptible hosts are most common (Adamson 1986). This means that many nematodes will have annual transmission cycles synchronized with the spawning activities of their hosts. Examination of seasonal variation in parasite stage abundance was somewhat hindered by the paucity of data from mid-November to end of March. *Apodichthys flavidus* became unavailable during these months because of their habit of moving from subtidal eelgrass beds into rocky subtidal areas to set up breeding territories. These rocky areas were impossible to seine and could only be sampled by rock tipping at extreme low tides, which are infrequent. The presence of adult to gravid parasite stages throughout those parts of the year sampled does not suggest annual cycles in transmission of *P. oligocotti.* Gravid stages may be relatively long-lived, at least long enough for *A. flavidus* individuals to acquire new infections that also become gravid: female worms in the hepatic sinus were often in different stages of development, from subgravid adult (L5) to larvigerous.

The development of experimentally transmitted third-stage larvae to the fourth stage by 61 days, and to adult by 231 days indicates that the life cycle takes a year to complete. Because only 11% of all parasites recovered were fourth stage, this stage is probably relatively transitory. *Pseudodelphis oligocotti* spends most of its life

as an adult (fifth-stage). These nematodes more than likely become adults well before 231 days. At this rate, *P. oligocotti* adults give rise to adult stages in the next fish host within a year. Most members of the superfamily Dracunculoidea typically complete their life cycle in a year: prepatent periods (time from acquisition of infection to appearance of gravid, transmitting stages) vary from 10 months to 2 years (Anderson 1992).

Table 4.1 Necropsy results showing percent of *Pseudodelphis oligocotti* worms by stage of development and tissue site in which each stage was recovered from fish host, *Apodichthys flavidus*.

Stage/sex:	L3 / L4	L5			Dead	Gravid (with dev-eloping embryos)	Gravid (with larvae)	L1
Tissue		Female	Male	un-known				
Body Cavity	99.7	23.6	99.4	98	99			
Ovary		0.5						
Heart/pericard-ium		5.1	0.6	0.3		3.8	1.5	
Hepatic sinus	0.3	70.8		1.7	1	95.3	97.3	
Common cardinal vein						0.9	1.2	
Thymus								10.6
Gills								89.4
Total number of worms	291	195	326	929	403	213	402	132

Table 4.2 Rates at which first-stage larvae were shed by *Apodichthys flavidus* infected with larvigerous females of *Pseudodelphis oligocotti.*

Fish Number	Fish Weight (g)	No. of Larvigerous Females	Elapsed Time (min)	Number of Larvae Shed	Shedding Rate (larvae/ minute/ female)	Average Shedding Rate
1566	-	12	13.0	3	0.02	0.02
			61.0	17	0.02	
1570	20.7	1	65.5	2	0.03	
			138.8	6	0.04	0.04
			51.7	3	0.06	
1576	20.9	1	82.0	0	0	
			101.0	0	0	
1591	52.9	2	51.0	0	0	
			14.0	0	0	
			112.0	0	0	
			32.0	0	0	

Table 4.3 Measurements of *Pseudodelphis oligocotti* from wild-caught and experimentally infected *Apodichthys flavidus*.

Stage	No. specimens	Sex	Length (mm)[1]	Width (mm)[1]
L1[2]	3		0.553 (0.531, 0.567)	0.027 (0.023, 0.030)
L1[3]	3 / 5		0.685 (0.663, 0.699)	0.038 (0.019, 0.052)
L3	2	unknown	2.33 (1.6, 2.99)	0.057 (0.047, 0.073)
L4	2	unknown	5.019 (4.95, 5.08)	0.076 (0.065, 0.087)
L4	1	Female	5.691	0.0853
L4[4]	2	Male	5.355 (5.34, 5.37)	0.064 (0.058, 0.070)
L5	1	Male	13.5	0.113
L5[4]	2	Male	15.77 (15.64, 15.91)	0.122 (0.116, 0.128)
L5	3	Female	21.18 (19.37, 22.79)	0.124 (0.115, 0.131)

[1] Measurements are the mean, followed by minimum and maximum in parentheses

[2] From uterus of larvigerous female

[3] From host gill region

[4] From experimentally infected fish killed at 8.5 and 33 weeks respectively

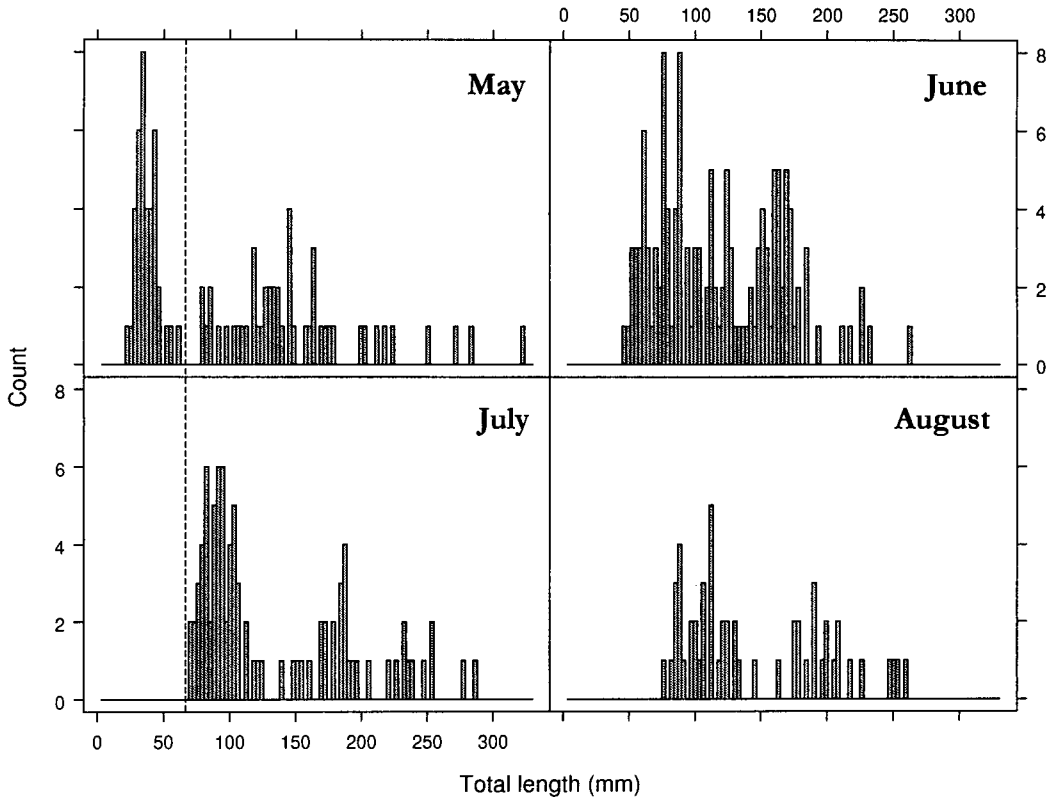

Figure 4.1 Frequency histogram of total length (mm) for all penpoint gunnel (*Apodichthys flavidus*) caught in sampling periods May, June, July, or August. Distinct modes in a sampling period indicate age groups (Devries and Frie 1996). The first age group (young of the year) is indicated in May by a dashed line.

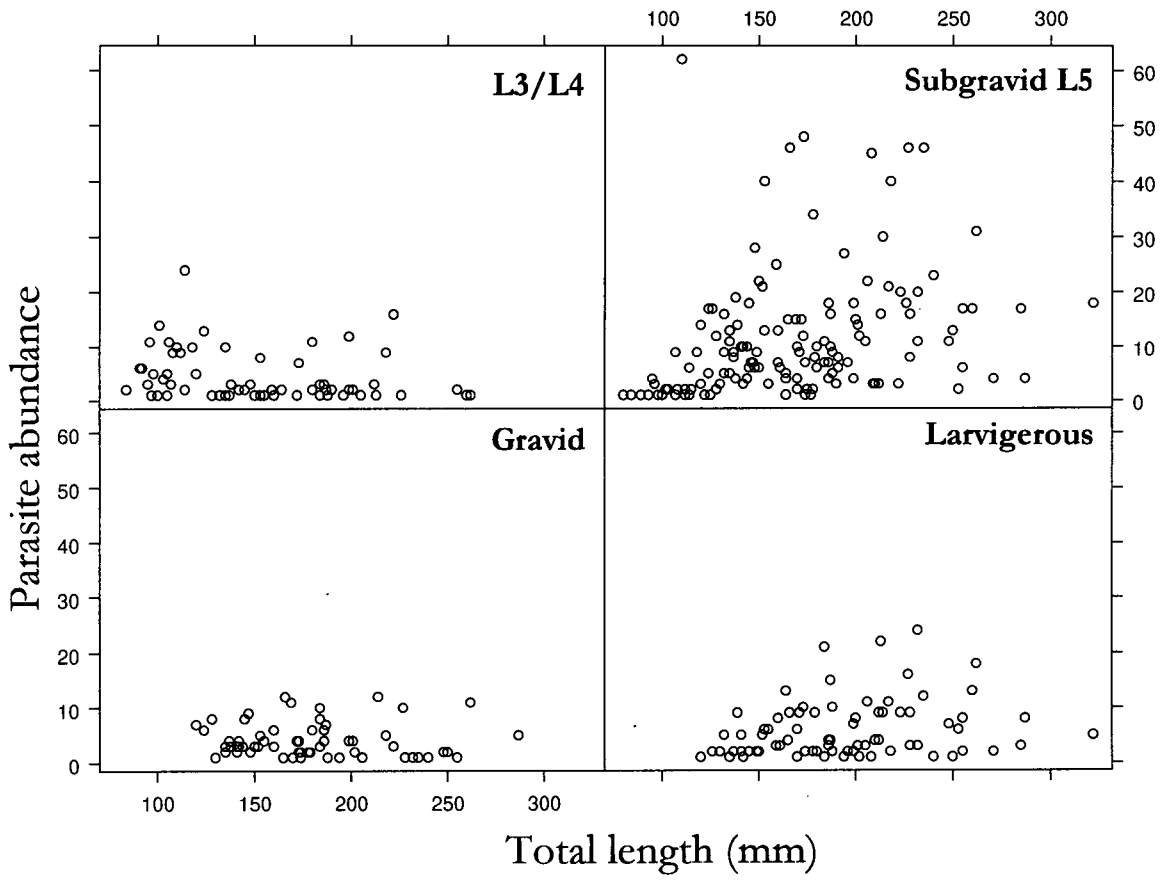

Figure 4.2 Abundance of various stages of *Pseudodelphis oligocotti* in hosts (penpoint gunnel, *Apodichthys flavidus*) of different lengths. Each subplot refers to a different stage of parasite development: "L3/L4" indicates third or fourth stage larvae, "Subgravid L5" indicates non-gravid adults, "Gravid" indicates female nematodes with developing embryos *in utero*, and "Larvigerous" indicates females with first-stage larvae *in utero*. Specimens of *A. flavidus* that contain more than one stage are represented by points in the appropriate window. No completely uninfected individuals were included.

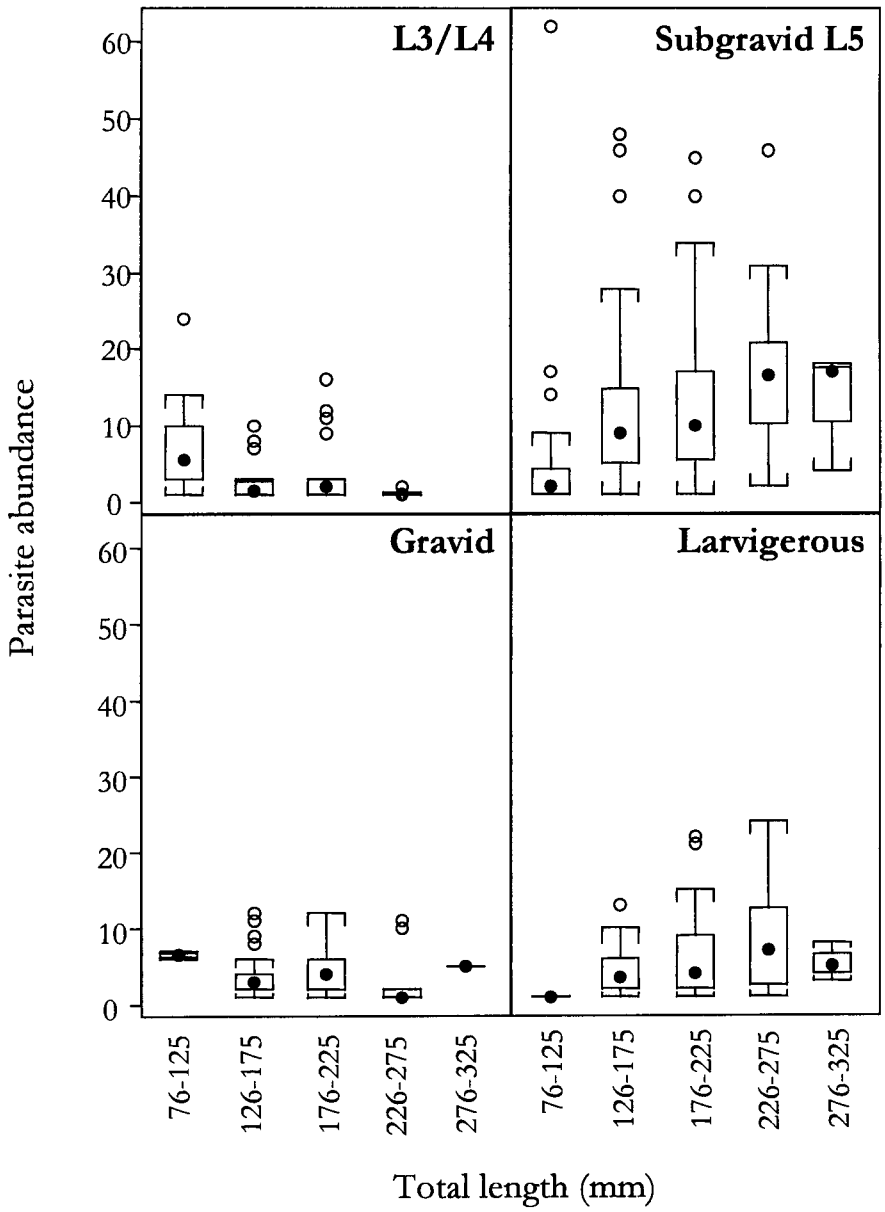

Figure 4.3 Size classes of penpoint gunnel (*Apodichthys flavidus*) and the number of *Pseudodelphis oligocotti* they host, according to stage of parasite development. Each subplot refers to a different stage of parasite development: "L3/L4" indicates third or fourth stage larvae, "Subgravid L5" indicates non-gravid adults, "Gravid" indicates female nematodes with developing embryos *in utero*, and "Larvigerous" indicates females with first-stage larvae *in utero*. Box diagram represents the upper and lower extremes of the data (excluding outliers) with whiskers, upper and lower quartiles with box borders, and median with a black dot. Outliers are drawn as open circles and refer to data outside 1.5 times the interquartile range.

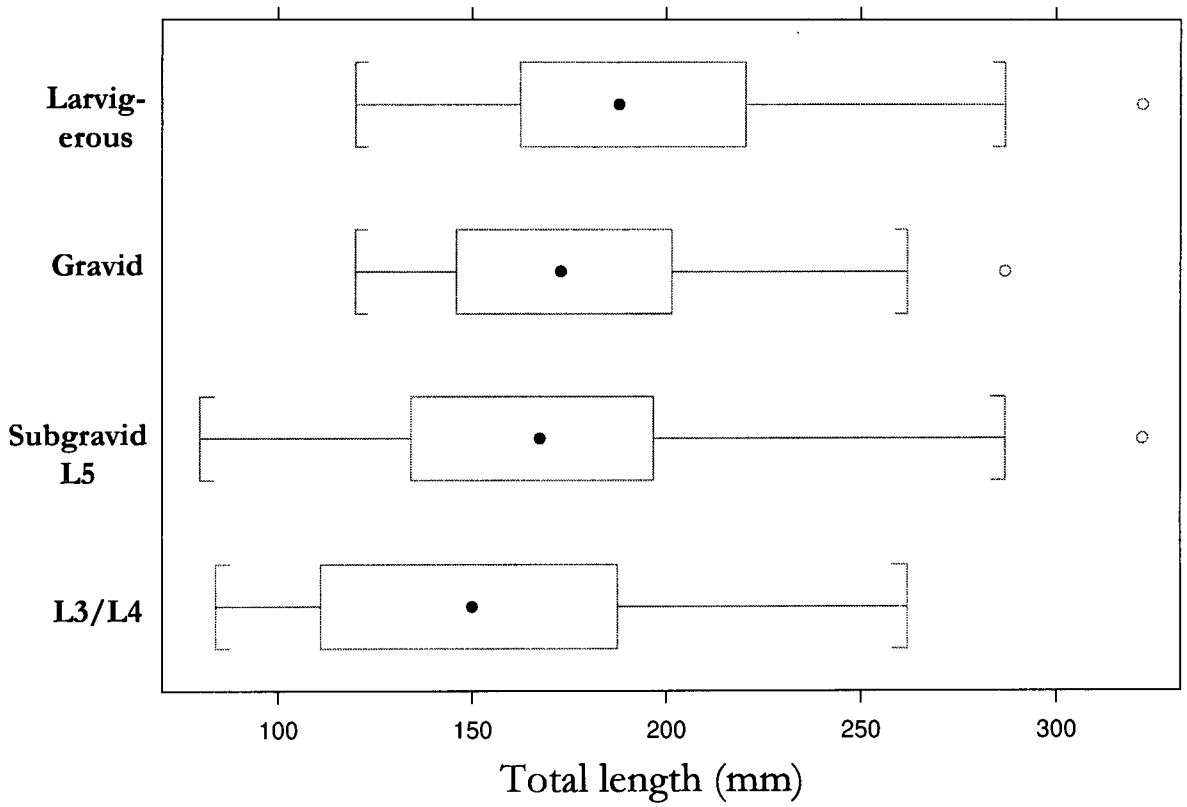

Figure 4.4 Distribution of sizes of penpoint gunnel (*Apodichthys flavidus*) infected by each stage of *Pseudodelphis oligocotti*. Box diagram represents the upper and lower extremes of the data (excluding outliers) with whiskers, upper and lower quartiles with box borders, and median with a black dot. Outliers are drawn as open circles and refer to data outside 1.5 times the interquartile range.

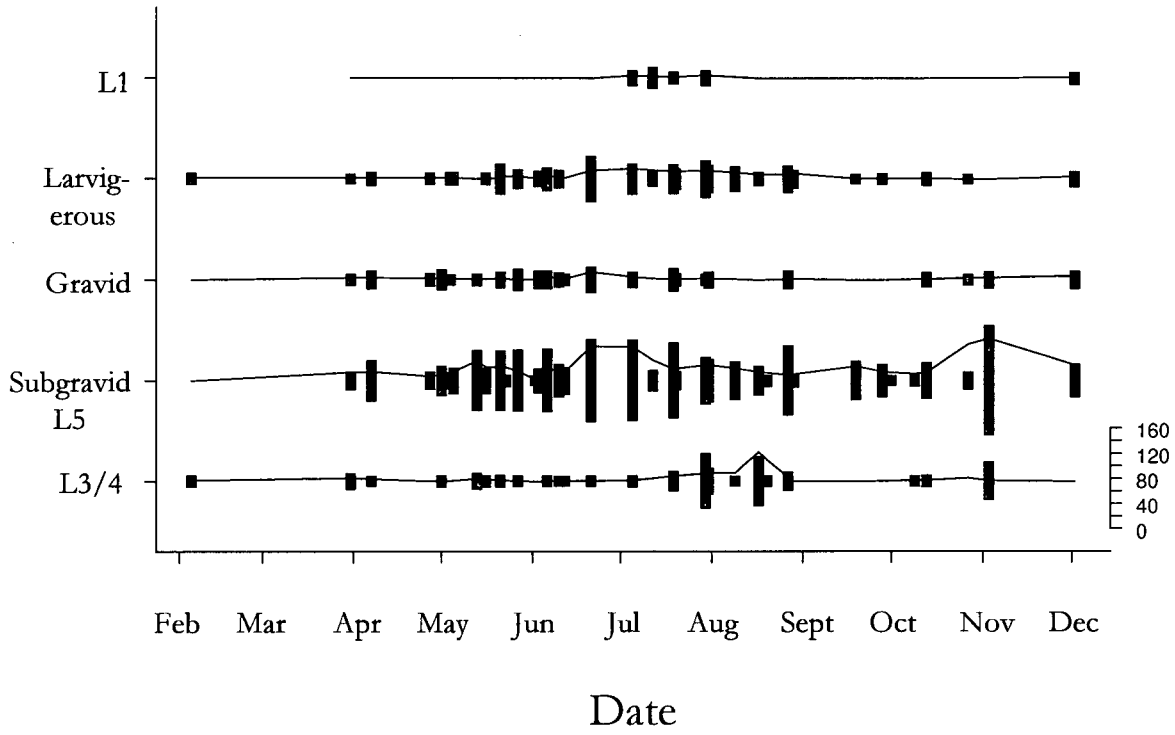

Figure 4.5 Seasonal distribution of *Pseudodelphis oligocotti* in *Apodichthys flavidus*. Stages are plotted on separate lines: "L3/L4" indicates third or fourth stage larvae, "Subgravid L5" indicates non-gravid adults, "Gravid" indicates female nematodes with developing embryos *in utero*, and "Larvigerous" indicates females with first-stage larvae *in utero*. Multiple years have been pooled and data plotted by time of year. Vertical bars represent number of worms. Right-hand scale bar represents abundance levels and applies to each row of the plot. Lines represent robust (3 iterations) estimates of each point using the nearest 0.1625 points of the data set to generate the estimate (lowess function, Mathsoft 1998)

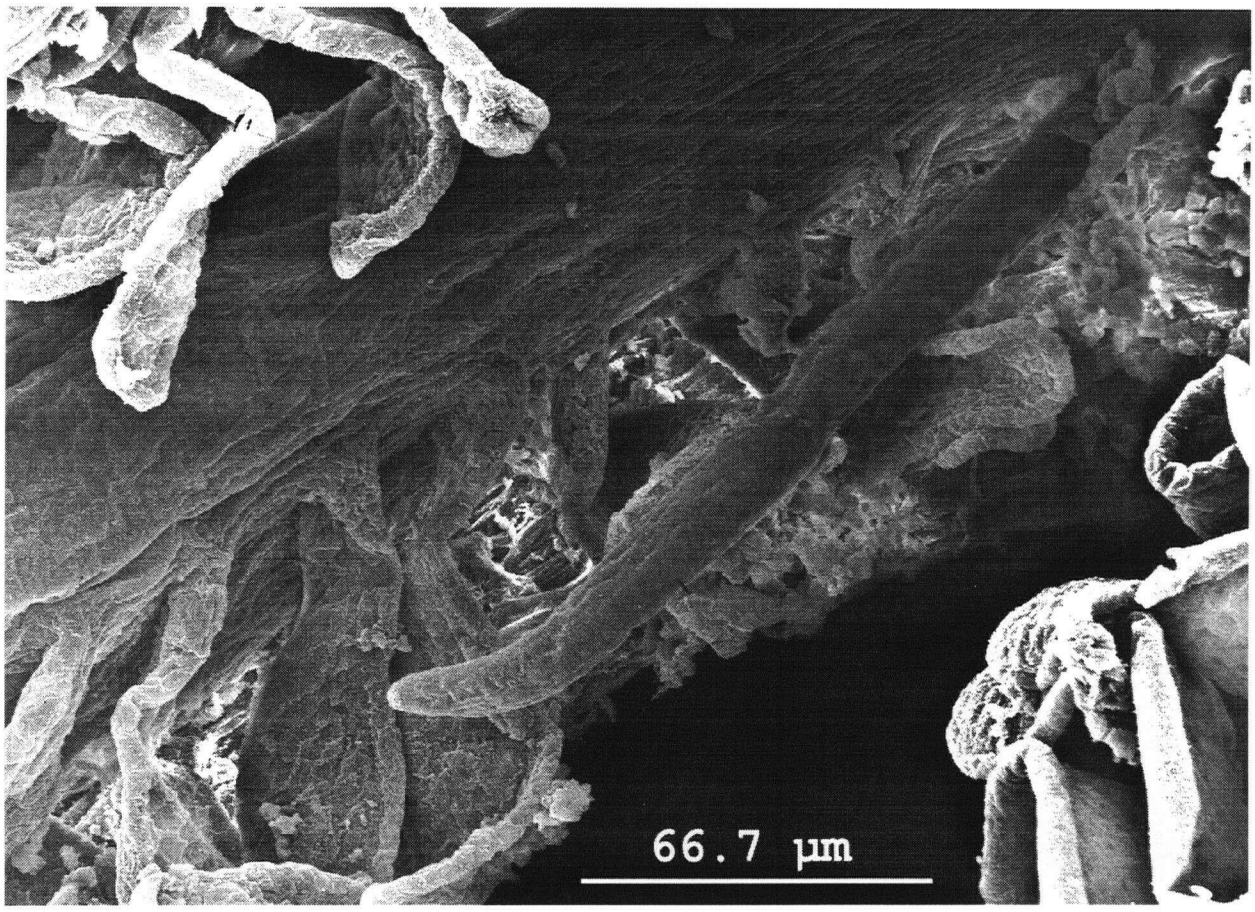

Figure 4.6 First-stage (L1) *Pseudodelphis oligocotti* larva as it leaves the gill primary lamella of penpoint gunnel, *Apodichthys flavidus*, fish host.

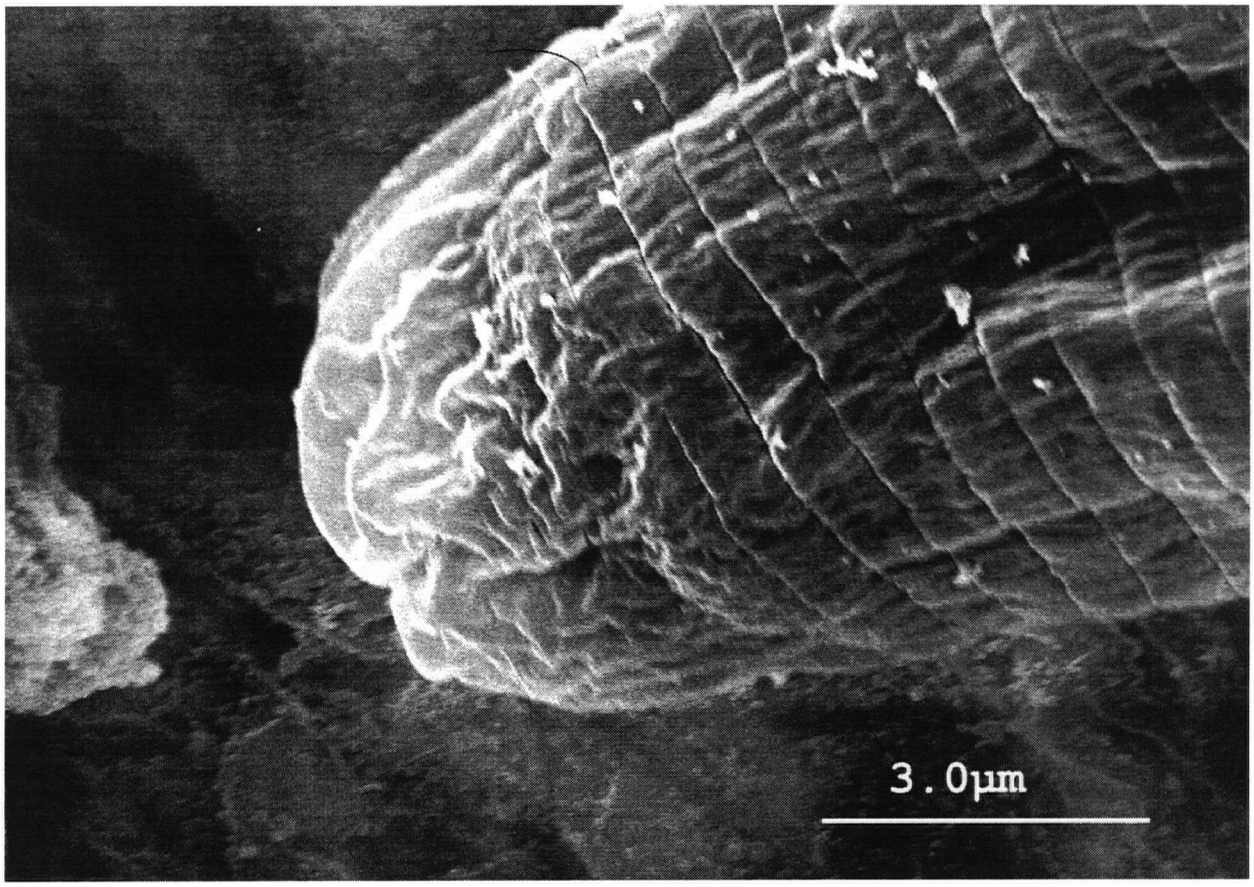

Figure 4.7 Closeup of head of first-stage (L1) *Pseudodelphis oligocotti* larva as it leaves the gill primary lamella of penpoint gunnel, *Apodichthys flavidus*, fish host. Examination of head reveals dorsal cephalic swelling (bottom of frame) diagnostic of the superfamily Dracunculoidea.

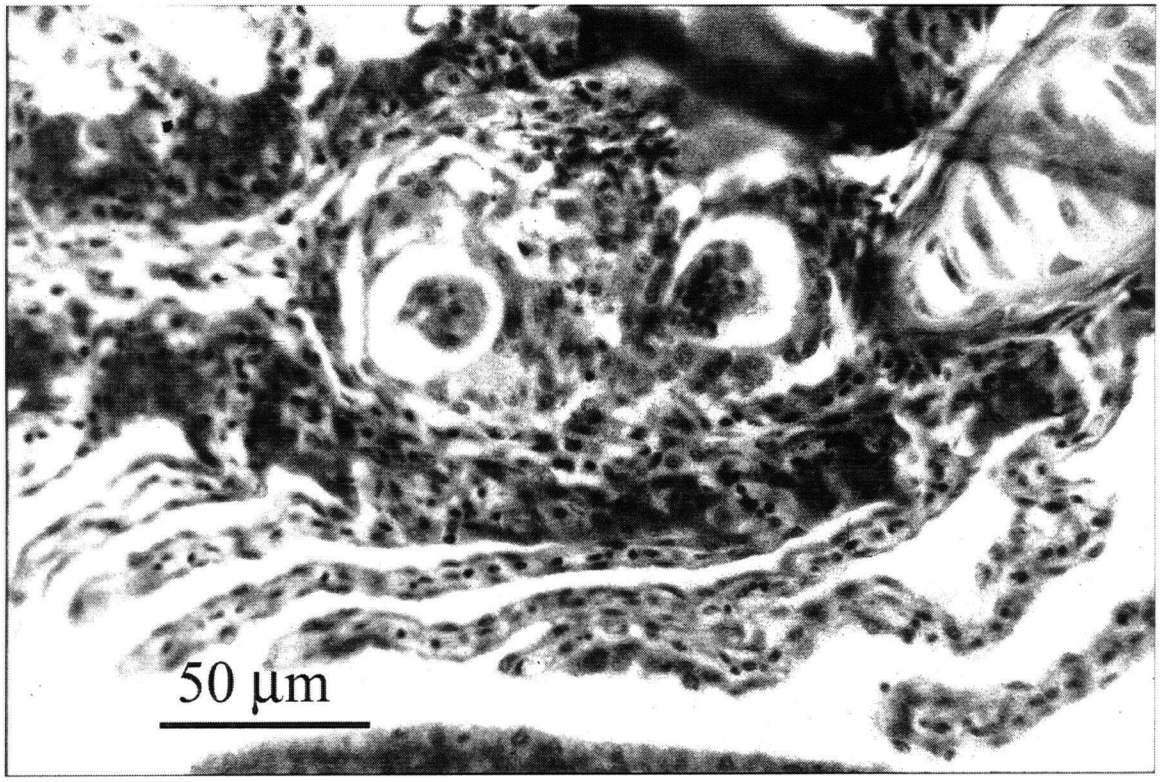

Figure 4.8 Histological cross-section of gills from penpoint gunnel, *Apodichthys flavidus*, infected with *Pseudodelphis oligocotti*. First-stage larva(e) of *P. oligocotti* sit in blood vessel(s) of gill primary lamella (center of photograph).

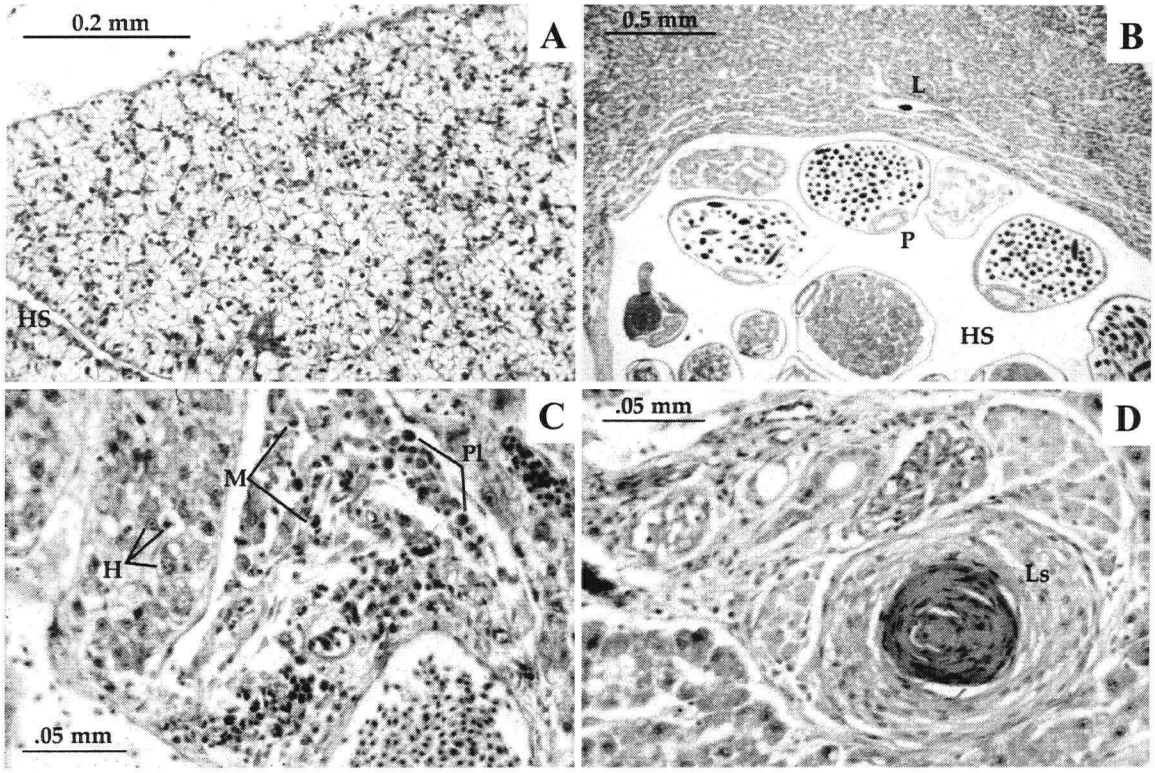

Figure 4.9 Comparison of liver tissue from penpoint gunnel, *Apodichthys flavidus*, uninfected and infected with nematode *Pseudodelphis oligocotti*. A. Liver tissue and hepatic sinus (HS) of an uninfected gunnel. B. Liver tissue and hepatic sinus infected with adult (P) and larval (L) *P. oligocotti*. Pressure atrophy has occurred in tissue surrounding the hepatic sinus. C. Area of inflammation of infected liver showing macrophages (M), plasma cells (Pl), and non-uniformly nucleated hepatocytes (H). D. Granulomatous lesion (Ls) in infected liver tissue.

CHAPTER 5: PREDICTORS OF SUITABILITY OF VARIOUS FINAL HOSTS FOR *PSEUDODELPHIS OLIGOCOTTI*

Introduction

Pseudodelphis oligocotti is an endoparasitic nematode inhabiting the body cavity and hepatic sinus of certain coastal marine fishes. Although it infects at least nine species of fishes, its course of development is variable and typically does not lead to transmission in most fish species.

Hosts vary in their suitability. Nutritional requirements of the parasite, the nature of the host immune response, and availability of hosts all play a role (Kennedy 1975). Variation in host suitability is reflected by variation in a parasite's ability to establish, the parasite's rate of development and number of progeny it produces depending on the host (Dogiel et al. 1964, Leong and Holmes 1981, Holmes 1976). Hosts in which parasite development is not actually completed are termed *unusual hosts* (Dogiel et al. 1964). Parasites vary in the range of host taxa in which they can establish and develop. The number of hosts and taxonomic diversity that comprise a parasite's host range can indicate a parasite's host specificity. Host specificity is defined as the "degree to which a parasite is able to mature in more than one host species" (Roberts and Janovy Jr. 1996).

Most parasites have relatively narrow host specificity, which often includes phylogenetically related hosts. Related hosts are more likely to have similar immune responses and biochemical make-up, partial determinants of a parasite's success (Wakelin and Apanius 1997), although it is also true that sometimes hosts share an essential element that does not follow taxonomic groupings (plant hosts of cabbage butterfly *Pieris rapae* in Barbosa 1988; anurans infected with pinworms in Inglis 1971). Parasite host range tends to include multiple

taxonomic groups when rate of encounter of different host species is high: rate of host encounter is a function of method of transmission and host ecological relationship with other potential hosts. Monogeneans in general are highly host specific: their transmission between fish hosts is restricted to direct contact between hosts (Rhode 1979). There are at least two exceptions, both Hawaiian species of monogenean. One species infects seven families of fish hosts, while the other infects 11 families of fish hosts. In both cases, host species live sympatrically in diverse coral reef systems (Rhode 1979). Certain pinworms of Anurans have broad host ranges when there are many sympatric hosts available (Adamson and Caira 1994, data from Adamson 1981). Parasites with passive (indiscriminate) transmission using food web relationships between hosts, particularly in aquatic habitats are often capable of maturing in multiple host taxa (Bush et al. 1990). One review concludes that parasites with actively swimming larvae penetrating or attaching to the host have a narrower range of suitable host species than do parasites entering the host through the mouth (Noble et al. 1989). Yet it is not clear how host range has evolved in response to ecological factors: it seems unlikely that parasites are in fact generalists that do not transfer to unusual hosts simply because they lack the ecological opportunity.

Parasites' ability to develop in a range of hosts determines potential ways in which parasites may speciate: by cospeciation with hosts, or by capturing unusual hosts (Chabaud 1959, Holmes and Price 1980). When a parasite completes its development in an unusual host while retaining its normal morphology, Chabaud (1959) refers to it as *parasite transfer*. If that parasite then becomes isolated in the newly acquired unusual host and subsequently speciates, *host capture* is said to have occurred (Chabaud 1959).

Pseudodelphis oligocotti, like many other members of the superfamily Dracunculoidea, transmit between fish hosts by way of an intermediate

crustacean host, usually a copepod, that is ingested (Chapter 3 and 4). It is not surprising that it establishes in many fish hosts that occur in eelgrass beds and adjacent rocky areas, since so many of these feed on copepods. Coastal waters, where the principal final host *Apodichthys flavidus* lives, are particularly diverse because many marine fishes use such areas as breeding/spawning grounds as well as feeding and nursery grounds for their young (Jones 1962). Nonetheless, *P. oligocotti*'s actual host range is extremely narrow.

Pseudodelphis oligocotti appears to be host specific to penpoint gunnel, *Apodichthys flavidus*, yet it demonstrates variation in development in other hosts from mere establishment to stages capable of transmission (Chapter 2). In this chapter I characterize fish hosts by their ecology and taxonomic relatedness using a variety of sources from the literature. I ask whether more appropriate hosts for *P. oligocotti*'s development are more ecologically associated than taxonomically related. This study supports the importance of a parasite's method of transmission and host ecology in expansion of host range (Adamson and Caira 1994). The results suggest mechanisms by which a host specialist might eventually transfer to unusual hosts, which may lead to a speciation phenomenon usually attributed to host generalists, that of host capture.

Methods

Nearshore fishes were collected from coastal British Columbia by pole seine and rock tipping. Collection sites occurred throughout the coastal mainland of British Columbia to West Coast Vancouver Island, British Columbia (Table 2.1). Sampling was as frequent as every extreme low tide, from early spring to fall of 1992 to 1996.

Once captured, fishes were placed in a holding tank and dissected for parasites in turn. Dissections follow standard methods detailed in Chapter 2. Nematodes

of the species *Pseudodelphis oligocotti* were collected and identified to stage and sex with the aid of a microscope. From parasite data I generated percent of worms gravid with developing embryos, and with first-stage larvae. I also coded the degree of *Pseudodelphis oligocotti* development within each host with a 1 if *Pseudodelphis oligocotti* never established, 2 if it established and reached adulthood, 3 if female nematodes became gravid with developing embryos, and 4 if females became gravid with first-stage larvae.

Host Information

Fish were identified to species, measured in total length and sexed if possible, in addition to being dissected for parasites. Fish species that were caught only once and do not normally occur in nearshore waters (that is, *Oncorhynchus nerka* and *Raja binoculata* from Chapter 2) were omitted from the following analysis. To determine the degree of ecological similarity between fishes potentially infected by *P. oligocotti*, data on diet and habitat of each fish species was amassed from the literature (Appendix 5.1A). Each species was represented by multiple citations to ensure complete coverage. To overcome inconsistencies between sources in units of measurement, level of detail, sampling effort, and ages of fish examined, I recorded only presence/absence of characteristics reported for a given species. Diet and habitat characters were expanded to the minimum level across all studies. For example, if a fish reportedly ate cirrulid tentacles, a placeholder record was created for "polychaete" and "invertebrate," since for some hosts only coarse-grained data were available. I compiled matrices where each row represented a fish species and each column represented a diet item or microhabitat. Each fish received a 1 if reported to have ingested said dietary item or occurred in said microhabitat, and a zero if a.) no one reported that they ate said dietary item or occurred in said microhabitat, and b.) if it was reported that they did not eat said item or occur in said microhabitat. I divided ecological similarity matrices into one based on diet (Appendix 5.1B), one based on habitat

(Appendix 5.1C), and one based on both groups of characteristics combined. I generated distance matrices (distance between rows, representing fishes) using the binary method, which evaluates the proportion of non-zero fields that two rows do not have in common, for every combination of rows (Mathsoft 1998). Groups of fishes were then determined using the single linkage (connected) method of clustering. Clustering methods begin by assigning each fish to its own group, then combining the "nearest" other fish to form larger groups until there is a single group. Groups were connected by their closest points (hence "single linkage", Becker et al. 1988). Another study measured percent biomass of each diet item and drew similarities between fishes using euclidean distance (root sum of squares difference) between rows (Edgar and Shaw 1995). They assigned groups using the average distance between points to link clusters. Again, because units of measurements used in my analysis were not consistent, this method would have been inappropriate.

Phylogenetic distance between fishes was quantified using established taxonomic data (Hart 1973). Although certain fish relationships have been studied using both ontogeny (Moser et al. 1984, Jamieson 1991) and ribosomal sequences, no two fish examined in this study have been compared using the same methods. After ordering fishes taxonomically, each fish was assigned 5 numbers, each number one in a sequence according to its superorder, order, family, genus, and species, respectively. Fish within the same taxonomic group received the same number (Appendix 5.2). A distance matrix between rows (fishes) was then assembled by first weighting the phylogenetic matrix of Appendix 5.2 by log(10,000) at the superorder level, log(1000) at the order level, log(100) at the family level, log(10) at the genus level, and log(1) at the species level. This has the effect of putting the most importance on differences in superorder and removing the redundancy of including the species level (since each species receives a unique number according to species). After weighting the phylogenetic

matrix, I used the Manhattan technique (sum of the absolute difference between rows) to generate the matrix of distances between each species (row) combination and assigned groupings according to single linkage (connected) method (Mathsoft 1998). Both the ecological and the phylogenetic distance matrices were condensed to vectors of distance relative to *A. flavidus*. Using only those fish species surveyed for *P. oligocotti* more than once, I modeled separately percentage of worms gravid, and coded response variable "Maximum stage of development" as functions of ecological distance and phylogenetic distance, considering separate and combined effects. To fulfill assumptions of the general linear model of equal variances, variables were transformed according to the following expressions: maximum stage of development and percent of worms gravid transformed by $\sqrt{}$(response variable+0.5); $\sqrt{}$(distance+4) for phylogenetic distance; and $\log_e$(distance) for ecological distance.

Members of an ecological grouping may be, but are frequently not, close phylogenetic relatives (Harvey and Pagel 1991). Since this study could not select which fish hosts became infected and to what extent, it was impossible to examine fishes of each taxon with sufficient variation in ecological traits, according to the comparative phylogenetic method (Harvey and Pagel 1991). To make allowances for a correlation between phylogenetic distance and ecological distance, a general linear model was proposed for partial correlation of parasite development as the dependent variable on both ecological and phylogenetic distance from *A. flavidus* where the two independent variables were allowed to interact.

Results

Of 1384 individuals from 22 coastal marine fishes surveyed (omitting *Oncorhynchus nerka* and *Raja binoculata*, represented by one individual each,

because they do not normally occur in nearshore waters), *Pseudodelphis oligocotti* established and reached adulthood in 9 species (Table 2.3). It was variable in the extent of its development beyond adulthood depending on the host species: in two species (*A. flavidus* and *Gobiesox meandricus*), female *P. oligocotti* developed to completion, becoming gravid with first-stage larvae; in three other species (*Syngnathus leptorhynchus*, *Pholis laeta*, and *Oligocottus maculosus*), female *P. oligocotti* developed until they were gravid with developing embryos (Chapter 2). Amongst fish in which *Pseudodelphis oligocotti* developed to an advanced stage, hosts varied in the percent of parasites recovered that were gravid. Other studies (Holmes 1976) gauge extent of development by measuring percent of the parasites that were gravid. However, an estimate of percent gravid females was unavailable for *Oligocottus maculosus* (Adamson and Roth 1990). I therefore focus on the coding variable, maximum extent of development, in my presentation of the results.

Ecological opportunity versus phylogenetic predisposition

When hosts are characterized according to diet and habitat (Figure 5.1A), their groupings based on similarities are different than the groups formed when they are characterized by taxonomic classification (Figure 5.1B). To examine the relative importance of host ecology versus phylogenetic relatedness on parasite development, I first excluded those host species that had been examined only once (Table 2.3), reducing the number of hosts in the analysis to 15 (*Gobiesox meandricus*, *Syngnathus leptorhynchus*, *Cymatogaster aggregata*, *Anoplarchis purpurescens*, *Lumpensus sagitta*, *Apodichthys flavidus*, *Pholis laeta*, *P. ornata*, *Hexagrammus decagrammus*, *Leptocottus armatus*, *Artedius fenestralis*, *A. lateralis*, *Oligocottus maculosus*, *Enophrys bison*, *Hippoglossoides elassodon*). Before correcting for interactions between host ecology and host taxonomy, trends indicate that host ecological association with the principal host, *A. flavidus*, is a better predictor of the maximum extent of parasite development in a given host species (Spearman's rank correlation coefficient $\rho = -0.5$, $p = 0.07$, Figure 5.2A) than host taxonomic relatedness

(Spearman's rank correlation coefficient $\rho = 0.22, p = 0.44$, Figure 5.2B). Analysis excludes *Apodichthys flavidus* itself because distance of fishes is measured from *A. flavidus*: it has zero distance. To take into account interactions between host ecology and host taxonomy, maximum stage of development was then modeled as a linear function of host ecological distance from *A. flavidus*, host phylogenetic distance from *A. flavidus* and a term for their interaction: development $\propto$ ecological distance + phylogenetic distance + ecological distance * phylogenetic distance. Host ecological distance from *A. flavidus*, taking into account the potentially confounding influence of phylogenetic relatedness, is negatively correlated with extent of parasite development ($F_{1,10} = 5.7, p = 0.038$, where 10 is the degrees of freedom for the residuals: 14 fish - three model terms - 1). Host phylogenetic distance from *A. flavidus*, taking into account the influence of ecological relatedness was not correlated with extent of parasite development ($F_{1,10} = 1.1, p = 0.32$). There was no interaction between host phylogenetic distance and host ecological distance ($F_{1,10} = 0.34, p = 0.57$). These results were robust to different variable transformations and different order of terms. To summarize, *Pseudodelphis oligocotti* appears to fail later along the path of development in hosts that are more similar ecologically to *A. flavidus* than in those hosts that are related phylogenetically.

Discussion

Fish parasites commonly infect two or more host families (data from Margolis and Arthur 1979, reviewed in Holmes and Price 1980). Presumably parasites acquired many of these hosts through ecological associations (Holmes and Price 1980). The results of this study indicate that the stronger the ecological association between hosts, the more likely *Pseudodelphis oligocotti* is to reach an advanced stage of development in that host. These results suggest mechanisms

by which unusual hosts may be acquired by a parasite through ecological associations between hosts.

The results are counterintuitive. Given that parasites spend the greater part of their lives in the host environment meeting nutritional demands and countering immune defenses, adaptation to the host environment might be expected to result in a positive correlation between a parasite's developmental success in an unusual host and the latter's taxonomic relatedness. This is not the case for *P. oligocotti*, which is a specialist of *A. flavidus*: even members of the same family as the host do not produce mature forms of the parasite, but *P. oligocotti* occasionally develops to maturity in distantly related fish (*Gobiesox meandricus*) of dramatically different morphology that are nonetheless sympatric with *A. flavidus*. *Pseudodelphis oligocotti* also reaches an advanced (but not quite fully mature) stage of development in type host *Oligocottus maculosus* (Adamson and Roth 1990), *Syngnathus leptorhynchus* and *Pholis laeta*. *Gobiesox meandricus* and *O. maculosus* live in rocky intertidal zones where *A. flavidus* overwinters to breed. *Syngnathus leptorhynchus* lives in eelgrass beds where *A. flavidus* also occurs, when not breeding. *Pholis laeta*'s distribution also appears to be similar to that of *A. flavidus* (Hart 1973, Wilkie 1966).

Parasite developmental success in a host requires the appropriate cues to initiate phases of development such as tissue migration, growth, or feeding. A liver fluke of herbivorous vertebrates, *Fasciola hepatica* has six different behavioural phases in the vertebrate host, each in a different host tissue where specific chemical cues induce specific responses (Sukhdeo 1990). The presence of a single type of bile salt (glycocholic acid) in the host intestine (formed by herbivores but not carnivores) cues excystment and begins a cascade of other parasite-host tissue interactions (Sukhdeo and Mettrick 1986). Unusual hosts of *P. oligocotti* may

require one or many chemical modifications to cue *P. oligocotti* along its developmental path.

It is not clear why parasite developmental success should be correlated with host ecological similarity. Hosts that live in close ecological association with *A. flavidus* probably encounter *P. oligocotti* often. Presumably, *P. oligocotti* and their potential host populations consist of variable genotypes. Individual larvae vary in their ability to establish and develop in different potential hosts, which are in turn genetically variable in susceptibility to *P. oligocotti* and suitability for *P. oligocotti* development. If enough attempts at successful combinations are made, eventually one will arise. When *P. oligocotti* larvae are frequently sampled by potential host species, eventually a variant larva will establish in a host in which it can develop. Thus the frequency at which *P. oligocotti* reaches advanced stages of development in potential host species would be higher in those hosts that are more ecologically similar to *A. flavidus*. Secord and Kareiva (1996) suggest that genetic heterogeneity in parasite and potential host populations, along with a change in ecological circumstances resulting in increased exposure, account for the transfer of parasite host-specialists to new host groups. Genetic heterogeneity in parasite and unusual host populations was demonstrated in the transfer of fish monogenean *Gyrodactylus turnbulli* (a taxon renowned for its specificity) to unusual hosts after an introduction had increased encounter rate between potential host and parasite (data from Leberg and Vrijenhoek 1994, in Secord and Kareiva 1996).

Artificially induced ecological association between an unusual host and the principal host may have resulted in one captive-held specimen of *Pholis ornata* producing larvigerous female *Pseudodelphis oligocotti* in the hepatic sinus. Wild-caught *Pholis ornata* never contained stages of *Pseudodelphis oligocotti* beyond subgravid adult (63 individual fish examined). Although the specimen of *Pholis*

ornata may have been infected before entering captivity, it was held at the Vancouver Public Aquarium in a seawater tank with infected *A. flavidus* for 34 months, and could have acquired the infection in captivity. Potential intermediate hosts may have been introduced into the water along with sand and coral by Aquarium staff. Direct transmission does not occur in this group of endoparasitic nematodes (Anderson 1992). However, since the source of infection is unknown, I cannot distinguish between two alternative explanations: 1) rates of transmission are artificially high in captivity, and of the greater number of attempts thus made by *Pseudodelphis oligocotti* to successfully infect *Pholis ornata*, one finally resulted in completed development due to the right combination of host and parasite genotypes. 2) parasite genotypes that can develop in genotypes of *Pholis ornata* occur in nature but I did not sample them, possibly because infected *Pholis ornata* die of advanced infection. In fact, the absence of advanced stages of development in many the unusual hosts sampled in this study may actually be the result of premature death on the part of the host rather than the actual inability of the parasite to develop. Regardless, parasite transmission, the successful endpoint of development, does not occur. Both explanations for the results of this study are in keeping with the suggestion that ecological associations take advantage of host and parasite genotypic heterogeneity, leading to host-parasite combinations in which parasite's develop and/or unusual hosts survive the infection to a point where the parasite can transmit.

Parasite transfer (when a parasite completes development in an unusual host) may result in a host capture event when the transferred parasite becomes isolated in the new host and undergoes speciation (Chabaud 1959). Host capture explains the presence of parasite lineages in very different host groups (Chabaud 1981). Isolation of *P. oligocotti* in *Gobiesox meandricus*, the only host it appears to have transferred to (although transmission from this host has not been confirmed)

would require some subsequent restriction in transmission, presumably a shift in habitat through which transmission occurs, or time of transmission, as a result of development in the unusual host. For example, sockeye salmon may have captured parasitic nematode *Philonema oncorhynchi* from landlocked salmonids harbouring *P. agubernaculum* because transmission, which is timed with host spawning (Ko and Adams 1969), is offset from the parent population by three years (M. L. Adamson, personal communication).

Use of ecological data from a variety of sources to characterize a fish's niche quantitatively has limitations. Ideally, comparison of niche overlap should include proportions of each prey category in the diet, and the proportion of individuals, or time spent, in each habitat category (Pianka 1975). These should be derived by standardized methods on fish of the same age. In addition, phylogenetic relationships between fishes were quantified using taxonomy, itself based on morphological and ontogenetic findings. These may be confounded with habitat use. Modern molecular biology techniques could provide percent genomic differences between fishes using neutral portions of the genome that are independent of niche (Harvey and Pagel 1991). Finally, Holmes (1976) uses percent of parasites that have reached maturity and are ready to transmit to quantify success of development. Data on percent of *Pseudodelphis oligocotti* individuals that contain developing embryos, for type host *Oligocottus maculosus*, would complete the data set.

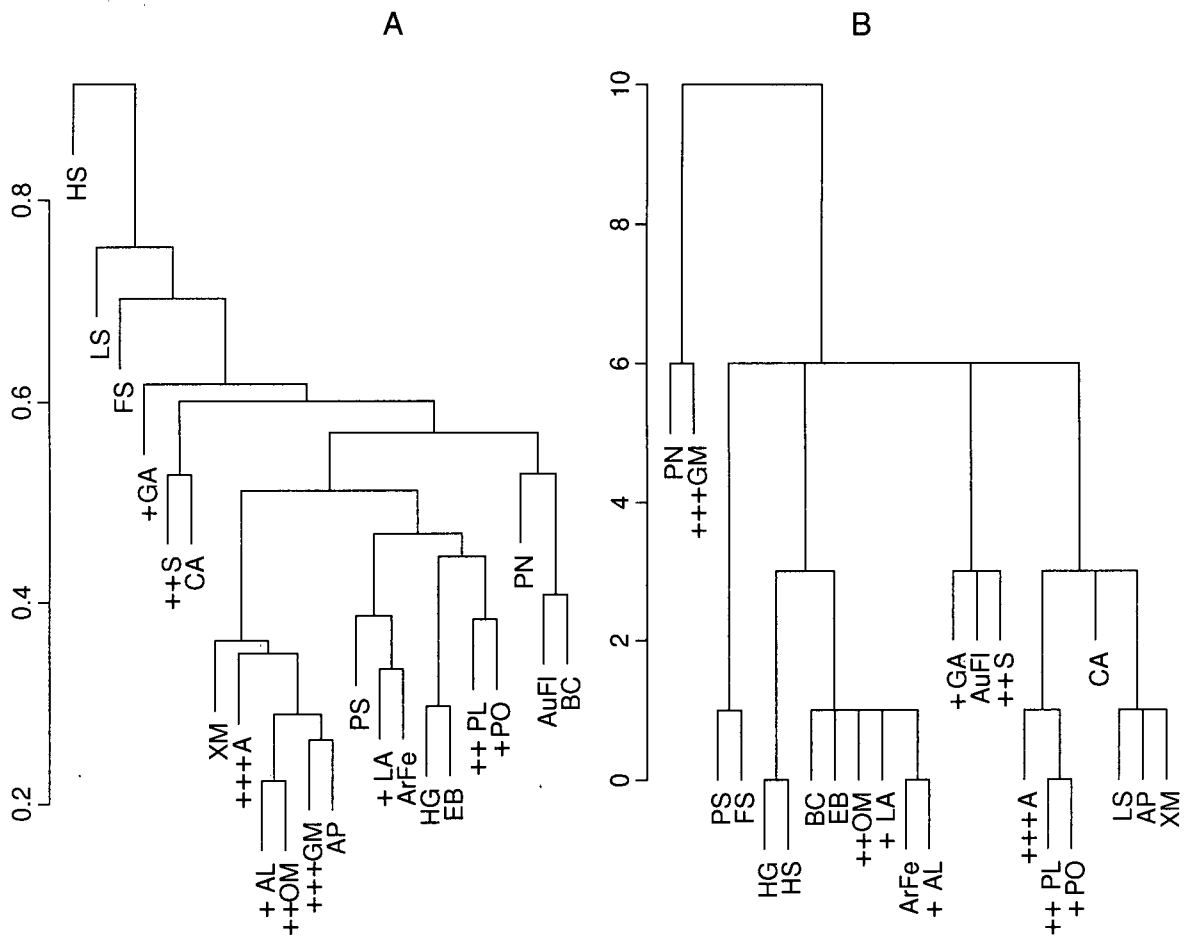

Figure 5.1 Dendograms representing the ecological (A) and taxonomic (B) relationships of the various fish hosts infected by *Pseudodelphis oligocotti* under natural conditions. Letters code for the different species (Table 2.2). Fishes marked with "+" are those that are infected with *P. oligocotti*, but in which parasites do not advance beyond subgravid adult. Those marked with "++" are fishes in which *P. oligocotti* becomes gravid with developing embryos. Fishes marked with "+++" are those in which *P. oligocotti* reaches an advanced state of development, becoming gravid with larvae.

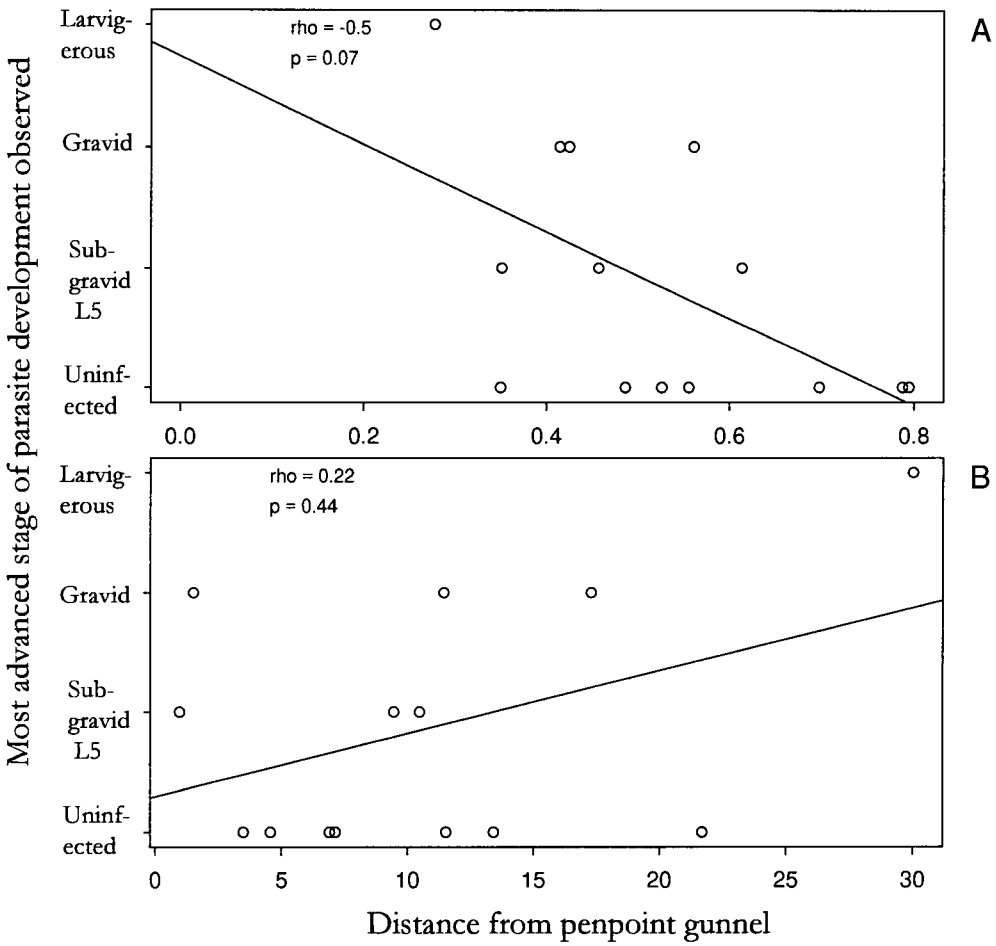

Figure 5.2 The ecological (A) and taxonomic (B) distances of potential fish hosts from *Apodichthys flavidus*, principal definitive host of *Pseudodelphis oligocotti*, are assessed as predictors of maximum stage of development reached by *P. oligocotti* in those hosts. Results of Spearman's Rank correlations appear in the upper left corner of each plot. Lines represent the least sum-of-squares best fit of the data.

Appendix 5.1A List of citations used for collecting ecological data presented in Appendix 5.1B and C. Fish species are represented by their alpha codes (see Table 2.2 for full name).

Fish species	Citations used to collect ecological data.
A	Bane and Bane 1971, Barton 1982b, Barton 1982a, Hubbard and Reeder 1965, Goodson 1988, Hay et al. 1989, Miller et al. 1980, Yatsu 1981, Yoshiyama 1981, Paulson 1998
AL	Bane and Bane 1971, Barton 1982a, Clemens and Wilby 1961, Hubbard and Reeder 1965, Goodson 1988, Green 1971b, Hay et al. 1989, Lamb and Edgell 1986, Marliave 1977, Miller et al. 1980, Paulson 1998, Yoshiyama 1980, Yoshiyama 1981
AP	Bane and Bane 1971, Barton 1982b, Barton 1982a, Goodson 1988, Hay et al. 1989, Lamb and Edgell 1986, Miller et al. 1980, Schultz and DeLacy 1932, Paulson 1998, Yoshiyama and Darling 1982, Yoshiyama 1981
ArFe	Clemens and Wilby 1961, Green 1971b, Hay et al. 1989, Lamb and Edgell 1986, Miller et al. 1980, Paulson 1998
AuFl	Carl 1964, Clemens and Wilby 1961, Goodson 1988, Hay et al. 1989, Lamb and Edgell 1986, Limbaugh 1962, Miller et al. 1980, Wang 1986, Marliave 1975, Paulson 1998
BC	Clemens and Wilby 1961, Green 1971b, Lamb and Edgell 1986, Miller et al. 1980, Paulson 1998
CA	Bane and Bane 1971, Clemens and Wilby 1961, Fitch and Lavenberg 1975, Hay et al. 1989, Jones 1962, Lamb and Edgell 1986, Miller et al. 1980, Bane and Robinson 1970, Boothe 1967, Paulson 1998
EB	Bane and Bane 1971, Barton 1982a, Clemens and Wilby 1961, Goodson 1988, Green 1971b, Miller et al. 1980, DeMartini 1978, Paulson 1998, Yoshiyama 1981
FS	Clemens and Wilby 1961, Lamb and Edgell 1986, Niggol 1982, Paulson 1998
GA	Bane and Bane 1971, Carl 1964, Clemens and Wilby 1961, Goodson 1988, Hay et al. 1989, Lamb and Edgell 1986, Wang 1986
GM	Bane and Bane 1971, Barton 1982a, Carl 1964, Clemens and Wilby 1961, Hay et al. 1989, Lamb and Edgell 1986, Marliave 1977, Miller et al. 1980, Schultz and DeLacy 1932, Wang 1986, Marliave 1975, Paulson 1998, Yoshiyama 1981
HG	Bane and Bane 1971, Barton 1982a, Goodson 1988, Hay et al. 1989, Lamb and Edgell 1986, Miller et al. 1980, Wang 1986, Barraclough 1967, Frey 1971, Gorbunova 1962, Marliave 1975, Paulson 1998, Yoshiyama 1981
HS	Miller et al. 1980
LA	Bane and Bane 1971, Carl 1964, Clemens and Wilby 1961, Fitch and Lavenberg 1975, Goodson 1988, Green 1971b, Hart 1973, Hay et al. 1989, Jones 1962, Lamb and Edgell 1986, Miller et al. 1980, Paulson 1998
LS	Gibson 1982, Hart 1973, Paulson 1998
OM	Bane and Bane 1971, Barton 1982a, Carl 1964, Clemens and Wilby 1961, Goodson 1988, Green 1971a, Green 1971b, Hay et al. 1989, Jones 1962, Lamb and Edgell 1986, Miller et al. 1980, Nakamura 1976, Wang 1986, Paulson 1998, Yoshiyama 1981
PL	Barton 1982a, Hay et al. 1989, Lamb and Edgell 1986, Marliave 1977, Miller et al. 1980, Peden and Hughes 1984, Schultz and DeLacy 1932, Paulson 1998, Yatsu 1981
PN	Bane and Bane 1971, Carl 1964, Hay et al. 1989, Jones 1962, Lamb and Edgell 1986, Wang 1986, Greene 1924, Hubbs 1920, Paulson 1998
PO	Bane and Bane 1971, Barton 1982a, Fitch and Lavenberg 1975, Goodson 1988, Hay et al. 1989, Jones 1962, Lamb and Edgell 1986, Miller et al. 1980, Peden and Hughes 1984, Schultz and DeLacy 1932, Paulson 1998, Yatsu 1981

PS	Bane and Bane 1971, Clemens and Wilby 1961, Goodson 1988, Hay et al. 1989, Jones 1962, Lamb and Edgell 1986, Miller et al. 1980, Barraclough 1967, Orcutt 1950, Porter 1964, Paulson 1998
S	Bane and Bane 1971, Bayer 1980, Carl 1964, Clemens and Wilby 1961, Goodson 1988, Howard and Koehn 1985, Jones 1962, Lamb and Edgell 1986, Miller et al. 1980, Steffe et al. 1989, Wang 1986, Moyle 1976, Paulson 1998
XM	Bane and Bane 1971, Barton 1982b, Barton 1982a, Fitch and Lavenberg 1975, Goodson 1988, Hay et al. 1989, Miller et al. 1980, Paulson 1998, Yoshiyama 1981

Appendix 5.1B Matrix of ecological traits (diet) for fishes potentially infected with *Pseudodelphis oligocotti*. An "x" indicates that a fish was reported ingesting a particular food item. Reference(s) for records are listed in Appendix 5.1A.

	Food items																															
					invertebrates																											
											molluscs					crustaceans																
																	copepods															
Potential Host Species	benthos	diatom	plant	fish	invertebrate	sponge	echinoderm	bryozoa	worm	annelid	mollusc	bivalve	limpet	snail	insect	crustacean	copepod	calanoid	cyclopoid	harpacticoid	branchiopod	barnacle	ostracod	mysid	cumacea	tanaid	amphipod	isopod	crab	shrimp	zooplankton
Porichthys notatus				x	x											x															
Gobiesox meandricus					x				x	x	x		x	x		x	x			x			x				x	x	x	x	
Aulorhynchus flavidus				x	x											x	x			x				x			x		x	x	x
Syngnathus leptorhynchus			x		x					x	x	x		x		x	x	x	x	x	x		x				x	x	x	x	x
Gasterosteus aculeatus			x		x										x	x															
Cymatogaster aggregata	x	x	x		x					x	x	x				x						x				x	x		x	x	x
Anoplarchis purpurescens			x	x	x				x	x	x	x		x		x	x			x		x					x	x	x	x	
Xiphister mucosus			x	x	x	x		x								x	x			x		x					x	x		x	
Lumpenus sagitta					x											x	x														
Apodichthys flavidus	x		x		x		x			x	x			x		x	x			x							x	x			
Pholis laeta		x			x					x	x	x				x	x	x		x							x	x	x	x	
P. ornata			x		x					x	x	x				x											x				
Hexagrammos stelleri			x																												
H. decagrammus			x	x	x				x	x	x	x				x	x										x		x	x	
Leptocottus armatus			x	x	x				x	x	x	x			x	x	x							x		x	x	x	x	x	
Artedius fenestralis			x	x	x					x	x	x				x										x	x	x	x	x	
A. lateralis				x	x					x						x	x			x						x	x	x	x	x	
Oligocottus maculosus				x	x					x	x		x		x	x	x	x		x		x	x			x	x	x	x	x	
Enophrys bison			x	x	x					x	x	x				x											x		x	x	
Blepsias cirrhosus					x											x								x			x	x		x	
Platichthys stellatus				x	x		x		x	x	x	x			x	x					x	x			x		x	x	x	x	
Hippoglossoides elassodon				x	x		x		x	x	x	x				x											x			x	

Appendix 5.1C Matrix of ecological traits (habitat) for fishes infected with *Pseudodelphis oligocotti*. An "x" indicates that a fish has been reported in a particular habitat. Reference(s) for records are listed in Appendix 5.1A.

	Habitat																														
							exposure							substrate											tidepool level						
Potential Host Species	coastal	pelagic	epibenthic	shallow	mid-depth	deep	high	medium	low	algal beds	eelgrass	rocky areas	beneath rocks	near structures	clay	mud	rocky	sand	shell	silty	intertidal	subtidal	tidepool	high-intermediate	high-nearshore	high-offshore	low	out-of-water			
Porichthys notatus	x		x	x	x	x			x			x	x			x	x	x			x	x									
Gobiesox meandricus	x		x	x				x	x		x	x	x				x	x			x	x	x	x	x		x	x			
Aulorhynchus flavidus	x	x	x	x	x			x	x	x	x	x		x			x	x			x	x									
Syngnathus leptorhynchus	x	x		x					x	x	x			x				x			x	x									
Gasterosteus aculeatus	x	x	x	x					x	x	x			x		x		x		x	x	x	x								
Cymatogaster aggregata	x	x		x		x	x		x	x				x		x		x					x								
Anoplarchis purpurescens	x		x	x			x	x	x		x	x	x			x	x				x	x	x	x	x	x	x	x			
Xiphister mucosus	x		x	x			x	x		x	x	x	x				x				x	x	x	x	x	x	x	x			
Lumpenus sagitta	x	x	x	x		x			x																						
Apodichthys flavidus	x		x	x				x	x	x	x	x	x				x	x	x		x	x	x	x	x		x	x			
Pholis laeta	x		x	x	x		x	x	x	x	x	x	x		x	x	x	x	x		x	x	x								
P. ornata	x		x	x	x				x	x	x	x	x			x		x	x	x	x	x	x								
Hexagrammos stelleri	x										x																				
H. decagrammus	x	x		x				x	x	x	x	x						x			x	x	x				x				
Leptocottus armatus	x		x	x	x	x	x		x		x					x		x		x	x	x	x								
Artedius fenestralis	x		x	x	x	x			x		x	x	x				x				x	x	x								
A. lateralis	x		x	x			x	x	x	x	x	x	x				x	x			x	x	x	x	x	x	x				
Oligocottus maculosus	x		x	x			x	x	x		x	x	x				x	x			x	x	x	x	x		x				
Enophrys bison	x		x	x			x	x		x		x						x			x	x	x								
Blepsias cirrhosus	x		x	x	x				x	x		x		x			x				x	x	x								
Platichthys stellatus	x		x	x	x	x			x		x			x		x	x	x			x	x									
Hippoglossoides elassodon			x		x	x			x							x				x											

Appendix 5.2 Matrix of taxonomic codes used to generate the taxonomic distances between fishes infected by *Pseudodelphis oligocotti* to varying degrees of parasite development.

Superorder: / Order: / Family	Species	Super order	Order	Family	Genus	Species
Paracanthopterygii:						
Batrachoidiformes:						
Batrachoididae	*Porichthys notatus*	1	1	1	1	1
Gobiesociformes:						
Gobiesocidae	*Gobiesox meandricus*	1	2	2	2	2
Acanthopterygii:						
Gasterosteiformes:						
Aulorhynchidae	*Aulorhynchus flavidus*	2	3	3	3	3
Syngnathidae	*Syngnathus leptorhynchus*	2	3	4	4	4
Gasterosteidae	*Gasterosteus aculeatus*	2	3	5	5	5
Perciformes:						
Embiotocidae	*Cymatogaster aggregata*	2	4	6	6	6
Stichaeidae	*Anoplarchis purpurescens*	2	4	7	7	7
Stichaeidae	*Xiphister mucosus*	2	4	7	8	8
Stichaeidae	*Lumpenus sagitta*	2	4	7	9	9
Pholidae	*Apodichthys flavidus*	2	4	8	10	10
Pholidae	*Pholis laeta*	2	4	8	11	11
Pholidae	*P. ornata*	2	4	8	11	12
Scorpaeniformes:						
Hexagrammidae	*Hexagrammas decagrammus*	2	5	9	12	13
Hexagrammidae	*H. stelleri*	2	5	9	12	14
Cottidae	*Leptocottus armatus*	2	5	10	13	15
Cottidae	*Artedius fenestralis*	2	5	10	14	16
Cottidae	*A. lateralis*	2	5	10	14	17
Cottidae	*Oligocottus maculosus*	2	5	10	15	18
Cottidae	*Enophrys bison*	2	5	10	16	19
Cottidae	*Blepsias cirrhosus*	2	5	10	17	20
Pleuronectiformes:						
Pleuronectidae	*Platichthys stellatus*	2	6	11	18	21
Pleuronectidae	*Hippoglossoides elassodon*	2	6	11	19	22

In this chapter I discuss the salient features of a parasitic life cycle with respect to how it influences host range. In doing so, I summarize the major results of each chapter, pointing out the limitations of this study and how to improve it.

Pseudodelphis oligocotti (Dracunculoidea: Guyanemidae) is an endoparasitic nematode originally described from *Oligocottus maculosus*, although larvigerous parasites were never recovered (Adamson and Roth 1990). Until this study almost nothing was known beyond *P. oligocotti*'s adult morphology and type host. I found *P. oligocotti* in 9 species of nearshore marine fishes in which it only reliably developed in one species, *Apodichthys flavidus*. Its development in the other fishes was variable but only led to completion in two incidences: one specimen of *Gobiesox meandricus*, and one specimen of *Pholis ornata* held in captivity with infected *A. flavidus*. This latter event was never observed in nature: wild-caught *Pholis ornata* individuals (63 sampled) never contained stages of *Pseudodelphis oligocotti* beyond subgravid adult. Although the specimen of *Pholis ornata* may have been infected before entering captivity, it may also have acquired the infection from captive *A. flavidus*. Direct transmission does not occur in this group of nematodes (Anderson 1992), but potential intermediate hosts may have been introduced into the aquarium with sand or coral by Vancouver Public Aquarium staff.

Pseudodelphis oligocotti's pattern of host distribution may in part be due to its method of transmission. In Chapter 2 I showed that *P. oligocotti* is transmitted by a free-living copepod intermediate host, common food item to small intertidal and subtidal fishes. Development in experimentally infected *Tigriopus californicus* progressed through three larval stages as expected. As the only representative of the Guyanemidae whose larvae have been described, *P. oligocotti* larvae are most similar in general body proportions to members of the genus *Philonema*

(Dracunculoidea: Philometridae; see descriptions by Ko and Adams 1969 and Vik 1964). Mortality of *T. californicus* due to infection was imperceptible. There are two alternative intermediate host types that transmit other members of Dracunculoidea that dwell in the blood: transmission by free-living intermediate host versus haematophagous arthropod vector (see Anderson 1992 for review). If *P. oligocotti* were transmitted by haematophagous arthropods, its final host distribution may have been different. Failure to infect wild-caught non-*Tigriopus californicus* species of copepods does not provide any information on the identity of *P. oligocotti*'s intermediate host in nature because copepods usually died before an infection attempt. Steps should be taken to improve conditions for wild copepod species in captivity before the question of identity of the intermediate host(s) in nature can be addressed. The specific identity of the intermediate host(s) of *P. oligocotti* would provide a more detailed explanation for its final host distribution.

Infected *Tigriopus californicus* were fed to 10 uninfected *Apodichthys flavidus*, two of which became infected, confirming both the role of a free-living copepod and *A. flavidus* in *P. oligocotti*'s transmission. Development in *A. flavidus* in nature consists of certain stages in different tissue sites. The final tissue site to be occupied by *P. oligocotti* larvigerous females is the hepatic sinus. Larvae are released into the blood stream and are swept downstream to the gills. They breach the gills and enter the water to be ingested by free-living copepods. This is the first demonstration of active gill exit by a dracunculoid larva. Other blood-dwelling dracunculoids either release larvae into the general circulation to be ingested by blood-sucking arthropods, or gravid females themselves migrate to, and breach, the gills (Tikhomirova 1971, Tikhomirova 1975, Tikhomirova 1980, Molnar 1976, Molnar 1980, Moravec 1978, Moravec and Dykova 1978, see Anderson 1992 for reviews).

Once in the copepod, there is no guarantee that larvae will find themselves back into a fish host in which they are capable of maturing. *Pseudodelphis oligocotti* infects many unusual fish hosts in which it follows a variable course of development that almost never results in transmission. However, Chapter 5 demonstrates that development appears to advance furthest in those hosts that are ecologically similar to *A. flavidus*, not necessarily taxonomically related.

It is postulated that one of the requirements that sets the stage for parasite speciation by host capture is a parasite's ability to develop in ecologically associated hosts: that is, it must have relaxed host requirements (Chabaud 1959, 1981). *Pseudodelphis oligocotti*'s development to the brink of maturity in ecologically associated hosts may require very few modifications on the part of the parasite or host to successfully develop to completion in unusual hosts. Alternative genotypes presumably exist in the population of *P. oligocotti* larvae, which is sampling genetically heterogeneous fish host populations in proportion to their ecological relatedness to *A. flavidus*. Parasite transfer to these unusual hosts is a requisite step in parasite speciation by host capture (Chabaud 1981). The results of this thesis indicate that parasite method of transmission and host ecology play an important role in the composition of parasite host range.

LITERATURE CITED

Adamson, M. L. 1980. *Gyrinicola batrachiensis* (Walton, 1929) n.comb. (Oxyuroidea; Nematoda) from tadpoles in eastern and central Canada. Canadian Journal of Zoology 59: 1344-1350.

---. 1981. Development and transmission of *Gyrinicola batrachiensis* (Walton, 1929) Adamson, 1981 (Pharyngodonidae; Oxyuroidea). Canadian Journal of Zoology 59: 1351-67.

---. 1986. Modes of transmission and evolution of life histories in zooparasitic nematodes. Canadian Journal of Zoology 64: 1375-1384.

Adamson, M. L. and Caira, J. N. 1994. Evolutionary factors influencing the nature of parasite specificity. Parasitology 109: S85-S95.

Adamson, M. L. and Roth, M. 1990. Prevalence and intensity of *Pseudodelphis oligocotti* n. gen., n. sp. (Dracunculoidea; Guyanemidae) in the tidepool sculpin, *Oligocottus maculosus* (Scorpaeniformes; Cottidae). Journal of Parasitology 76(4): 509-514.

Anderson, R. C. 1984. The origins of zooparasitic nematodes. Canadian Journal of Zoology 62: 317-328.

---. 1992. Nematode parasites of vertebrates: their development and transmission. Cambridge: C. A. B. International.

Appy, R. G., Anderson, R. C., and Khan, R. A. 1985. *Ichthyofilaria canadensis* n. sp. (Nematoda: Dracunculoidea) from eelpouts (*Lycodes* spp.). Canadian Journal of Zoology 63: 1590-1592.

Bane, G. W. and Bane, A. W. 1971. Bay fishes of Northern California (with emphasis on the Bodega-Tomales Bay Area). Southampton, New York: Mariscos Publications; 143 pp.

Bane, G. and Robinson, M. 1970. Studies on shiner perch, *Cymatogaster aggregata* Gibbons, in upper Newport Bay, California. Wasmann Journal of Biology 28(2): 259-268.

Barbosa, P. 1988. Some thoughts on "the evolution of host range." Ecology 69(4): 912-915.

Barraclough, W. E. 1967. Number, size composition and food of larval and juvenile fish caught with a two-boat surface trawl in the Strait of Georgia, June 6-8, 1966. Fisheries Research Board of Canada Report Series 928: 58 pp.

Barton, M. G. 1982a. Comparative distribution and habitat preferences of two species of stichaeoid fishes in Yaquina Bay, Oregon. Journal of Experimental Marine Biology and Ecology 59: 77-87.

---. 1982b. Intertidal vertical distribution and diets of five species of central California stichaeoid fishes. California Fish and Game 68(3): 174-182.

Bashirullah, A. K. M. and Ahmed, B. 1976. Development of *Camallanus adamsi* Bashirullah, 1974 (Nematoda: Camallanidae) in cyclopoid copepods. Canadian Journal of Zoology 54: 2055-2060.

Bayer, R. D. 1980. Size, seasonality, and sex ratios of the bay pipefish (*Syngnathus leptorhynchus*) in Oregon. Northwest Science. 54(3):161-166.

Becker, R. A., Chambers, J. M., and Wilks, A. R. 1988. The new S Language, a programming environment for data analysis and graphics. Pacific Grove, CA: Wadsworth & Brooks/Cole Advanced Books & Software; 702 pp.

Bennett, S. N., Adamson, M. L., and Margolis, L. 1998. Long-term changes in parasites of sockeye salmon (*Oncorhynchus nerka*) smolts. Canadian Journal of Fisheries and Aquatic Sciences 55(4): 977-986.

Boothe, P. 1967. The food and feeding habits of four species of San Francisco Bay fish. California Fish and Game. MRO Ref. Ser. (Amer.) 67-13: 1-151.

Bush, A. O., Aho, J. M., and Kennedy, C. R. 1990. Ecological versus phylogenetic determinants of helminth parasite community richness. Evolutionary Ecology. 4: 1-20.

Carl, G. C. 1964. Some common marine fishes of British Columbia. Victoria, B.C.: A. Sutton; 86 pp.

Chabaud, A. G. 1957. Spécificité parasitaire chez les nématodes parasites de vertébrés. In: Baer, J. G. Premier symposium sur la spécificité parasitaire des parasites de vertébrés. Zoology, Univ. Neuchatel: Imprimerie Paul Attinger S.A.; pp. 230-243.

---. 1959. Remarques sur l'evolution et la taxonomie chez les nematodes parasites de vertebres. Proceedings. International Congress of Zoology 15: 679-680.

---. 1981. Host range and evolution of nematode parasites of vertebrates. Parasitology 82: 169-170.

Clark, W. C.1994. Origins of the parasitic habit in the Nematoda. International Journal for Parasitology. 24 (8): 1117-1129.

Clemens, W. A. and Wilby, G. V. 1961. Fishes of the Pacific coast of Canada. Bulletin of the Fisheries Research Board of Canada 68: 1-443.

Costa, H. M. A., Moreira, N. I. B., and Oliveira, C. L. 1991. *Travassosnema* gen. n. with the description of *T. travassosi* sp. n. (Dracunculoidea, Guyanemidae) parasite of *Acestrorhynchus lacustris* Reinhardt, 1874 (Characidae) from Três Marias Reservoir, MG, Brazil. Mem. Inst. Oswaldo Cruz, Rio De Janeiro 86(4): 437-439.

Crichton, V. F. and Beverley-Burton, M. 1975. Migration, growth, and morphogenesis of *Dracunculus insignis* (Nematoda: Dracunculoidea). Canadian Journal of Zoology 53(2): 105-113.

DeMartini, E. E. 1978. Spatial aspects of reproduction in buffalo sculpin, *Enophrys bison.* Environmental Biology of Fishes 3(4): 331-336.

Dethier, M. N. 1980. Tidepools as refuges: predation and the limits of the harpacticoid copepod *Tigriopus californicus.* Journal of Experimental Marine Biology and Ecology 42: 99-111.

Devries, D. R. and Frie, R. V. 1996. Determination of age and growth. in: Murphy, B. R. and Willis, D. W., editors. Fisheries Techniques. 2nd edition ed. Bethesda, Maryland: American Fisheries Society; pp. 483-512.

Dogiel, V. A., Polyanski, Y. I., and Kheisin, E. M. 1964. General Parasitology.

Kabata, Z., translator. Edinburgh: Oliver and Boyd.

Edgar, G. J. and Shaw, C. 1995. The production and trophic ecology of shallow-water fish assemblages in southern Australia II. Diets of fishes and trophic relationships between fishes and benthos at Western Port, Victoria. Journal of Experimental Marine Biology and Ecology 194: 83-106.

Fedchenko, A. P. 1871. Concerning the structure and reproduction of the guinea worm (*Filaria medinensis* L.). American Journal of Tropical Medicine and Hygiene 20: 511-523.

Fitch, J. E. and Lavenberg, R. J. 1975. Tidepool and nearshore fishes of California. Berkeley, CA: University of California Press; 156 pp.

Frey, H. W. 1971. California's living marine resources and their utilization. California: California Department of Fish and Game; 148 pp.

Gibson, R. N. 1982. Recent studies on the biology of intertidal fishes. Oceanography and Marine Biology Annual Review 20: 363-414.

Goodson, G. 1988. Fishes of the Pacific coast: Alaska to Peru including the Gulf of California and the Galapagos. Stanford, California: Stanford University Press; 267 pp.

Gorbunova, N. N. 1962. Spawning and development of greenlings (Hexagrammidae). in: Rass, T. S., editor. Greenlings: Taxonomy, Biology, Interoceanic Transplantation. pp. 121-183.

Green, J. M. 1971a. Field and laboratory activity patterns of the tidepool cottid *Oligocottus maculosus* Girard. Canadian Journal of Zoology 49: 255-264.

---. 1971b. Local distribution of *Oligocottus maculosus* Girard and other tidepool cottids of the west coast of Vancouver Island, British Columbia. Canadian Journal of Zoology 49: 1111-1128.

Greene, C. W. 1924. Physiological reactions and structures of the vocal apparatus of the California singing fish, *Porichthys notatus.* Am. J. Physiol. 70(3): 496-499.

Hart, J. L. 1973. Pacific fishes of Canada. Bull. Fish. Res. Board Can. 180: 740 pp.

Harvey, P. H. and Pagel, M. D. 1991. The comparative method in evolutionary biology. New York: Oxford University Press; 239 pp.

Hay, D. E., Healey, M. C., Richards, L. J., and Marliave, J. B. 1989. Distribution, abundance and habitat of prey fishes in the Strait of Georgia. In: Vermeer, K. and Butler, R. W., editors. The ecology and status of marine and shoreline birds in the Strait of Georgia, British Columbia. Ottawa: Spec. Publ. Can. Wildl. Serv.; pp. 37-49.

Holmes, J. C. 1976. Host selection and its consequences. in: Kennedy, C. R., editor. Ecological aspects of parasitology. Amsterdam: North-Holland Publishing Company; pp. 21-39.

Holmes, J. C. and Price, P. W. 1980. Parasite communities: the roles of phylogeny and ecology. Systematic Zoology 29(2): 203-213.

Horn, M. H. and Gibson, R. N. 1988. Intertidal Fishes. Scientific American 258(Jan.): 64-70.

Howard, R. K. and Koehn, J. D. 1985. Population dynamics and feeding ecology of pipefish (Syngnathidae) associated with eelgrass beds of Western Port, Victoria. Australian Journal of Marine and Freshwater Research 36: 361-370.

Hubbard, J. D. and Reeder, W. G. 1965. New locality records for Alaskan Fishes. Copeia 4: 506-508.

Hubbs, C. L. 1920. The bionomics of *Porichthys notatus* Girard. The American Naturalist 54(633): 380-384.

Inglis, W. G. 1965. Patterns of evolution in parasitic nematodes. In: Evolution of parasites., Editor. Taylor, A.E.R. Oxford: Blackwell Scientific Publications; pp. 79-124.

---. 1971. Speciation in Parasitic Nematodes. Advances in Parasitology 9: 185-223.

Jamieson, Barrie G. M. 1991. Fish Evolution and Systematics: Evidence of

Spermatozoa. Cambridge: Cambridge University Press; ISBN: 0521413044.

Jones, A. C. 1962. The biology of the euryhaline fish *Leptocottus armatus armatus* Girard (Cottidae). University of California Publications (in) Zoology 67: 321-368.

Kennedy, C. R. 1975. Ecological Animal Parasitology. London: Blackwell Scientific Publications; 163 pp.

Ko, R. C. and Adams, J. R. 1969. The development of *Philonema oncorhynchi* (Nematoda: Philometridae) in *Cyclops bicuspidatus* in relation to temperature. Canadian Journal of Zoology 47: 307-312.

Lagler, K. F., Bardach, J. E., and Miller, R. R. 1962. Ichthyology. Ann Arbor, Michigan: John Wiley & Sons, Inc.

Lamb, A. and Edgell, P. 1986. Coastal fishes of the Pacific Northwest. Madeira Park, B.C.: Harbour Publishing.

Leberg, P. L. and Vrijenhoek, R. C. 1994. Variation among desert topminnows in their susceptibility to attack by exotic parasites. Conservation Biology 8: 419-424.

Leong, T. S.1975.Metazoan parasites of fishes of Cold Lake, Alberta: a community analysis. [Ph.D. Thesis]. Edmonton, Alberta: University of Alberta. 200 pages.

Leong, T. S. and Holmes, J. C. 1981. Communities of metazoan parasites in open water fishes of Cold Lake, Alberta. Journal of Fisheries Biology 18: 693-713.

Lewis, A. G., Chatters, L., and Raudsepp, M. 1998. Feeding structures and their functions in adult and preadult *Tigriopus californicus* (Copepoda: Harpacticoida). Journal of the Marine Biological Association of the United Kingdom 78(2): 451-466.

Limbaugh, C. 1962. Life history and ecological notes on the tubenose, *Aulorhynchus flavidus*, a hemibranch fish of western North America. Copeia 1962(3): 549-555.

MacInnis, A. J. 1976. How parasites find hosts: some thoughts on the inception of host-parasite integration. in: Kennedy, C. R., editor. Ecological aspects of parasitology. Amsterdam: North-Holland Publishing Company; pp. 3-20.

Margolis, L. and Arthur, J. R. 1979. Synopsis of the parasites of the fishes of Canada. Bulletin of the Fisheries Research Board of Canada. 199. 269 pages

Margolis, L., Esch, G. W., Holmes, J. C., Kuris, A. M., and Shad, G. A. 1982. The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). Journal of Parasitology 68: 131-133.

Marliave, J. B.1975.The behavioral transformation from the planktonic larval stage of some marine fishes reared in the laboratory. [Doctoral Thesis]. Vancouver, B.C: University of British Columbia, Zoology.

---. 1977. Substratum preferences of settling larvae of marine fishes reared in the laboratory. Journal of Experimental Marine Biology and Ecology 27: 47-60.

---. 1986. Lack of planktonic dispersal of rocky intertidal fish larvae. Transactions of the American Fisheries Society 115: 149-154.

Mathsoft, Data Analysis Products Division. 1998. Splus4 Guide to Statistics. Seattle, WA: Mathsoft.

Metaxas, A. and Scheibling, R. E. 1993. Community structure and organization of tidepools. Marine Ecology Progress Series 98: 187-198.

Miller, B. S., Simenstad, C. A., Cross, J. N., Fresh, K. L., and Steinfort, S. N. 1980. Nearshore fish and macroinvertebrate assemblages along the Strait of Juan de Fuca including food habits of the common nearshore fish: final report of three years' sampling, 1976-1979. Washington, D.C.: Office of Environmental Engineering and Technology, Office of Research and Development, U.S. Environmental Protection Agency.

Molnar, K. 1976. Data on the developmental cycle of *Philometra obturans* (Prenant, 1886) (Nematoda: Philometridae). Acta Veterinaria Academiae Scientiarium Hungaricae 26: 183-188.

---. 1980. Recent observations on the developmental cycle of *Philometra obturans* (Prenant, 1886) (Nematoda: Philometridae). Parasitologia Hungarica 13: 65-66.

Moorthy, V. N. 1938. Observation on the development of *Dracunculus medinensis* larvae in *Cyclops*. American Journal of Hygiene 27: 437-460.

Moravec, F. 1977. The development of the nematode *Philometra abdominalis* Nybelin, 1928 in the intermediate host. Folia Parasitologica 24: 237-245.

---. 1978. The development of the nematode *Philometra obturans* (Prenant, 1886) in the intermediate host. Folia Parasitologica 25: 303-315.

---. 1980. Development of the nematode *Philometra ovata* (Zeder, 1803) in the copepod intermediate host. Folia Parasitologica 27: 29-37.

---. 1994. Parasitic Nematodes of Freshwater Fishes of Europe. Prague: Kluwer Academic Publishers.

Moravec, F. and Dykova, I. 1978. On the biology of the nematode *Philometra obturans* (Prenant, 1886) in the fishpond system of Macha Lake, Czechoslovakia. Folia Parasitologica 25: 231-240.

Moravec, F. and Scholz, T. 1990. First record of *Avioserpens* larvae (Nematoda) from the naturally infected intermediate host. Folia Parasitologica 37: 93-94.

Moser, H. G., Richards, W. J., Cohen, D. M., Fahay, M. P., Kendall Jr., A. W., and Richardson, S. L. 1984. Ontogeny and Systematics of Fishes. Lawrence, KA: Allen Press.

Moyle, P. B. 1976. Inland fishes of California. Berkeley, CA: University of California Press; 405 pp.

Nakamura, R. 1976. Experimental assessment of factors influencing microhabitat selection by the two tidepool fishes *Oligocottus maculosus* and *O. snyderi*. Marine Biology 37: 97-104.

Niggol, K.1982. Data of fish species from Bering Sea and Gulf of Alaska. Seattle, WA: NOAA/NMFS.

Noble, E. R., Noble, G. A., Schad, G. A., and MacInnes, A. J. 1989. Parasitology: the biology of animal parasites. sixth ed. Philadelphia: Lea & Febiger.

Orcutt, H. G. 1950. The life history of the starry flounder *Platichthys stellatus* (Pallus). California Department of Fish and Game Bulletin 78: 64 pp.

Paulson, D. 1998. Washington Marine Fishes [Web Page]. Accessed 1998. Available at: www.ups.edu/biology/museum/WAmarfishes.html.

Peden, A. E. and Hughes, G. W. 1984. Distribution, morphological variation, and systematic relationship of *Pholis laeta* and *P. ornata* (Pisces: Pholididae) with a description of the related form *P. nea* n. sp. Canadian Journal of Zoology 62: 291-305.

Petter, A. J. 1974. Deux nouvelles espèces du Camallanina, parasites de Hoplerythrinus unitaeniatus (Characidae, Cypriniformes) en Guyane; création d'une nouvelle famille: les Guyanemidae (Dracunculoidea). Bulletin De Musée National D'Histoire Natural, Paris, 3^{e} Sér., N° 232, Zoologie 156: 803-812.

---. 1987. Nématodes de poissons de l'equateur. Revue Suisse Zool. 94(1): 61-76.

Petter, A. J., Cassone, J., and Le Belle, N. 1990. Observations sur la biologie des premiers stades larvaires d'*Anguillicola crassus*, nématode parasite d'anguille. Annales De Parasitologie Humaine Et Comparée 65(1): 28-31.

Petter, A. J. and Dlouhy, C. 1985. Nématodes de poissons du Paraguay. III. *Camallanina.* Description d'une espèce et d'une sous-espèce nouvelles de la famille des Guyanemidae. Revue Suisse Zoology 92(1): 165-175.

Pianka, E. R. 1975. Niche relations of desert lizards. in: Cody, M. L. and Diamond, J. M., editors. Ecology and evolution of communities. Cambridge, Mass.: Belknap Press; pp. 292-314.

Porter, R. G. 1964.Food and feeding of staghorn sculpin (*Leptocottus armatus* Girard) and starry flounder (*Platichthys stellatus* Pallus) in euryhaline environments. [M.Sc. Thesis]. Oregon: Humboldt State College. 84 pp.

Powlik, J. J., Lewis, A. G., and Spaeth, M. 1997. Development, body length, and feeding of *Tigriopus californicus* (Copepoda, Harpacticoida) in laboratory and field populations. Crustaceana 70(3): 324-343.

Read, A. F. and Skorping, A. 1995. The evolution of tissue migration by parasitic nematode larvae. Parasitology 111(3): 359.

Rhode, K. 1979. A critical evaluation of intrinsic and extrinsic factors responsible for niche restriction in parasites. The American Naturalist 114: 648-671.

Roberts, L. S. and Janovy Jr., J. 1996. Gerald D. Schmidt & Larry S. Roberts' Foundations of Parasitology. 5 ed. Dubuque, IA, USA: Wm. C. Brown Publishers.

Schultz, L. P. and DeLacy, A. C. 1932. The eggs and nesting habits of the crested blenny, *Anoplarchus*. Copeia 3: 143-147.

Secord, D. and Kareiva, P. 1996. Perils and pitfalls in the host specificity paradigm. BioScience 46(6): 448-453.

Slauson, D. O. and Cooper, B. J. 1990. Mechanisms of Disease: a textbook of comparative general pathology. 2nd ed. Baltimore, MD: Williams and Wilkins.

Solter, L. F. and Maddox, J. V. 1998. Physiological host specificity of Microsporidia as an indicator of ecological host specificity. Journal of Invertebrate Pathology 71(3): 207-216.

Steffe, A. S., Westoby, M., and Bell, J. D. 1989. Habitat selection and diet in two species of pipefish from seagrass: sex differences. Marine Ecology Progress Series 55: 23-30.

Sukhdeo, M. V. K. 1990. Habitat selection by helminths: a hypothesis. Parasitology Today 6(7): 234-237.

Sukhdeo, M. V. K. and Mettrick, D. F. 1986. The behavior of juvenile *Fasciola hepatica*. Journal of Parasitology 72(4): 492-497.

Supryaga, A. M. 1965. On the recognition of the developmental cycle of *Avioserpens mosgovoyi* n.sp. (Camallanata: Dracunculidae), a nematode of

birds. Mater. Nauchn. Konf. VOG 4: 275-277.

---. 1971. Life cycle of *Avioserpens mosgovoyi* (Camallanata, Dracunculidae), a nematode of waterbirds. Sbor. Rabot po gelmintologii posvyashch. Kolos, Moscow: 90-letiyu Akad. K.I. Skryabina, Izd.; pp. 374-383.

Tikhomirova, V. A.1971. Biology of Skrjabillanidae (Camallanata). [In Russian]. Trudi Gelmintologische Laboratorii. 22: 208-211.

---. 1975. The life cycle of nematodes of the family Skrjabillanidae. Voprosy ekologii zhivotnykh Vypush 2. Kalinin, USSR; pp. 100-113.

---. 1980. Nematodes of the family Skrjabillanidae (Nematoda: Camallanata). Parazitologiya 14: 258-262.

Vik, R. 1964. Notes on the life history of *Philonema agubernaculum* Simon et Simon, 1936 (Nematoda). Canadian Journal of Zoology 42(511-513).

Wakelin, D. and Apanius, V. 1997. Immune defense: genetic control. in: Clayton, D. H. and Moore, J., editors. Host-parasite evolution. Oxford: Oxford University Press; pp. 30-58.

Wang, J. C. S. 1986. Fishes of the Sacramento-San Joaquin Estuary and adjacent waters, California: a guide to the early life histories. [Web Page]. Accessed 1998. Available at: elib.cs.berkeley.edu/kopec/tr9.

Wang, P., Sun, Y., and Zhoa, Y. 1983. Studies on the life history and epidemiology of *Avioserpens taiwana* (Sugimoto, 1919) of the domestic duck in Fugian. Acta Zoologica Sinica 29: 350-357.

Wilkie, D. W.1966.Colour pigments in the penpoint gunnel *Apodichthys flavidus* and their ecological significance. [M. Sc. thesis]. Vancouver, B.C.: Zoology, University of British Columbia. 143 pp.

Wilson, C. B.1932. The Copepods of the Woods Hole Region Massachusetts. U. S. National Museum Bulletin. 158.

Woolhouse, M. E. J. 1998. Patterns in parasite epidemiology. Parasitology Today 14(10): 429-434.

Yasutake, W. T. and Wales, J. H. 1983. Microscopic Anatomy of Salmonids:

an Atlas. Washington, D.C.: US Fish and Wildlife Service.

Yatsu, A. 1981. A revision of the gunnel family Pholididae (Pisces, Blennioidei). Bulletin of the National Science Museum, Tokyo, Series A 7(4): 166-190.

Yoshiyama, R. M. 1981. Distribution and abundance patterns of rocky intertidal fishes in central California. Environmental Biology of Fishes 6(3/4): 315-332.

Yoshiyama, R. M. and Darling, D. S. 1982. Grazing by the intertidal fish *Anoplarchis purpurescens* upon a distasteful polychaete worm. Environmental Biology of Fishes 7(1): 39-45.

Yoshiyama, R. N. 1980. Food habits of three species of rocky intertidal sculpins (Cottidae) in central California. Copeia 1980(3): 515-525.

Zar, J. H. 1996. Biostatistical Analysis. 3rd ed. Englewood Cliffs New Jersey: Prentice-Hall, Inc.