HATCHING, COPEPODID SURVIVAL AND LARVAL DEVELOPMENT OF *SALMINCOLA EDWARDSII* (CRUSTACEA: COPEPODA) ON BROOK TROUT (*SALVELINUS FONTINALIS*)

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SUGGESTED SHORT TITLE:

EARLY LIFE CYCLE OF THE PARASITE SALMINCOLA EDWARDSII ON BROOK TROUT

ABSTRACT

Salmincola edwardsii is an ectoparasitic copepod typically found on the gills of brook trout (Salvelinus fontinalis). Laboratory experiments were conducted to determine: i) the effects of temperature and photoperiod on early life cycle events, and ii) the rate of larval development to adult. Egg incubation time, duration of copepodid swimming activity and copepodid survival time all decreased with increasing temperature. Different photoperiods had no effect. Hatching duration and hatching success were not influenced by either temperature or photoperiod. Copepodids remained alive and active for up to 16 days at 8° and 5 days at 20°C. Adult male copepods were observed at 3 to 8 days after host exposure. They lived for up to 3 days. Adult females became permanently attached to hosts between 11 and 16 days post-infection.

ABREGE

Salmincola edwardsii est un copépode ectoparasitaire généralement retrouvé dur les branchies de l'omble de fontaine (Salvelinus fontinalis). Des expériences en laboratoire furent effectuées afin de mesurer i) les effets de la température et de la photopériode sur les étapes initiales du cycle vital, et ii) le taux de développement larvaire jusqu'au stade adulte. Le temps d'incubation des oeufs, la durée de la période d'activité natatoire des copépodites, et leur temps de survie on tous diminué suite à une hausse de température. Les différentes photopériodes n'eurent aucun effet. La durée de la période d'éclosion et le succès d'éclosion ne furent pas influencés par la température ou la photopériode. Les copépodites demeurèrent vivants et actifs jusqu'à 16 jours à 8° et 5 jours à 20°C. Des copépodes adultes mâles furent observés de 3 à 8 jours suivant l'exposition de l'hôte aux parasites. Ils survécurent jusqu'à 3 jours. Des femelles adultes devinrent attachées en permanence à leurs hôtes entre 11 et 16 jours suivant l'infection.

DEDICATION

To Cynthia,

For her love and support.

And for giving me
the most beautiful little daughter.

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CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Parasitic copepods of the genus Salmincola are considered serious fish pathogens when found in freshwater salmonid culture facilities (Kabata 1970). Reports of their adverse effects on cultured fish populations have appeared in the literature since the beginning of this century (Wilson 1911, 1915; Fasten 1912, 1918, 1921a; Smallwood 1918; Savage 1935; Davis 1953; Kabata 1970; Hare and Frantsi 1974; Sutherland and Wittrock 1985).

The literature on Salmincola prior to 1940 is often unscientific in the modern sense, giving information which is anecdotal, speculative, and sometimes erroneous. Many statements, unsupported by data, have become entrenched assumptions. Kabata (1969) undertook to review the entire genus because of "the inadequate quality of the earlier work," prior to investigating the biology and host-parasite relationship of S. californiensis. Although species of the genus have been known for over 200 years (Kabata 1969), the complete life cycle of Salmincola has only been described within the last 20 years by Kabata and Cousens (1973).

Considering their importance as parasitic pathogens in fish culture facilities, more needs to be known concerning the biology and life history of these ectoparasitic crustaceans in order to develop practical and cost-effective control strategies, none of which exist at the present time (Kabata 1970; Hoffman and Meyer 1974; Cousens 1977). Moreover, the results of such basic research may provide new approaches for future studies on parasitic crustaceans in general.

Three Salmincola species are particularly important parasites of commercially cultured salmonids: S. salmoneus, commonly on Atlantic salmon (Salmo salar); S. californiensis, commonly on members of the genus Oncorhynchus; and S. edwardsii, commonly on members of the genus

Salvelinus (Nabata 1969).

Brook trout (Salvelinus fontinalis) has long been cultured in Quebec for the purpose of stocking lakes and streams for angling. Provincial legislation passed in 1989 approved the production of brook trout for public consumption as a food fish with the result that brook trout can now be farmed and sold to processors, wholesalers and retailers. The creation of this new market for trout producers will stimulate more intensive cultivation of the fish resulting in greater opportunity for the occurrence of disease outbreaks.

Epizootics of S. edwardsii were reported on several commercial trout farms in Quebec during the summer of 1987. These ectoparasitic crustaceans have a direct life cycle which, when combined with the high densities of potential hosts present in fish culture facilities, can rapidly create a serious fish health problem.

As a result of discussions with a Quebec Government fish pathologist, Dr. Robert Péloquin, (Ministère de l'Agriculture, de la Pêche et de l'Alimentation, Rock Forest, Quebec), laboratory experiments were undertaken at the Institute of Parasitology in an effort to learn more about the biology and life history of S. edwardsii and its association with brook trout.

The objectives of my research were i) to determine the effects of temperature and photoperiod on duration of hatching, duration of swimming activity and survival of the infective stage (copepodid), and ii) to monitor the rate of development from larvae to adult on brook trout. The results of experiments in these specific areas are presented in chapters 3 and 4, respectively.

The opportunity to explore the fascinating biology and behavior of this species was limited, by necessity, and there remains a great amount of study to be done. It is hoped that the work reported in this thesis will encourage others to carry on more in-depth investigations of these interesting creatures.

LITERATURE REVIEW

Classification:

Based upon the system set forth by Kabata (1979), S. edwardsii is classified as follows: Phylum: Arthropoda; Class: Crustacea; Order: Copepoda; Suborder: Siphonostomatoida; Family: Lernaeopodidae; Genus: Salmincola Wilson, 1915; Species: edwardsii (Olsson 1869).

Taxonomy:

The genus Salmincola was established by Wilson (1915) for the freshwater species of Lernaeopoda. The name has its roots in "Salmo, the host family and incola, a dweller".

S. edwardsii is one of 17 species of the genus Salmincola (Kabata 1988). Kabata's (1969) revision of the genus Salmincola Wilson, 1915 indicated a long list of species synonyms for S. edwardsii (Olsson 1869): Lernaeopoda salmonea Mayor, 1824; Basanistes salmonea Milne Edwards, 1840; Lernaeopoda edwardsii Olsson, 1869; Lernaeopoda fontinalis Smith, 1874; Lernaeopoda alpina Olsson, 1877; Lernaeopoda arcturi Miers, 1877; Lernaeopoda bicauliculata Wilson, 1908; Salmincola arcturi (Miers 1877), Wilson, 1915; Salmincola bicauliculata (Wilson 1908), Wilson, 1915; Salmincola oquassa Wilson, 1915; Salmincola mattheyi Dedie, 1940; Salmincola exsanguinata Sandeman and Pippy, 1967.

Host associations:

S. edwardsii has been reported primarily on fishes of the genus Salvelinus - brook trout (brook char) (S. fontinalis), lake trout (lake char), (S. namaycush), Arctic char (S. alpinus) and Dolly Varden (S. malma), but also on mountain whitefish Prosopium williamsoni. Reports of its occurrence on rainbow trout O. mykiss and cutthroat trout O. clarki, (formerly Salmo gairdneri and S. clarki, respectively), are considered doubtful (Margolis and Arthur 1979; Kabata 1988).

Distribution and host range:

S. edwardsii is circumpolar in distribution, living in freshwater habitats of the northern hemisphere. According to Kabata (1969), its range coincides with that of its common hosts, fishes of the genus Salvelinus - brook trout, lake trout, Arctic char and Dolly Varden.

Brook trout are endemic to northeastern North America from Hudson Bay drainages to the Atlantic seaboard as far south as Cape Cod, in the Appalachian Mountains to Georgia and westward in the Great Lakes drainages and upper Mississippi basin to Minnesota (Scott and Crossman 1973; Lee et al. 1980).

Lake trout are native to and widely distributed in northern North America. Its natural range extends in a great arc from Alaska, the Yukon Territory and interior British Columbia eastward through the Northwest Territories and many arctic islands, from southern Alberta and the northern portions of the prairie provinces through the Great Lakes region and the New England states to Nova Scotia and north to the tip of Quebec. Pockets of lake trout occur in Montana and Idaho but it is completely absent from the southern portions of the prairie provinces (Scott and Crossman 1973; Lee et al. 1980).

Arctic char is the most northerly freshwater species, has a circumpolar distribution and occurs in nearshore marine waters as well as in lakes and rivers. Its range extends across northern North America from Alaska through the arctic islands to northern Quebec and Newfoundland. This range extends to Greenland, Iceland and northern Europe (especially Scandinavia), through the northern Soviet Union to northern Asia and south to Kamchatka. More southerly landlocked populations occur in Europe, including parts of the Alps, and in the British Isles, as well as in pockets in North America in eastern Canada, Maine and New Hampshire (Scott and Crossman 1973; Lee et al. 1980; Fryer 1981).

Dolly Varden are found in both fresh and salt waters of western North America and eastern Asia. Their native range extends from northern

California, western Montana, Nevada and Idaho north to the Seward Peninsula in Alaska on the North American side. In Asia it occurs from the Sea of Japan, north through the Kuril Islands and Kamchatka to the Anadyr River in the USSR and across the Aleutian chain. Dolly Varden also occur in drainages of the Chukchi and Beaufort Seas (Scott and Crossman 1973; Lee et al. 1980).

Salvelinus species, mainly brook and lake trout, have been introduced widely outside their natural range; see MacCrimmon and Campbell (1969), MacCrimmon et al. (1971) and Balon (1980) for extended ranges.

It is quite possible that S. edwardsii has been transferred with introduced populations of Salvelinus species, just as S. californiensis has been transferred with stocks of rainbow trout (Oncorhynchus mykiss) (Buttner and Heidinger 1979; Hoffman 1984; Sutherland and Wittrock 1985). Hoffman (1984) suggested that Salmincola can be transferred on live fish as well as with shipments of trout eggs.

Biology and life history:

Although S. edwardsii lives in freshwater habitats, adult females may survive and grow for undetermined periods (1-2+ years) on anadromous fish in marine environments (Friend 1941; Black et al. 1983; Bailey et al. 1989). However, mating and the production of eggs is unlikely while still at sea (Friend 1941). Free-swimming copepodids are killed when placed in a solution of sodium chloride at a concentration of 20,000 ppm (20 ppt) (Fasten 1912; Hoffman and Meyer 1974) and when placed in sea water (Friend 1941), proving it impossible for infections to occur in marine environments.

The life cycles of the various species of Salmincola are similar (see Fig. 1.1), consisting of six distinct stages, separated by five molts (Kabata and Cousens 1973). Hatching of the egg results in the simultaneous molt of the nauplius into the copepodid. This first stage is free-swimming and must contact a suitable host within 24-48 hours (Fasten 1912; Wilson

1915; Kabata and Cousens 1973), in order to survive and undergo further development. However, Friend (1941) reported copepodids of S. salmoneus surviving up to 6 days in the laboratory. Kabata and Cousens (1977) suggested that host-seeking behavior is initiated by shadows and disturbances in the surrounding water and Poulin et al. (1990b) confirmed this hypothesis.

Upon contact, the copepodid moves over the surface of the fish searching for a suitable location to implant the frontal filament, an appendage located between the anterior dorsal margin and medial eye of the copepodid (see Fig. 1.2). Kabata and Cousens (1973, 1977) were unable to determine the factors which influence the choice of an attachment site, but the presence of solid subdermal support, such as a fin ray, the rod of a gill filament, a scale, or a bone, is important. The copepodid then excavates a small cavity into which the terminal plug of the frontal filament is cemented. Successful attachment is followed by the commencement of the first molt, characterized by the loss of swimming appendages and body segmentation of the copepodid.

Four chalimus instars follow, each chalimus molt involving a complicated series of maneuvers in which the frontal filament is detached and reattached to the second maxillae. After the fourth chalimus molt, the young adult male (0.7 mm long; see Fig. 1.3) does not reattach but breaks free and sets off in search of a female. The late fourth chalimus female breaks free of the frontal filament and using its maxillipeds and antennae seeks a site for permanent attachment. It rasps out a hole for implantation of the bulla, completes the final molt, then everts the bulla from the frontal region and places it in the excavation. Permanent attachment is completed when the linked tips of the second maxillae are cemented into small ducts in the implanted bulla (for illustrated details see Kabata and Cousens 1973).

Mating takes place when a male locates and attaches to the genital region of a chalimus IV or adult female and fertilizes it by inserting

paired spermatophores into the vaginal openings. After fertilization, the vaginae are plugged by a cement extruded from the male and the female cannot be fertilized again (Kabata and Cousens 1973). Having performed its function, the male then drops off and dies. The life span of the adult male is still unknown.

The pale yellowish adult female, now permanently attached to the host by the bulla (see Fig. 1.4), grazes on the epithelium, increases in size, (reaching between 2-4 mm in length), and produces eggs which become visible within the trunk. Two slender egg sacs appear at the base of the trunk and the ripe ova are fertilized as they pass down the oviduct into the egg sacs, past the stored spermatozoa in the spermathecae. The embryos complete development in the 2-3 mm long egg sacs, with egg maturation being marked by changes in pigmentation from light to dark brown. The eggs are ready to hatch about 30 days after fertilization, at 12°C. The female produces two batches of between 60-200 eggs (Fasten 1921a; Savage 1935) during its lifetime. Fasten (1912) speculated the normal life span to be about 2.5 months but taking into account seasonal factors regulating development, 6 months (Smallwood 1918; Hoffman 1977) to a year (Bailey et al. 1989) or more (Friend 1941) may be possible. After death, the body of the copepod gradually disintegrates while still attached by the bulla to the tissue of the host.

Figure 1.1. Life cycle diagram of Salmincola edwardsii.
(Redrawn by M. LaDuke. After Kabata and Cousens 1973).

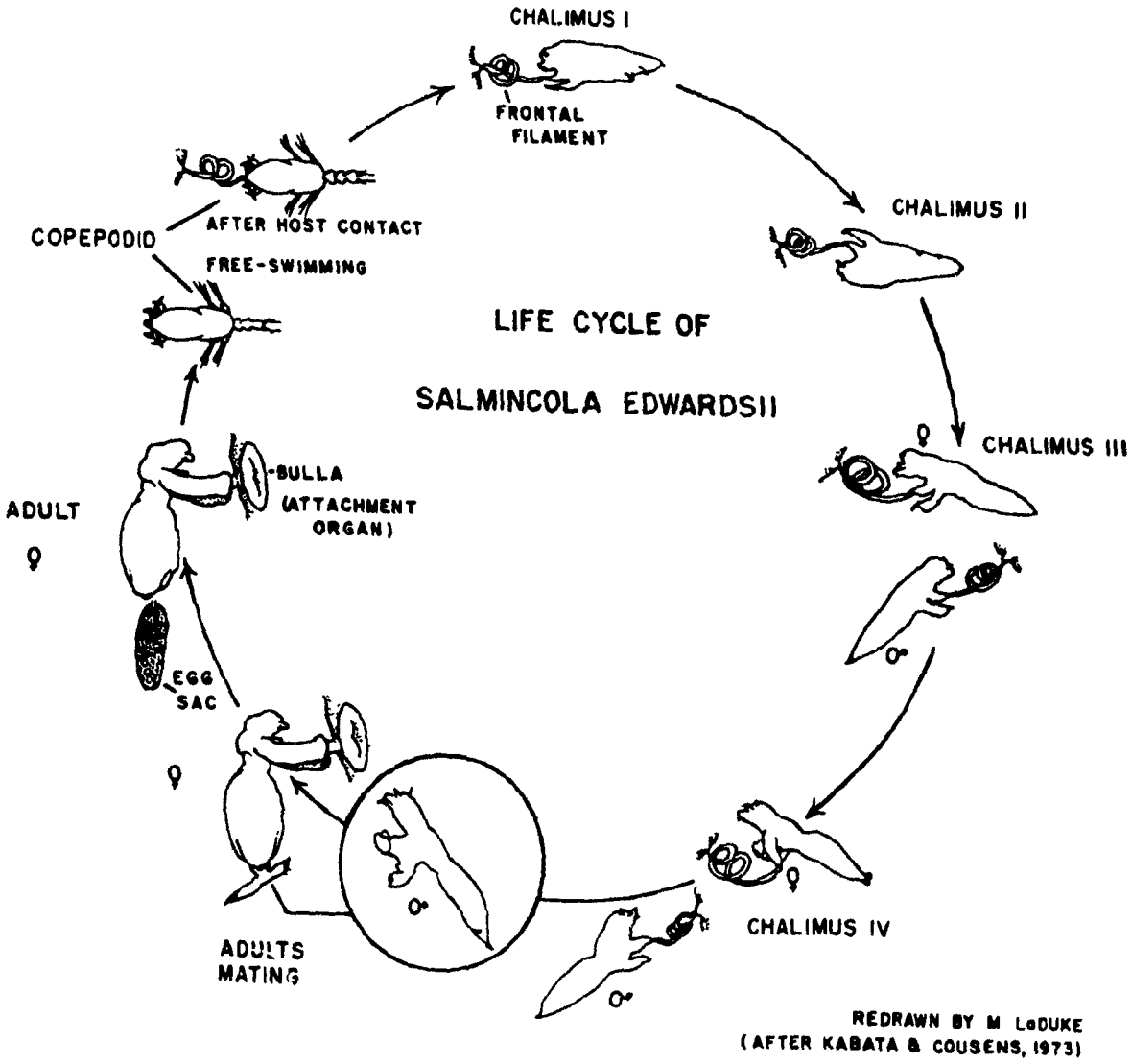

CHALIMUS I
FRONTAL FILAMENT
CHALIMUS II
CHALIMUS III
CHALIMUS IV
ADULTS MATING
EGG SAC
ADULT
BULLA (ATTACHMENT ORGAN)
COPEPODID
AFTER HOST CONTACT
FREE-SWIMMING
LIFE CYCLE OF
SALMINCOLA EDWARDSII
REDRAWN BY M LaDUKE
(AFTER KABATA & COUSENS, 1973)

Figure 1.2. Copepodid of S. edwardsii.

Dorsal view and lateral view. FF - frontal filament.

(Redrawn by C. Bonnell. After Kabata and Cousens 1973).

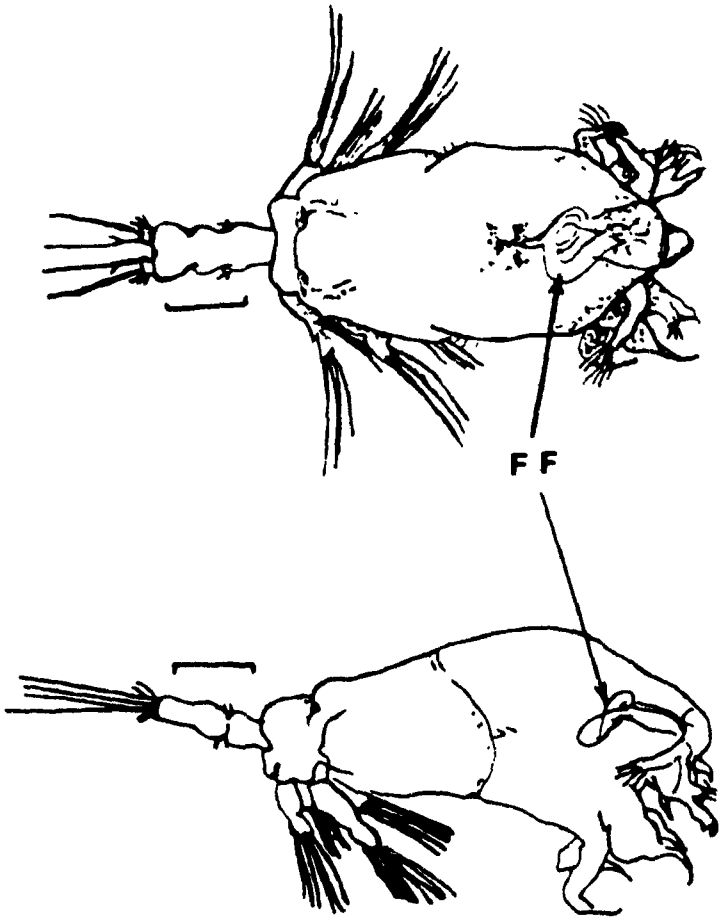

F F

Figure 1.3. Adult male S. edwardsii.

Lateral view and ventral view.

(Redrawn by C. Bonnell. After Kabata and Cousens 1973).

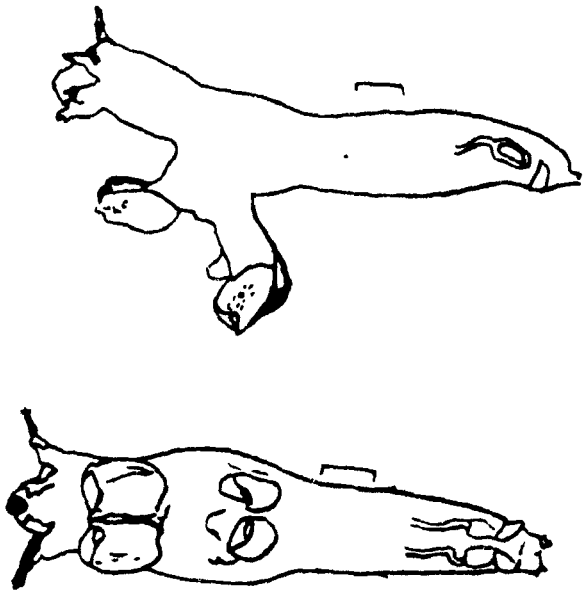
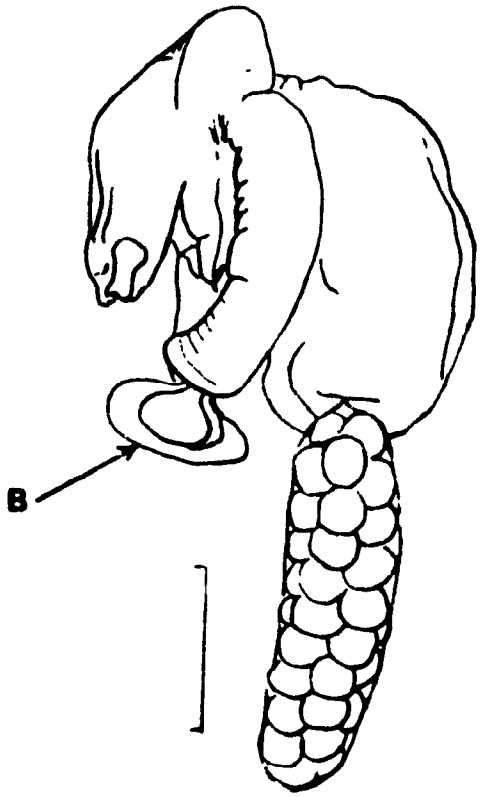


Figure 1.4. Adult female S. edwardsii.
Lateral view. B - bulla.
(Redrawn by C. Bonnell. After Fryer 1981).


B

Critical periods in the survival and development of Salmincola occur at the time of attachment of copepodids to the host by the frontal filament and at the time of each successive molt. Copepods which fail to successfully molt cease development and drop off within hours (Kabata and Cousens 1973). Implantation of the bulla by females and its coupling to the second maxillae is also accompanied by hazards. It is not surprising that Kabata and Cousens (1973) found a decline in attached copepods over the course of development on the host.

In reviewing the literature on S. edwardsii, it becomes obvious how little is really known about these organisms in spite of their long associations with economically important salmonids of the genus Salvelinus. Identifying and classifying these ectoparasitic crustaceans created many problems for early taxonomists, as evidenced by the long list of synonyms. This in turn contributed to many errors in recording host associations (Hoffman 1967; Kabata 1969; Margolis and Arthur 1979; Kabata 1988).

Although the life cycle and approximate developmental rate for S. californiensis at 11-12°C has now been described by Kabata and Cousens (1973), it remains to be proven that other members of the genus follow the same pattern.

It is also important to investigate the length of the free-swimming period and copepodid survival to determine the duration of infectivity.

The life spans of the adult male and female are also unknown. No one has followed the life cycle closely enough or long enough to determine their duration under natural conditions. The effect of seasonal temperature variation on physiological and metabolic processes is recognised in other organisms, and it remains to be shown what effect it may have on Salmincola's survival and reproduction. Also the number of egg batches per female during her lifetime may be more than the two stated in the literature.

Linked to this matter of longevity is the question of how long the

adult female copepod can survive on anadromous fish in marine environments and whether or not this experience adversely affects reproductive performance.

Host associations are another area which needs clarification. To my knowledge (Z. Kabata, pers. comm.), no studies have been attempted to show the degree of host specificity of Salmincola species. The growth of intensive salmonid aquaculture and the highly artifical environments created to maximise fish production may facilitate opportunities for ectoparasites such as Salmincola to exploit the new niches arising in such novel environments.

Filling these gaps in our knowledge of Salmincola would provide a sound basis for further studies of this genus and related genera.

Effects on host fish:

In natural populations of salmonid fishes, the prevalence and intensity of Salmincola infestations are low (<10 adults/fish), resulting in little or no effect on condition, fecundity and disease susceptibility (Friend 1941; Pippy 1969; Allison and Latta 1969), and may even be self-limiting (McGladdery and Johnston 1988). However, the presence of Salmincola species in salmonid culture facilities is potentially dangerous because of their direct life cycles and their ability to cause great mortality (Fasten 1918). Dense fish populations and low water flow rates enhance parasite transmission (Fasten 1912; Friend 1941), resulting in the rapid development of a serious fish health problem.

The gills are the major site of attachment although the mouth, branchial cavity, opercula, fins and body surface can also be infested (Fasten 1912; Savage 1935; Kabata 1969, 1970; Kabata and Cousens 1977). Parasite prevalence and intensity increases with host size (Friend 1941; Kabata and Cousens 1977) and both sexes appear equally susceptible (Black 1982). Fish with heavy infestations (>30 adult females) on their gills suffer particularly during periods of high temperature and low dissolved

oxygen (Sutherland and Wittrock 1985; Vaughan and Coble 1975) and severe infestations (>120 adult females) are potentially lethal (McGladdery and Johnston 1988). Retarded growth and sexual maturation of the host also occurs as a result of heavy infestations (Hoffman 1977; Sutherland and Wittrock 1985). Gall et al. (1972) reported that S. californiensis can reduce fecundity of rainbow trout but that it does not appear to affect egg size. On the other hand, Savage (1935) and Vaughan and Coble (1975) noted no decline in egg yield in brook trout but Savage (1935) observed that eggs were slightly smaller than normal. He also observed a lack of vitality in fry spawned from heavily infested brood stock.

Salmincola infestations produce mechanical injuries to gills, skin, muscle, and even bone (Kabata 1970; Sutherland and Wittrock 1985). The "burrowing phenomenon" of S. californiensis is most spectacular: prolonged excavation of a cavity for implantation of the frontal filament can result in perforation of the body wall and penetration of the viscera (Kabata and Cousens 1977).

Injuries to fish tissues result from the breaching of the integument caused by the attachment and feeding activities of the parasite. Damage is also caused by the pressure exerted on soft tissues by the copepod in the confined spaces of the gill cavity. Other injuries occur indirectly by the stimulation of host behavior such as chaffing or rubbing of fins and skin to dislodge the parasites. The extent of damage depends on the number and size of the copepods (Kabata and Cousens 1977).

Implantation of the frontal filament and bulla stimulate vigorous tissue responses of the epithelium resulting in firmer attachment as well as serving as a source of nutrients for the parasite (Sutherland and Wittrock 1985). Kabata and Cousens (1972, 1977) and Cousens (1977) suggested the possibility of metabolic exchanges between parasite and host through the bulla but were unable to substantiate this in vivo.

Feeding activities, which consist of browsing on epithelial cells and blood, create a feeding cavity in affected tissues. Ingested blood can

be observed in the gut, being a reddish to dark brown. Shredding of tissues by the feeding appendages leads to epithelial hyperplasia and loss of mucous cells and the scale layer. Pressure exerted by the parasite results in partial or complete disappearance of affected tissues. Friend (1941) and Kabata and Cousens (1977) used the term "crypting" to describe how the presence of the adult female causes atrophy and/or disappearance of the tips of affected gill filaments. Adjacent parts of the gill affected by tissue reactions are rendered non-functional for respiration, excretion and osmoregulation. Rubbing by the fish results in frayed or lost fins (Kabata 1970, 1984; Kabata and Cousens 1977; Sutherland and Wittrock 1985). Secondary infections by viruses, bacteria or fungi are frequent dangers (Kabata 1970; Cusack and Cone 1986; Donoghue 1986). Kabata (1970, 1984) provides comprehensive reviews of injuries caused by parasitic crustaceans.

Juvenile sockeye salmon (O. nerka) parasitised with S. californiensis showed reduced weight and developed anemia, due to damage of gill and skin epithelia. Infested fish were also less able to cope with increases in temperature and their ability to swim was reduced as they became quickly fatigued; the ability of these fish to tolerate increased salinity was also reduced (Pawaputanon 1980). Kabata and Cousens (1977) reported a 33% loss of respiratory surface on gills of heavily parasitised fish. A 30% reduction in gill area can lead to a considerable reduction in maximum sustainable swimming speed (Duthie and Hughes 1982).

Savage (1935) and Friend (1941) commented on the possible development of immunity to a second encounter of Salmincola after recovery from the primary infestation. More recently, Shields and Goode (1978) suggested immunity as a contributing factor in host rejection of Lernaea cyprinacea (Copepoda) by goldfish Carassius auratus and Shariff et al. (1986) reported on the possible development of immunity in several species of fish to L. cyprinacea following a previous infestation. Woo and Shariff (1990) reporting on the dynamics of recovery of Helostoma temmincki

infested with L. cyprinacea also suggested acquired protective immunity in recovered fish. In addition, the authors reported a greater loss of egg sacs from parasites on recovered fish than on naive fish and concluded that the protective mechanism was also directed against parasite reproduction. These immunological investigations may lead to the development of vaccines in the future.

It appears that fish hosts are severely affected by heavy infestations of Salmincola, a most likely result should the parasite be allowed to enter a fish culture facility. Due to the lack of an approved chemical therapy at present, the best control method is to prevent parasite entry by practicing proper quarantine and fish husbandry procedures as well as applying appropriate water quality control measures.

Effects of temperature and photoperiod on parasitic copepods:

The influence of temperature on biological processes is well known. Free-living copepods have been extensively studied regarding the positive effects of increasing water temperature on their rate of development (Corkett 1972; Bottrell 1975; Landry 1975a; Cooley 1978; Cooley and Minns 1978; Sarvala 1979; Palmer and Coull 1980; McLaren et al. 1989). Photoperiod effects have received less attention (Barnes 1963; Landry 1975b) possibly due to their secondary importance.

Kabata (1981) reported that the effect of temperature on the development of parasitic copepods has been well examined. It has been established that increasing water temperature results in faster development and growth within optimal limits, beyond which it becomes retardant or lethal (Friend 1941; Kabata and Cousens 1973; Kabata 1981).

Johnston and Dykeman (1987) found that colder water temperatures (1-6°C) significantly reduced body size and the number and size of eggs of S. salmoneus, whereas warmer temperatures (7-12°C) significantly increased them. However, photoperiod did not seem to have any effect. Friend (1941) noted variations in egg sac length and the number of eggs per sac on

female S. salmoneus from different geographic areas of England and Scotland which Johnston and Dykeman (1987) suggested could have been temperature-dependent.

McGladdery and Johnston (1988) found that low temperatures (1-4 C) slowed S. salmoneus egg development while warmer water temperatures (7-12°C) resulted in faster development. In fact, they reported water temperature to be the most important environmental variable affecting egg production. Larval transmission was also observed to be greater at warmer than colder temperatures. However, it was not clear whether this was due to poor hatching success, lower numbers of detectable larvae or poor survival of free-swimming copepodids at the colder water temperatures (<6°C).

Shields and Tidd (1968) found that temperature affected the rate of development of all stages of Lernaea cyprinacea. Low temperatures not only slowed developmental rate, increased developmental time and prevented egg sac production but also impaired secure parasite attachment.

Kabata (1981) summarized the work of Kuperman and Shulman (1972, 1977) who studied the effects of temperature and photoperiod on parasitic copepod development, relative to seasonal changes. They found that copepods were adapted to spring and summer when slight increases in ambient temperature stimulated development and egg production. Day length became important in late summer and early autumn when the effect of temperature waned. The authors postulated the existence of a diapause such that the copepods arrested development in late autumn and over the winter but were stimulated by rising spring temperatures.

Friend (1941) observed a seasonal decline in copepod infections; fewer larval stages over winter and reduced numbers of adults in spring. Shields and Tidd (1968) also found a loss of parasites over winter resulting in low levels of infection in the spring which then increased to a maximum in the autumn. Friend (1941) and Shields and Tidd (1968) noted that rising spring temperatures stimulated egg development resulting in a

shortening of the generation interval during the summer months. Johnston and Dykeman (1987) confirmed this when they showed females were capable of producing 3 broods per year at 12°C but only 2 broods at 1-7°C.

CHAPTER 2: GENERAL MATERIALS AND METHODS

The materials and methods outlined in this chapter form the basis for more than one experiment. More detailed methods specific to individual experiments are described in the appropriate chapter.

Source of parasites:

Brook trout infested with S. edwardsii were obtained from a lake located 160 km northeast of Montreal which had unknowingly been stocked with infested fish from a commercial hatchery later discovered to have had an epizootic. The infested fish were caught using barbless hooks and live bait and transported to the Institute of Parasitology in live-haul tanks with supplemental aeration. No mortalities during transit ever occurred. Fish ranged in size from 150 to 400 mm fork length and from 45 to 540 g in weight.

Infested fish were also obtained during the winter months by fishing through the ice of the lake. The captured fish were then transferred to the lab in live-haul tanks.

Source of fish:

Healthy brook trout fingerlings (100-160 mm fork length) were obtained from a commercial hatchery near Montreal (Réserve de la Petite Nation Inc., Montebello, QC.). These fish were transported to the lab in plastic bags containing water and pure oxygen and acclimated in the fish holding facility.

Fish holding facility:

The fish holding facility consisted of 2 series of 3 tanks arranged on 3 levels, each tank with a capacity of 600 liters (see schematic diagram in Fig. 2.1). The 2 series of tanks formed two completely separate systems, one system being a mirror image of the other. Each system had its

own biofilter, sump, water pump, pre-filter, cartridge filter, chiller unit and UV filter. A double layer of plastic sheet (4 mil; 0.1016 mm) isolated one system from the other, and prevented cross-contamination; it allowed the holding of uninfected fish on one side and infected fish on the other. Standard practices of hygiene were observed whereby no equipment or products were transferred from the infected side to the uninfected side. All equipment was disinfected with 6 ml/liter Bactol (brand of n-Alkyl dimethyl benzyl ammonium chloride, Avmor Chemical) after use and allowed to air-dry.

The fish facility was supplied with unfiltered and untreated well water. The system had been designed to recirculate the water with only slight daily additions to compensate for losses due to evaporation and to tank cleanings. Aeration was supplied to the biofilters and each holding tank to enhance circulation. Fish were fed a commercial fish ration (Zeigler Bros. Inc.) of appropriate size and at recommended rates to maintain health.

Water temperature in the fish facility was set at 13°C and ranged between 11.5-14.5°C. Water quality was monitored twice weekly using standard test kits (LaMotte Chemical) to measure ammonia nitrogen, nitrite nitrogen, alkalinity and hardness. Dissolved oxygen and pH were monitored daily with an oxygen meter (Otterbine/Barebo Sentry III) and pH meter (Hanna Piccolo, ATC), respectively. Water temperature was monitored daily using mercury thermometers in stainless steel cases suspended in each sump. Records of all water quality readings are maintained in the logbook of the fish facility.

Brook trout, brown trout, rainbow trout, Arctic char and Atlantic salmon have been maintained in the fish facility since December 1988. Some of the original brook trout acquired to condition the biofilters are still being held in the facility to keep the biofilter bacteria populations going. These fish are now 3-4+ years in age and range between 360-450 mm fork length and from 595-1215 g.

Figure 2.1. Schematic diagram of fish holding facility showing side view and end view.

A - biofilter (1 x 600 L. per system)

B - fish tank (2 x 300 L. per system)

C - fish tank (1 x 600 L. per system)

PS - plastic sheets

W - incoming water from well

S - sump

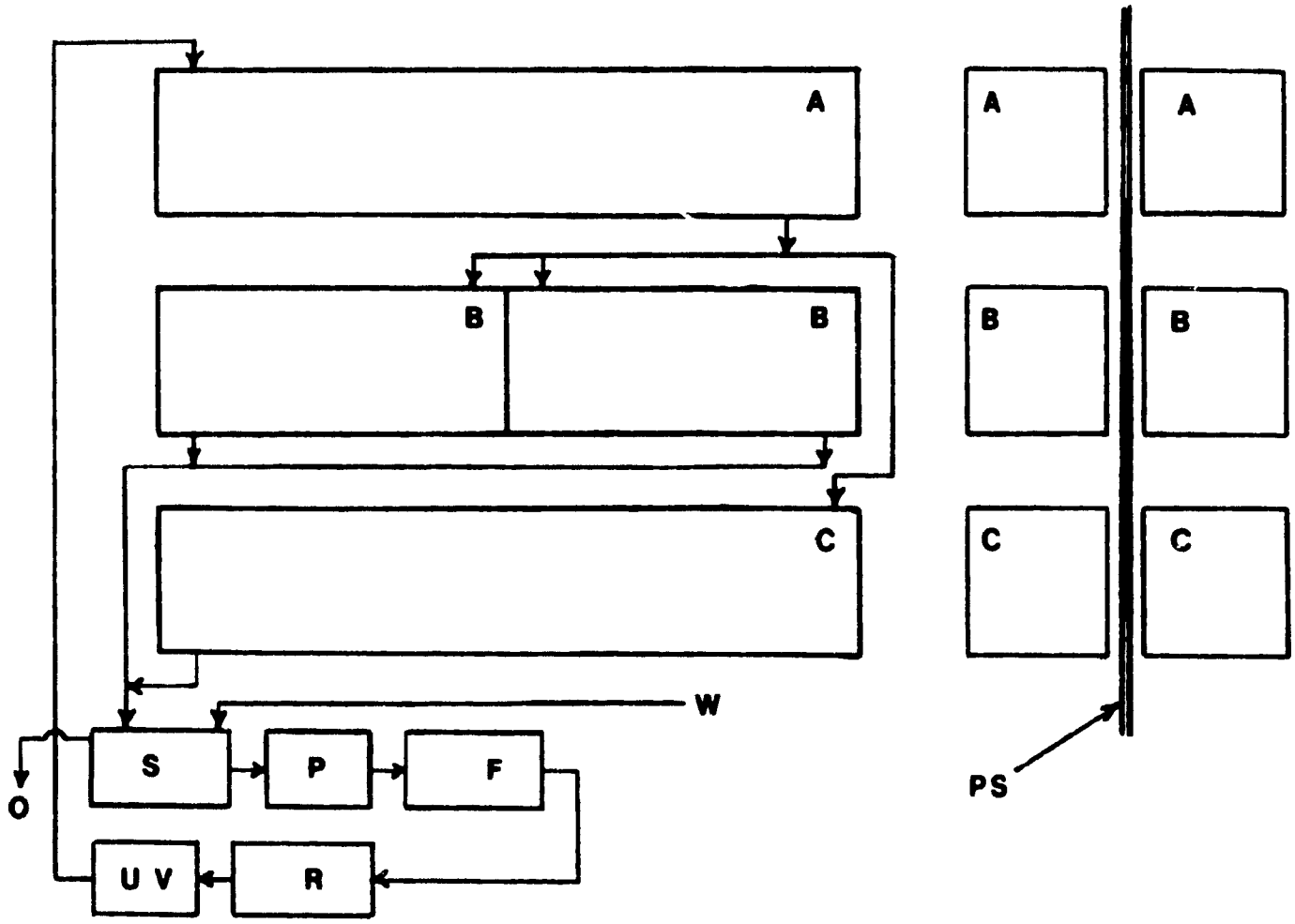
P - pump

F - mechanical filters

R - refrigeration unit (chiller)

UV - ultra-violet filters

O - overflow to drain


A
B
B
C
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P
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O
U V
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A
A
B
B
C
C
PS

Egg sac removal and hatching of copepodids:

Egg sacs and copepodids were required for the various experiments. The methods outlined here were used to obtain egg sacs and to hatch sufficient numbers of copepodids for experimental purposes.

Naturally infested fish were either sacrificed or sedated and the adult female copepods with egg sacs removed. Sacrificed fish received a blow to the head; fish to be sedated were placed in either a 10 or 30-liter pail containing 50 mg/l (50 ppm) of Finquel (brand of tricaine methanesulfonate, (MS-222), Argent Chemical Laboratories) until loss of equilibrium occurred (Ross and Ross 1984). Sacrificed and/or sedated fish were placed in a holder (see Fig. 2.2) constructed of rigid styrofoam with an elliptical slot cut in it. The slot was filled with either cold water (for sacrificed fish) or sedative solution (for sedated fish) to facilitate removal of the copepods. Adult copepods with egg sacs at all stages of development were removed using fine forceps and placed in Petri dishes filled with aerated, dechlorinated tapwater at 8°C. Pigmented egg sacs were then detached with fine forceps, by grasping the narrow portion of the sac where it attaches to the base of the female, and used in the hatching and survival experiments. The remaining sacs were placed, 1 pair per well, in 24-well plastic culture plates (Falcon Multiwell # 3047, well volume: 3 ml) filled with aerated, dechlorinated tapwater. These plates were incubated at 8°C under low light conditions and examined daily for hatched copepodids for use in other experiments. The total number of adult copepods removed from each fish and fish weight were recorded.

Using this method, all stages of developing egg sacs could be removed from the copepods and incubated to hatching. However, over long periods (1-2 months) it was observed that eggs in immediate contact with the bottom of the plate (as the sac lay on its side) were slower to develop to the pigmented stage, possibly due to the lack of aeration. Fungal infection was the most common cause of loss of egg sacs during incubation but this was effectively controlled by using the well plates,

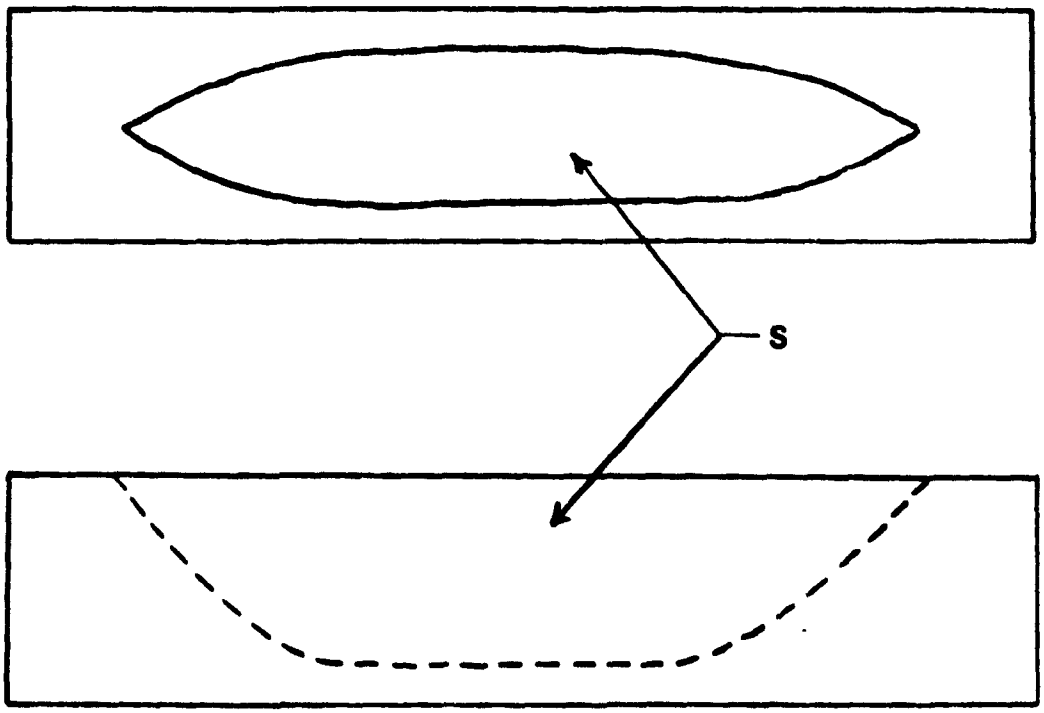
which isolated pairs from one another. The incubation temperature of 8°C gave a consistent rate of hatching over a long period. Egg sac development to hatching could be accelerated by increasing the temperature, which was done on occasion by placing culture plates in waterbaths at 12-16°C.

Aerated, dechlorinated tapwater was obtained from a 30-liter pail kept at 8°C and aerated with two airstones connected to a standard aquarium air pump. The pail was covered to prevent contamination of the water. This water was used in all protocols involving the incubation and hatching of egg sacs.

The use of MS-222 on fish did not appear to have any effect on subsequent hatching of the egg sacs, however, egg sacs used in hatching and survival experiments were removed from sacrificed fish as a precaution.

Fish which had been sedated and the adult copepods, but not the chalimus stages, removed were put in one of the holding tanks on the side of the fish facility used for infection experiments. These fish were kept for up to a year to determine if the infection could be maintained in the lab.

Figure 2.2. Diagram of styrofoam fish holder with elliptical slot (S). Dimensions: 5 x 5 x 20 cm. Top view and side view.


S

Infection of fish:

The experimental infection of fish was required for studies on larval development, generation interval and host specificity. This was done by placing groups of fish in 5, 10 or 20-liter aquaria and exposing them to large doses of recently hatched copepodids for various periods of time. Fish were selected such that all fish in the same group were relatively the same size. Infection of the fish was accomplished by setting the aquaria on supports in a tank of the holding facility, such that the rims of the aquaria were above the surface of the water in the holding tank, and the hatched copepodids poured into the aquaria (see Fig. 2.3). Copepodids were obtained by pipetting them from the culture plates, as outlined above, into 50 ml beakers. The aquaria were aerated and covered at all times to prevent fish escapes.

No attempt was made to count the number of hatched copepodids used in these experiments but sometimes an estimate was made by using the average number of eggs per sac (e.g., 50) times the number of sacs harvested.

At the end of the exposure period, the aquaria were flushed with water from the tank inflow (see Fig. 2.3) to remove unattached copepodids and then set in another holding tank such that water in the covered aquaria would be freely exchanged with water in the holding tank (see Fig. 2.3).

This method had several advantages: i) the fish experienced no temperature stress either going from one holding tank to another or during the exposure period; ii) the fish were confined in a small space during exposure to enhance infection; iii) the copepodids could be flushed from the aquaria without the necessity of handling the fish and inadvertantly removing some attached copepodids; iv) different groups of infected fish could be maintained in the same holding tank thus ensuring uniformity of temperature and water quality over the course of the experiments.

Figure 2.3. Experimental infection set-up.

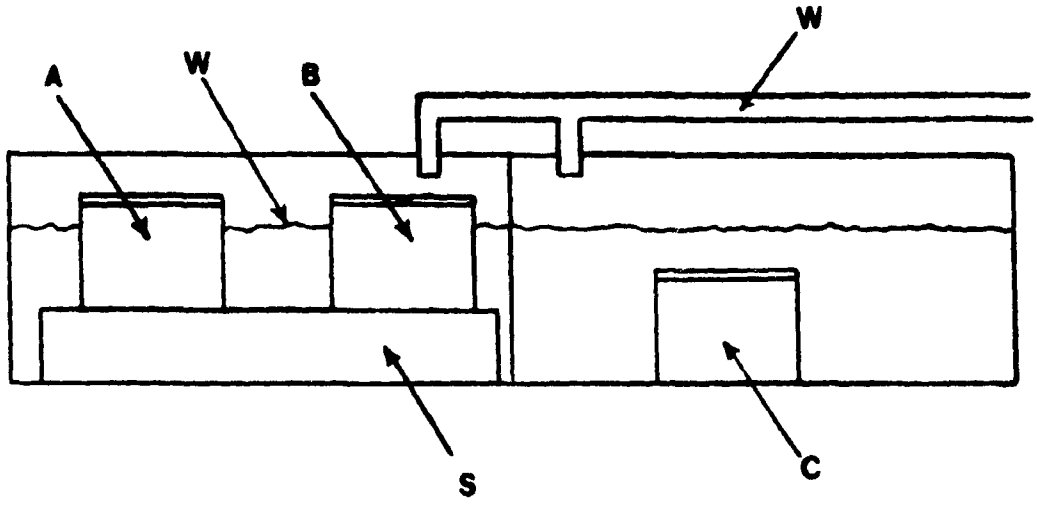
A - aquarium containing fish during exposure to copepodids

S - aquarium support

W - water

B - aquarium containing fish during flushing

C - aquarium containing infected fish in holding tank


W
A
W
B
S
C

Sedation and fish examination:

The sedation of fish was a common neces ty and was routinely done using water from the fish facility to avoid temperature-stressing the fish. A suitable container was filled with water to which was added 50 mg/l (50 ppm) of MS-222. Using a dipnet, fish were transferred to the sedative solution and left until total loss of equilibrium occurred (Stage II, deep anaesthesia; see Ross and Ross 1984). It was observed that fish could be left in the solution with no aeration for up to 20-30 minutes without mortalities.

Sedated fish were transferred by hand to a holder, as described above (see Fig. 2.2), containing sedative solution from the pail. The use of rigid styrofoam for the holder had several benefits: i) simple fabrication; ii) excellent insulating properties; iii) light-weight and easily maneuvered for observations; iv) easily cleaned and disinfected; v) custom sizing possible for various size-ranges of fish. The elliptical slot in the holder was cut out using a sharp knife, the size of slot determined by the size of fish to be observed.

Fish were normally placed belly-up in the holder to facilitate observation of the fins, opercula and gills but they could be easily turned over to examine dorsal areas. The holder was placed upon the stage of a dissection microscope during observations.

Fish were revived by putting them in a pail of fresh water from the fish facility and providing plenty of aeration. Normal recovery was achieved within 2-3 minutes. Fish were then returned to the appropriate aquaria which were set back in the holding tank.

The repeated use of MS-222 did not appear to have any detrimental effect on the fish, copepodids or attached stages during the course of the experiments. However, no tests were done to compare copepods from sedated and non-sedated fish. On one occasion about 50 copepodids were placed in a Petri dish containing 50 ppm MS-222 and left for 4 hours on a bench in the cold room and then examined. There was no perceptible change in

behavior nor was there any mortality.

Statistical analyses:

Data analyses were performed using SYSTAT software (Version 5.0, 1990). Procedures used were "STATISTICS", "TTEST" and "MGLH: Regression" (Wilkinson 1990).

CHAPTER 3: EFFECTS OF TEMPERATURE AND PHOTOPERIOD ON DURATION OF HATCHING, SWIMMING AND COPEPODID SURVIVAL OF SALMINCOLA EDWARDSII.

INTRODUCTION

Previous studies have focused on various aspects of Salmincola, not only to determine the basic biology and life cycle (Fasten 1912, 1913, 1914, 1916, 1918, 1919, 1921a, 1921b; Savage 1935; Zandt 1935; Dedie 1940; Friend 1941; Kabata and Cousens 1973) but also to seek information relevant to the development of control strategies (Fasten 1912, 1913; Savage 1935; Hoffman and Meyer 1974; Johnston and Dykeman 1987; McGladdery and Johnston 1988). To date, only the infective larval stage (or copepodid) has been found susceptible to chemical treatment; the adult copepods have an impermeable cuticle rendering them more resistant than their host to common chemical agents. Consequently, the fish often suffers more than the parasite from the control treatments (Fasten 1912; Friend 1941; Cousens 1977).

Due to the susceptibility of the copepodid to chemical control treatments, it would be of great value to know more about the hatching, free- swimming activity and survival time of this stage.

A review of the literature revealed no precise information on the length of time the copepodid lives. It is this stage which is free-swimming and seeking to infect a suitable host. Many authors have reported that the copepodid has about 2 days after hatching to contact a host in order to survive and undergo further development (Wilson 1911, 1915; Fasten 1912, 1913, 1918, 1921b; Savage 1935; Davis 1953; Hoffman 1977; Kabata and Cousens 1973), however, Friend (1941) reported copepodids surviving up to 6 days in the laboratory. Consequently, there appears to be some question as to the life-span of copepodids due to the lack of data.

Many of the previous studies did not specify the temperatures at which they were conducted (Fasten 1912, 1913; Savage 1935; Friend 1941). Although Kabata and Cousens (1973) did their work at temperatures between 11-12°C, they did not investigate survival of copepodids other than to say that, "Without finding a suitable host, the copepodid can survive for about 2 days, though many die before reaching that age."

No mention is made in the literature about the effect of temperature on survival of Salmincola copepodids, only on larval development once it has made contact with a suitable host (Kabata and Cousens 1973; Kabata 1981). Fasten (1913) exposed copepodids to increasing water temperature to see if it had any affect on phototaxis, it did not. Consequently, it appeared that an investigation of the effect of temperature on survival of the copepodid should be undertaken.

The effect of photoperiod on hatching, swimming and survival constitutes another area of study about Salmincola where little is known. An investigation of the effect of photoperiod on copepodids could easily be incorporated into the same temperature experiments.

In view of these considerations, I designed experiments to test whether various temperature and photoperiod regimes had any effect on duration of egg hatching, swimming activity and copepodid survival. Fortunately, adult Salmincola females produce pairs of egg sacs which are ideal controls. By using the paired egg sacs, a comparison of genetically related individuals developed under the same conditions could be made to determine the effects of the treatments.

MATERIALS AND METHODS

Due to the inherent difficulties of working with early life cycle stages of parasitic copepods it is useful to provide the following details on the experimental apparatus and procedures.

Experimental Apparatus.

The temperatures chosen to test for effects were 8, 12, 16 and 20°C because they represent the natural range over which Salmincola infestations are most likely to occur. To carry out the experiments over this range of temperature it was necessary to use a room which had constant temperature control in order to compensate for the heat generated by the high-intensity light of the dissection microscope. The most suitable location was a walk-in cold room.

Two static waterbath incubators were set up in the cold room (see Fig. 3.1) which had a stable temperature of 8°C. The incubators were placed side-by-side and covered with plexiglass sheets. A 33-watt fluorescent light fixture was suspended 30 cm above the water surface of the incubators and mercury thermometers were placed in them to monitor temperature. Each bath, with its full complement of plates, was adjusted to the appropriate experimental temperature using rheostats and the experiments began after 3 days of consistent temperature observations.

Figure 3.1. Schematic diagram of temperature and photoperiod experimental apparatus.

Top view - arrangement of plates in incubators.

A - plates exposed to light

B - plates wrapped in black plastic

C - thermometer

Side view - arrangement of incubators and light.

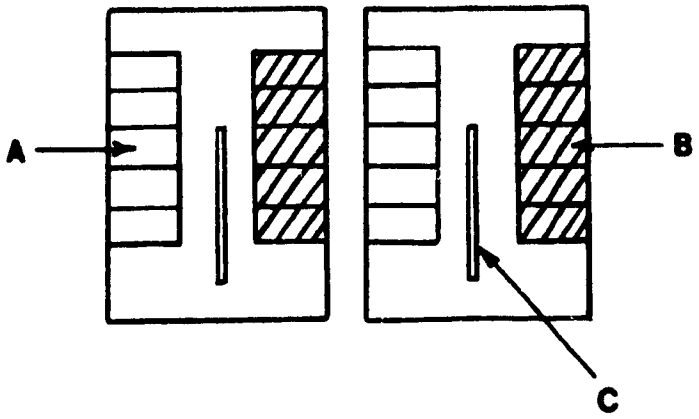
D - fluorescent light fixture

A - plate position in incubator

E - water level in incubator

F - waterbath incubator

G - rheostat


A
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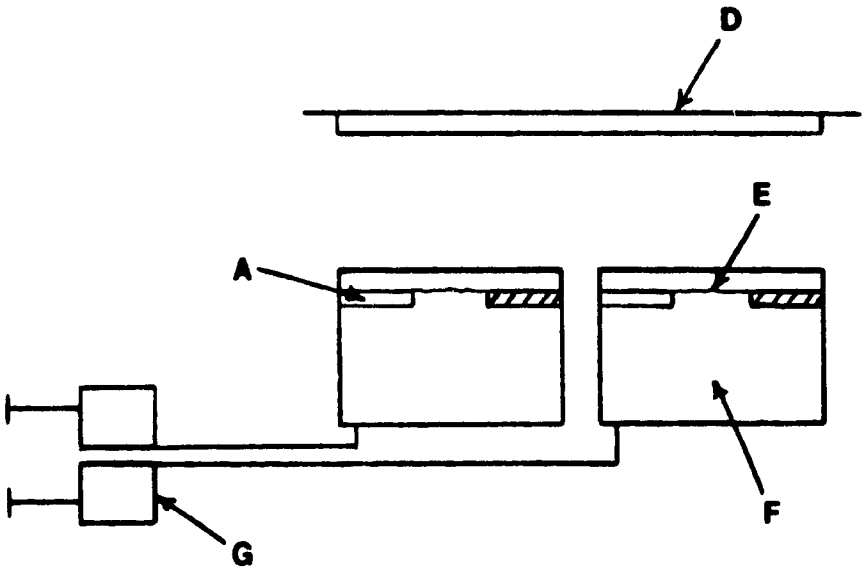
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G

Temperature / Photoperiod Experiments.

Adult female copepods with pigmented egg sacs were removed from the gills of naturally infested brook trout. The paired pigmented egg sacs were detached from the female copepods and separated, with each sac of a pair being placed in the first well of 6-well plastic culture plates (Corning # 25810-6, well volume: 16.8 ml). Wells had been filled with aerated, dechlorinated tapwater at 8°C from a plastic pail in the cold room. Plates containing one sac of a pair were placed in a waterbath at either 8, 12, 16 or 20°C. Plates containing the other sac of a pair were wrapped in 6 mil (0.1524 mm) black plastic to exclude light and placed in the same waterbath as their mates (see Fig. 3.1). Plates were labelled with a waterproof pen for identification of pairs.

Photoperiod was either 23 h L:1 h D or 1 h L:23 h D. This regime was chosen to give extremes for determination of effects, and to allow for a 1-hour window for observation purposes. Plates exposed to 23 h of light were removed from their respective baths, observed with a dissection microscope and then wrapped in black plastic and returned to their waterbath for 1h. The plastic wrapping was removed after 1h and the plates returned to their respective baths. Plates exposed to 23 h of darkness had the black plastic removed, were observed and then returned to their bath for the duration of the hour. After the hour, the plates were rewrapped and returned to the respective baths.

Each experiment at the 4 selected temperatures consisted of 10 plates; 5 pairs of sacs with 1 sac exposed to 23 h of light and 1 sac in 23 h of darkness.

Observations were made at 24-hour intervals and consisted of viewing each plate with a dissection microscope to determine the number of copepodids hatched, swimming, non-swimming, and dead. Swimming and non-swimming copepodids were determined by passing a hand between the source of light and the plate and/or nudging the copepodids with a blunt probe. Dead copepodids were removed daily with a Pasteur pipette.

To facilitate determination of the duration of hatching after hatching commenced, the egg sac was moved, using fine forceps, from the first well to the next well of the culture plate, leaving behind all previously hatched copepodids. This was done each day until hatching ceased.

Constant Light / Constant Dark Hatching Experiment.

Prior to the commencement of the temperature / photoperiod experiments described above, a trial was undertaken to determine whether or not eggs would hatch if kept completely in the dark during the last few weeks of maturation. This experiment consisted of removing paired egg sacs from adult female copepods as described previously. One sac of the pair was placed in 1 well of a 24-well plastic culture plate and the other sac in the corresponding well of another 24-well plate. Twenty-four pairs of egg sacs were used ranging from lightly pigmented to heavily pigmented, representing various stages of maturation. One plate was exposed to constant light while the other plate was wrapped in 6 mil black plastic to simulate constant darkness. The plates were placed in the cold room at 8°C, and the non-wrapped plate observed daily for signs of hatching. When all the egg sacs in the plate exposed to constant light had hatched, the other plate was unwrapped and the numbers of hatched and unhatched eggs in both plates counted and compared.

RESULTS

Temperature / Photoperiod Experiments.

Table 3.1 contains a summary of the statistical data for each temperature and photoperiod regime. One pair of egg sacs failed to hatch in the 20°C regime and was discarded from analyses.

The number of days elapsed from the start of each experiment until hatching commenced differed between egg sac pairs. Therefore, the commencement of hatching of each sac was considered to be day 1 for comparison purposes. The time between the start of each experiement and the onset of hatching was considered the incubation period.

The life history variables quantified on a daily basis for each culture plate at each temperature and photoperiod were mean cumulative percentage hatched, mean percentage swimming and mean percentage survival. Cumulative percentage hatched is the sum of the percentage of eggs hatched per day; percentage swimming is the percentage of copepodids swimming out of the total number of copepodids alive per day; percentage survival is the percentage of copepodids still alive out of the cumulative sum of eggs hatched per day.

Statistical analyses revealed no significant difference ($P > 0.05$) in duration of hatching, swimming and survival due to photoperiod. Therefore, data for each photoperiod at the same temperature were pooled and analysed for differences due to temperature.

Means ($\pm$ SD) of cumulative percentage hatched, percentage swimming and percentage survival by day at 8, 12, 16 and 20°C are shown in Fig. 3.2, Fig. 3.3, Fig. 3.4 and Fig. 3.5 respectively. Fig. 3.6 summarises the results for all four experimental temperatures.

Hatching

The majority of eggs hatched within 2 days and amounted to 93%, 79%, 95% and 89% for egg sacs at 8, 12, 16 and 20°C respectively (see Fig.

3.6a). Final mean percentage of eggs hatched reached 97%, 90%, 96% and 90% at 8, 12, 16 and 20°C respectively, and hatching success was not found to be significantly affected ($P > 0.05$) by differences in temperature. Hatching was completed in 6 days, 10 days, 3 days and 4 days at 8, 12, 16 and 20°C respectively. Time to 50% hatched and to 80% hatched at the experimental temperatures were compared and found not to be significantly different ($P > 0.05$).

The means ($\pm$ SD) of incubation periods, which were the number of days elapsed from the start of each experiment until hatching commenced, are shown in Fig. 3.7a. When compared (see Fig. 3.7b) they were found to be significantly affected by temperature ($P < 0.01$) but not by photoperiod ($P > 0.05$).

Swimming

Duration of swimming activity was inversely related to increasing water temperature (see Fig. 3.6b). Although copepodids exhibited swimming ability up to 17 days, 13 days, 9 days and 6 days after initial hatching of egg sacs at 8, 12, 16 and 20°C respectively, approximately 50% of copepodids were still capable of swimming at 7.5 days, 6 days, 5 days and 2.5 days after hatching commenced. Swimming activity averaged 74%, 76%, 65% and 59% at 8, 12, 16 and 20°C respectively, on the day after hatching began. At 16°, swimming activity peaked at 70% on day 3 and then declined. The duration of time until 50% stopped swimming and until 100% stopped swimming were found to be significantly affected by temperature ($P < 0.05$ and $P < 0.01$ respectively; see Fig. 3.8a and 3.8b).

Survival

Copepodid survival followed the same pattern as swimming activity, being inversely affected by higher water temperature (see Fig. 3.6c). Although all copepodids were dead 29 days, 20 days, 13 days and 8 days after hatching commenced at 8, 12, 16 and 20°C respectively, approximately

50% of copepodids were still alive 11.5 days, 9.5 days, 6.5 days and 3.5 days after initial hatching of egg sacs. The mean percentage survival of copepodids on the first day of hatching was 98%, 93%, 100% and 91% for 8, 12, 16 and 20°C respectively. The duration of time until 50% were dead and until 100% were dead were also significantly affected by temperature ($P < 0.05$ and $P < 0.01$ respectively; see Fig. 3.9a and 3.9b).

Constant Light / Constant Dark Hatching Experiment.

The results of hatching in constant light vs. constant dark conditions are shown in Table 3.2. Hatching of all egg sacs was completed over an 11-day period. Four egg sacs belonging to the same 2 pairs failed to hatch in both treatments for unknown reasons and were discarded from statistical analyses. No significant difference ($P > 0.05$) between hatching in either treatment was found, indicating that onset of hatching and hatching success does not appear to be affected by light conditions.

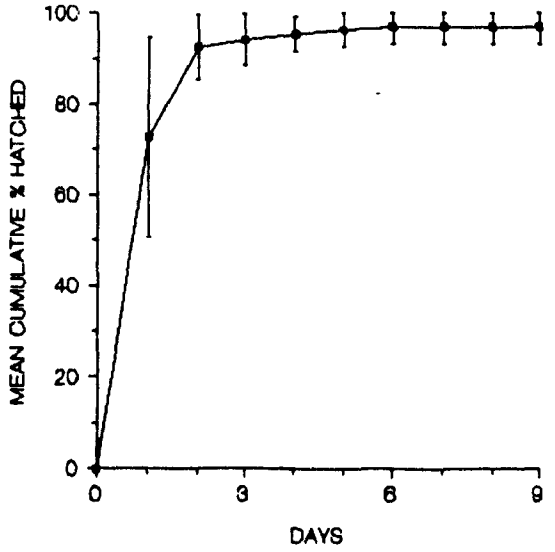
Table 3.1. Summary of statistical data for temperature / photoperiod experiments showing number of egg sacs, number of eggs/sac (mean ± SD), range and total number of eggs, per photoperiod and per temperature.

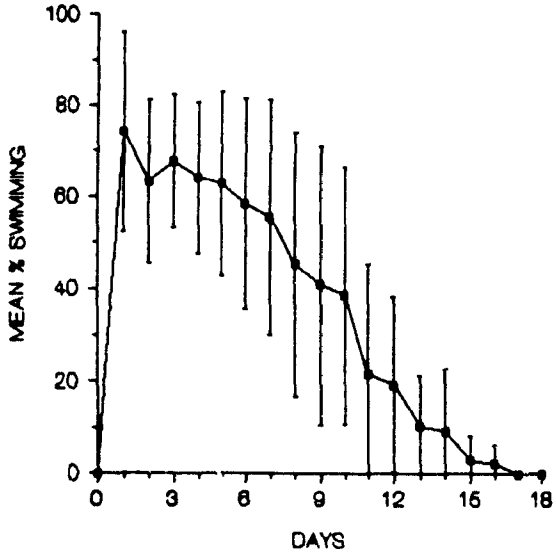
Temp.	Photo.	Egg Sacs	Eggs/Sac	Range	Total Eggs
8	23L:1D	5	59.0 ± 13.2	41-73	295
8	1L:23D	5	61.4 ± 11.9	46-74	307
8	TOTAL	10	60.2 ± 12.5	41-74	602
12	23L:1D	5	70.2 ± 7.7	63-81	351
12	1L:23D	5	69.4 ± 15.6	51-87	347
12	TOTAL	10	69.8 ± 11.6	51-87	698
16	23L:1D	5	54.2 ± 18.1	41-86	271
16	1L:23D	5	53.0 ± 17.1	36-81	265
16	TOTAL	10	53.6 ± 17.6	36-86	538
20	23L:1D	4	47.5 ± 10.4	38-60	190
20	1L:23D	4	50.2 ± 10.7	36-59	201
20	TOTAL	8	48.8 ± 10.5	36-60	391

Figure 3.2a. Mean (± SD) cumulative percentage of eggs hatched by day at 8°C.

Figure 3.2b. Mean (± SD) percentage of copepodids swimming by day at 8°C.

Figure 3.2c. Mean (± SD) percentage survival of copepodids by day at 8°C.


MEAN CUMULATIVE % HATCHED
100
80
60
40
20
0
0
3
6
9
DAYS


MEAN % SWIMMING
100
80
60
40
20
0
0
3
6
9
12
15
18
DAYS

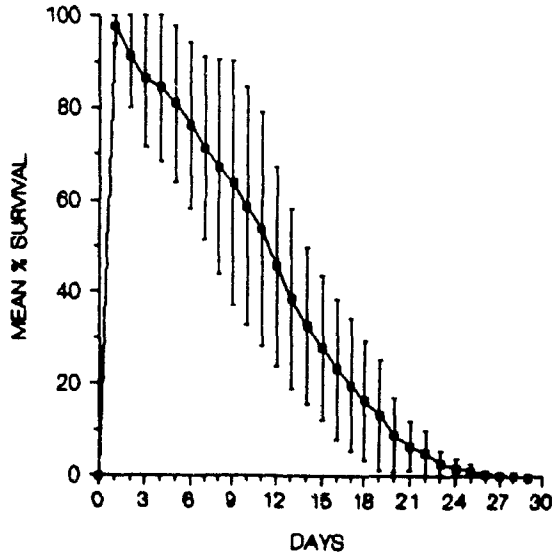
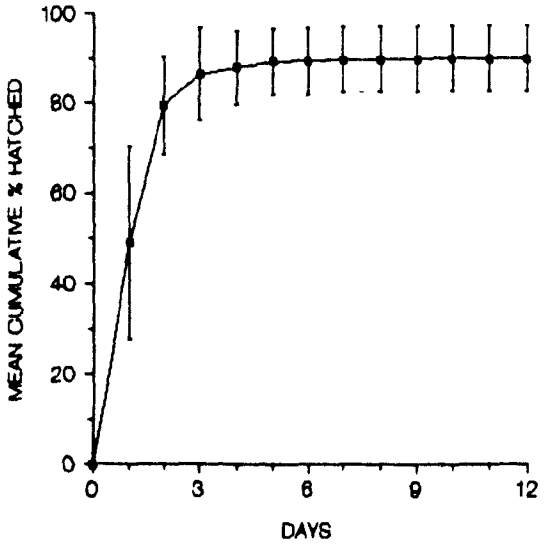
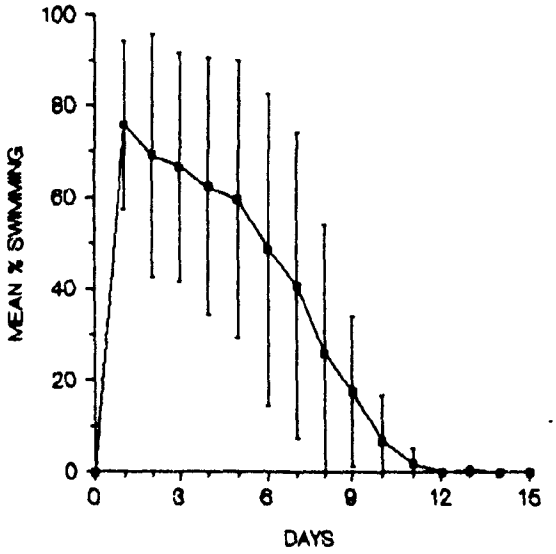

MEAN % SURVIVAL
100
80
60
40
20
0
0
3
6
9
12
15
18
21
24
27
30
DAYS

Figure 3.3a. Mean (± SD) cumulative percentage of eggs hatched by day at 12°C.

Figure 3.3b. Mean (± SD) percentage of copepodids swimming by day at 12°C.

Figure 3.3c. Mean (± SD) percentage survival of copepodids by day at 12°C.


MEAN CUMULATIVE % HATCHED
100
80
60
40
20
0
0
3
6
9
12
DAYS


MEAN % SWIMMING
100
80
60
40
20
0
0
3
6
9
12
15
DAYS

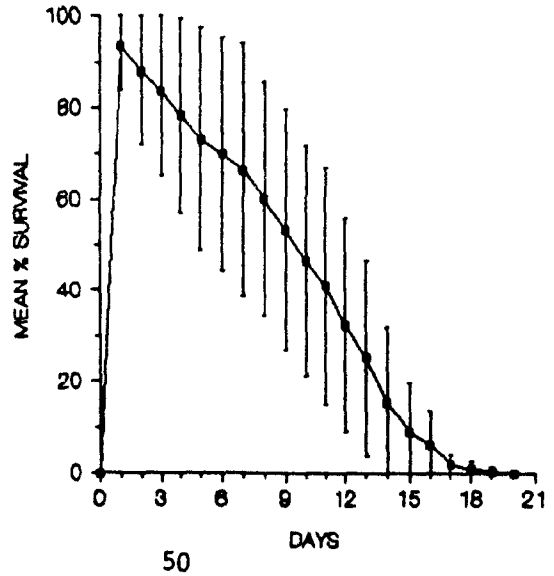
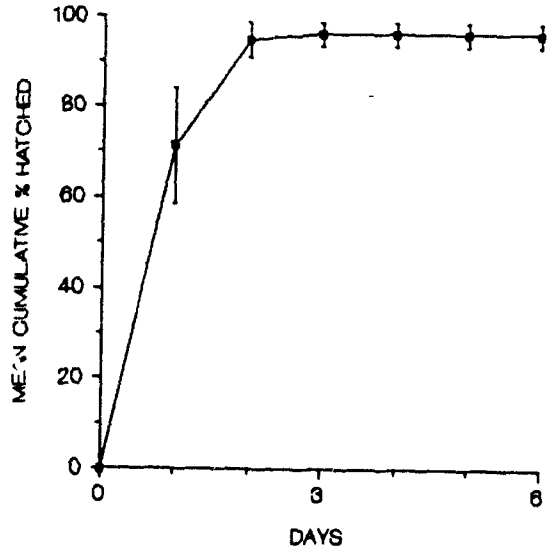
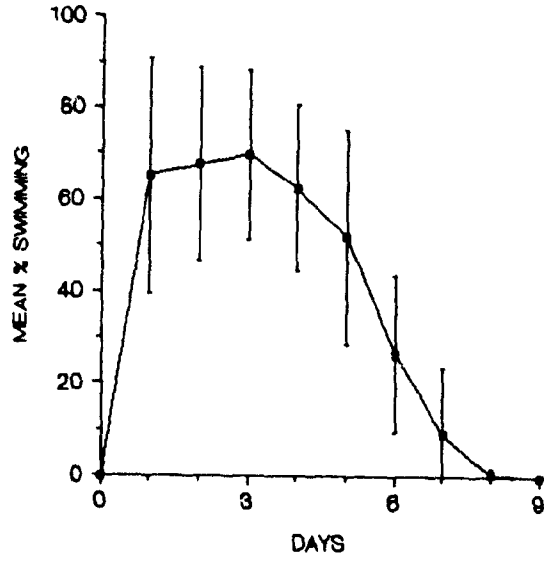

MEAN % SURVIVAL
100
80
60
40
20
0
0
3
6
9
12
15
18
21
DAYS

Figure 3.4a. Mean (± SD) cumulative percentage of eggs hatched by day at 16°C.

Figure 3.4b. Mean (± SD) percentage of copepodids swimming by day at 16°C.

Figure 3.4c. Mean (± SD) percentage survival of copepodids by day at 16°C.


MEAN CUMULATIVE % HATCHED
100
80
60
40
20
0
0
3
6
DAYS


MEAN % SWIMMING
100
80
60
40
20
0
0
3
6
9
DAYS

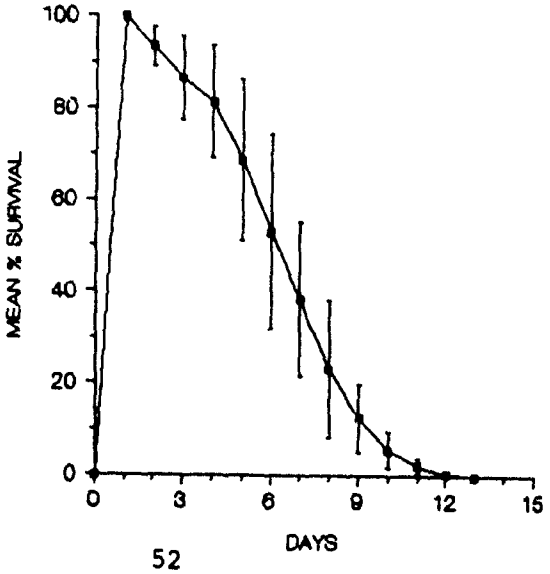
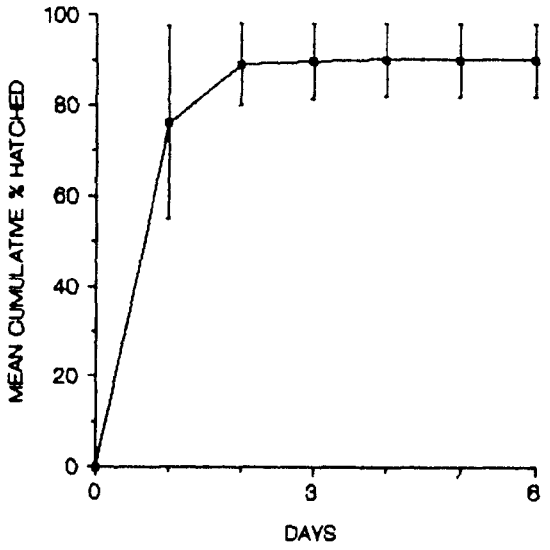
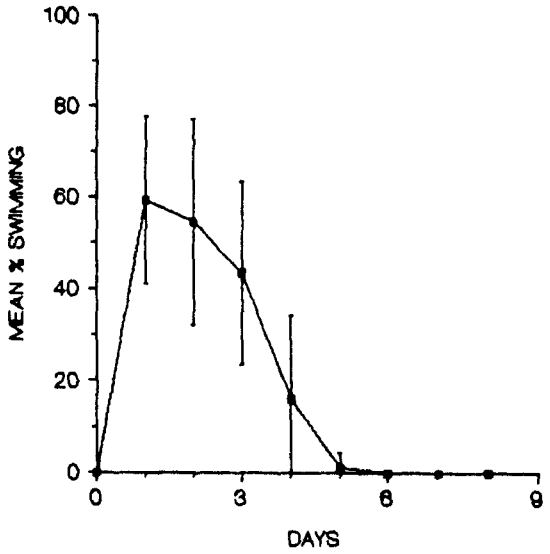

MEAN % SURVIVAL
100
80
60
40
20
0
0
3
6
9
12
15
DAYS

Figure 3.5a. Mean (± SD) cumulative percentage of eggs hatched by day at 20°C.

Figure 3.5b. Mean (± SD) percentage of copepodids swimming by day at 20°C.

Figure 3.5c. Mean (± SD) percentage survival of copepodids by day at 20°C.


MEAN CUMULATIVE % HATCHED
100
80
60
40
20
0
0
3
6
DAYS


MEAN % SWIMMING
100
80
60
40
20
0
0
3
6
9
DAYS

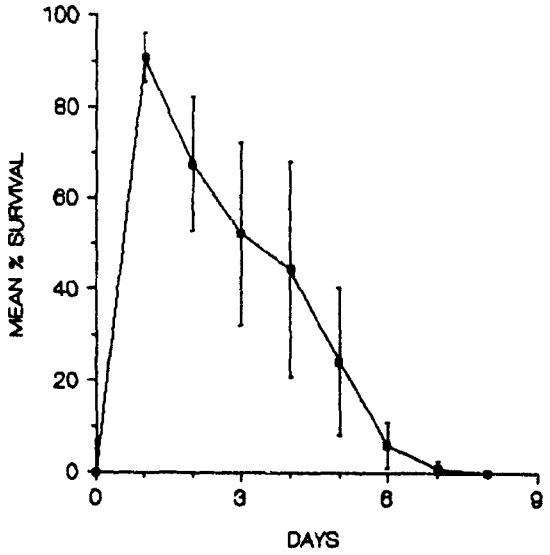
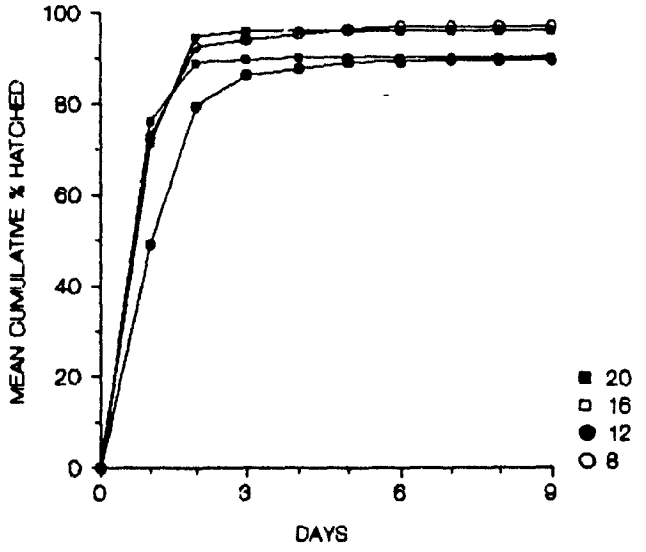
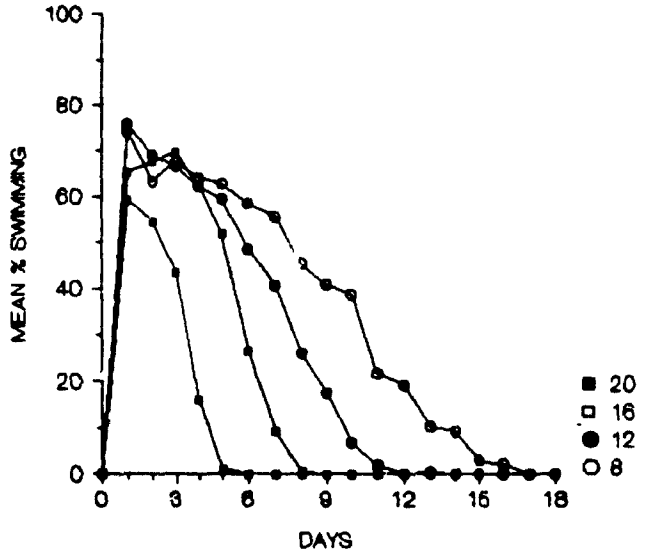

MEAN % SURVIVAL
100
80
60
40
20
0
0
3
6
9
DAYS

Figure 3.6a. Mean cumulative percentage of eggs hatched by day at 8, 12, 16 and 20°C.

Figure 3.6b. Mean percentage of copepodids swimming by day at 8, 12, 16 and 20°C.

Figure 3.6c. Mean percentage survival of copepodids by day at 8, 12, 16 and 20°C.


MEAN CUMULATIVE % HATCHED
100
80
60
40
20
0
0
3
6
9
DAYS
20
16
12
8


MEAN % SWIMMING
100
80
60
40
20
0
0
3
6
9
12
15
18
DAYS
20
16
12
8

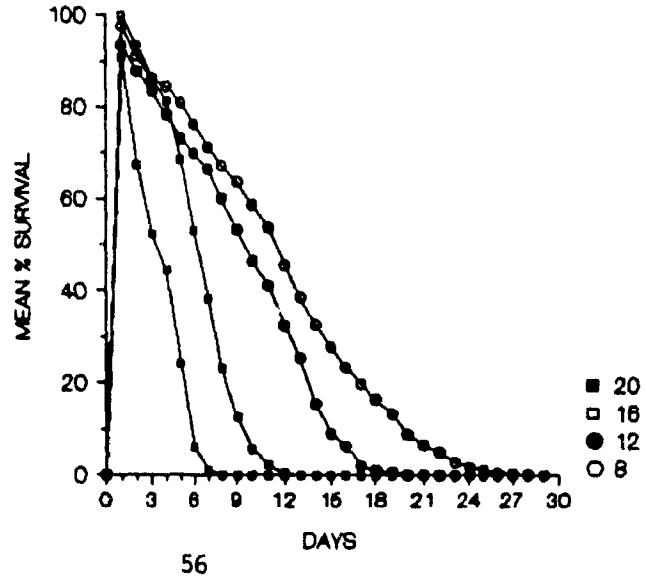
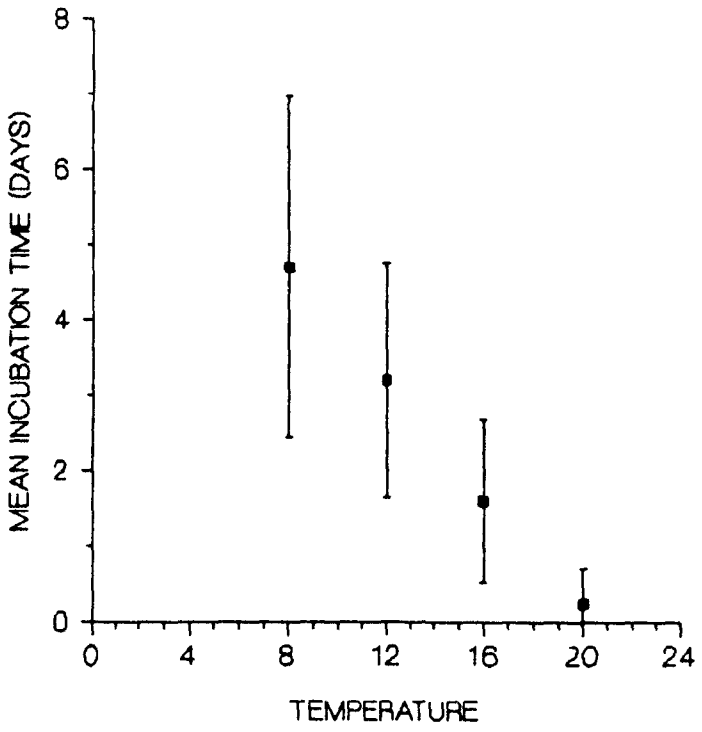

MEAN % SURVIVAL
100
80
60
40
20
0
0
3
6
9
12
15
18
21
24
27
30
DAYS
20
16
12
8

Figure 3.7a. Mean (± SD) incubation period in days from the start of each experiment until commencement of hatching at 8, 12, 16 and 20°C.

Figure 3.7b. Regression plot of incubation period from the start of each experiment until commencement of hatching as a function of temperature. (r^2=0.99, df=3, P< 0.01). Regression equation: Y = 7.67 - 0.374 T


MEAN INCUBATION TIME (DAYS)
0
2
4
6
8
0
4
8
12
16
20
24
TEMPERATURE

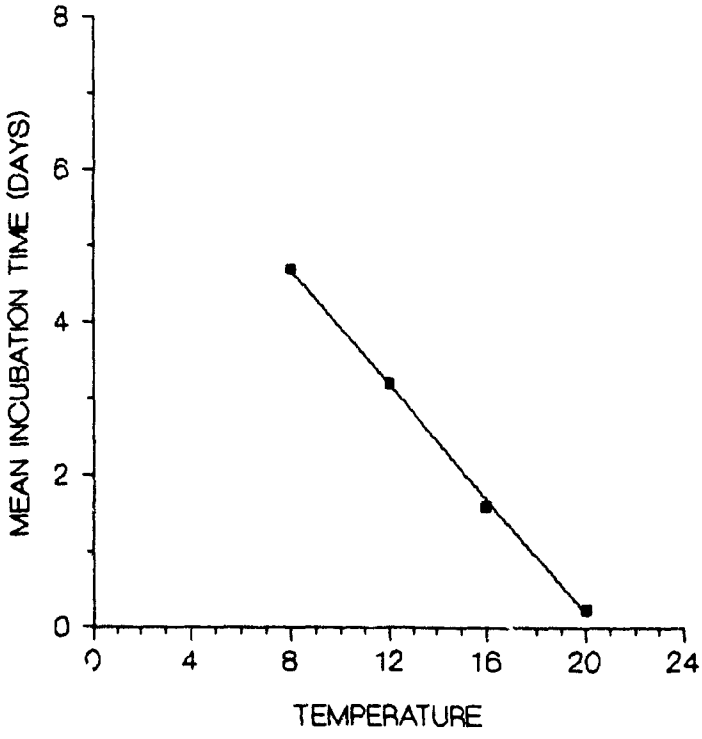
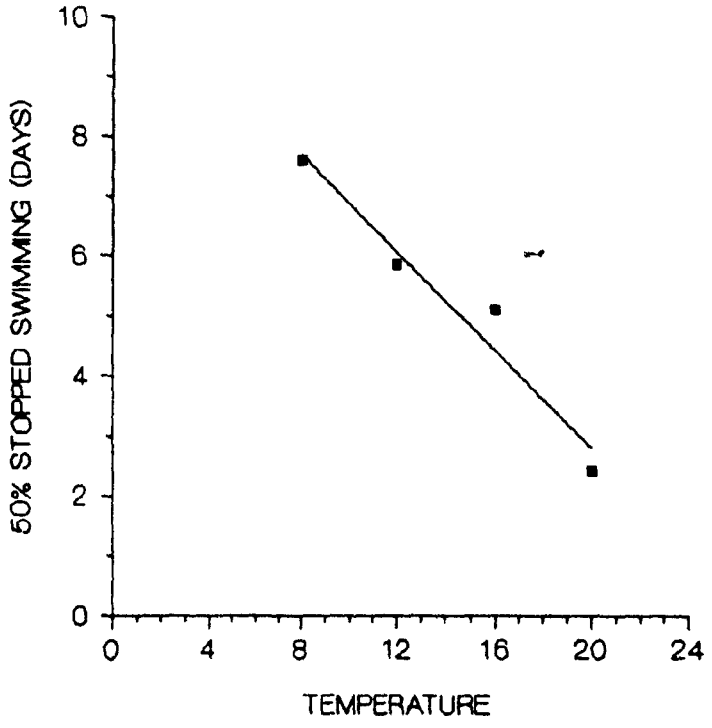

MEAN INCUBATION TIME (DAYS)
0
2
4
6
8
0
4
8
12
16
20
24
TEMPERATURE

Figure 3.8a. Regression plot of time in days until 50% of copepodids have stopped swimming as a function of temperature. (r^2=0.95, df=3, P< 0.05).
Regression equation: Y = 10.926 - 0.407 T

Figure 3.8b. Regression plot of time in days until 100% of copepodids have stopped swimming as a function of temperature. (r^2=0.99, df=3, P< 0.01).
Regression equation: Y = 24.2 - 0.925 T


50% STOPPED SWIMMING (DAYS)
10
8
6
4
2
0
0
4
8
12
16
20
24
TEMPERATURE

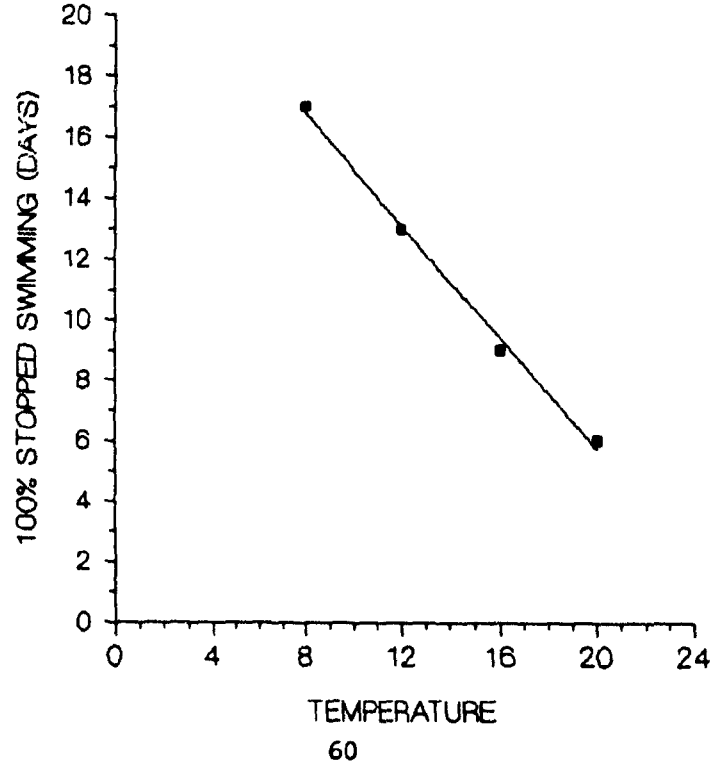
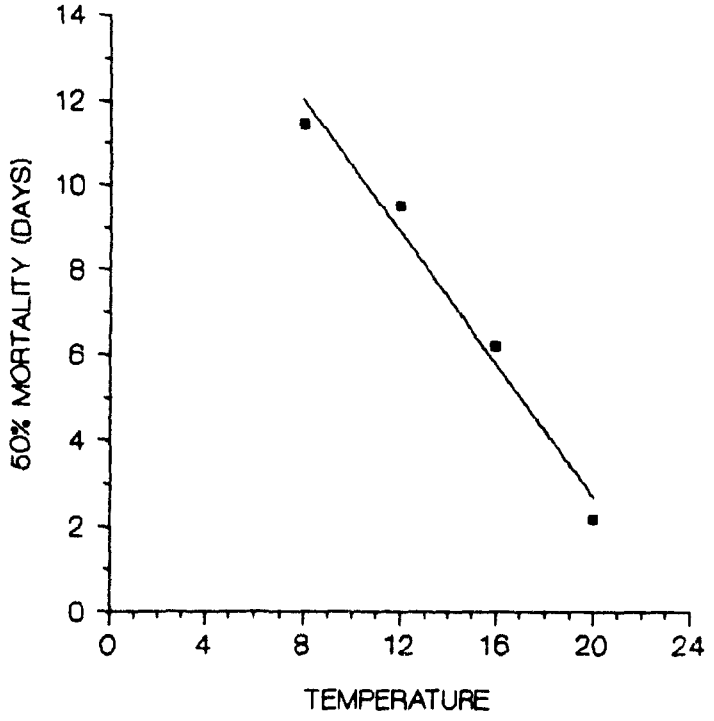

100% STOPPED SWIMMING (DAYS)
20
18
16
14
12
10
8
6
4
2
0
0
4
8
12
16
20
24
TEMPERATURE

Figure 3.9a. Regression plot of time in days until 50% of copepodids have died as a function of temperature.
(r^2=0.98, df=3, P< 0.05).
Regression equation: Y = 18.208 - 0.777 T

Figure 3.9b. Regression plot of time in days until 100% of copepodids have died as a function of temperature.
(r^2=0.98, df=3, P< 0.01).
Regression equation: Y = 42.0 - 1.75 T


14
12
10
8
6
4
2
0
50% MORTALITY (DAYS)
0
4
8
12
16
20
24
TEMPERATURE

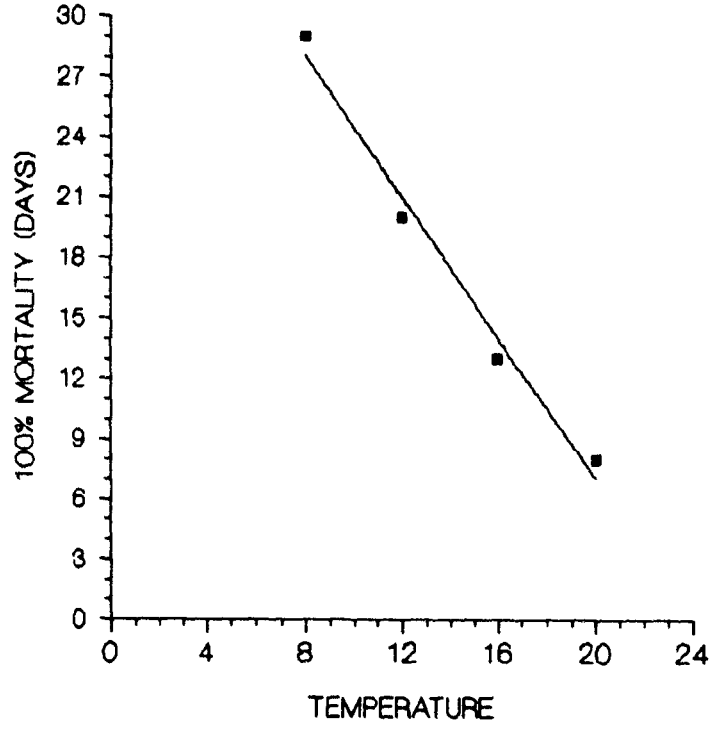

30
27
24
21
18
15
12
9
6
3
0
100% MORTALITY (DAYS)
0
4
8
12
16
20
24
TEMPERATURE

Table 3.2. Summary of statistical data for constant light / constant dark hatching experiments showing temperature, photoperiod, number of egg sacs used (N), number of eggs/sac (mean ± SD, range in parentheses) and percentage (%) for hatched and unhatched, and total number of eggs.

Temp.	Photo.	N	Hatched	%	Unhatched	%	Total Eggs
8	24 h L	22	45.0 ± 10.0* (31-71)	94	2.8 ± 4.7	6	1050
8	24 h D	22	44.3 ± 11.9* (27-75)	92	4.0 ± 5.1	8	1063

* paired t-test, t=0.192, df=42, P> 0.05

DISCUSSION

The influence of temperature on biological processes is well documented. It is an accepted fact that organisms function within an optimum range of temperature, outside of which there are serious repercussions for survival. Consequently, the lack of data in the literature on Salmincola relating the survival of the free-swimming copepodid to temperature is surprising. The results of this study clearly show that temperature has a significant effect on copepodid swimming activity and survival and that the infective stage lives much longer than the 2 days commonly stated. On the other hand, photoperiod has no detectable effect on the variables quantified.

The onset of egg sac hatching was directly related to increasing water temperature. However, duration of hatching and hatching success were not affected. Photoperiod had no effect on hatching success, hatching duration, swimming activity or copepodid survival. Previous studies on Salmincola salmoneus (Johnston and Dykeman 1987; McGladdery and Johnston 1988) found temperature to be the most significant environmental variable affecting egg production and development. Poulin et al. (1990a) reported temperature to be a significant factor in egg hatching for S. edwardsii. An increase in water temperature, not temperature or photoperiod fluctuations, resulted in an earlier onset of hatching and a greater hatching rate.

The most dramatic results of this study were the temperature-dependent relationships of copepodid swimming activity and survival. These results clearly indicate that copepodids are capable of swimming and presumably infecting fish for a considerable period of time, especially at lower temperatures. For example, at 8° the last copepodid stopped swimming by day 17, indicating a maximum swimming duration of 16 days. For 12, 16 and 20°C, maximum swimming durations were 12 days, 8 days and 5 days respectively.

When we consider survival, the copepodid is functionally dead when it is no longer capable of infecting a host. Why then did the copepodids continue to live so long after all ability to swim had ceased. At 8° the last copepodid was dead by day 29, 12 days after swimming activity had ceased. At 12, 16 and 20°, the last copepodid died 7 days, 4 days and 2 days respectively, after swimming ended. Perhaps it is still capable of infecting a fish if chance favours it. More likely, this longevity is linked to temperature-dependent metabolic processes.

I considered the possibility that my method of stimulation using shadows and a blunt probe may have been inadequate to provoke swimming activity. However, the most striking feature I observed of copepodid behavior was the response to shadows. For several days prior to death, even when it was incapable of moving, the passing of a shadow provoked a reflexive twitch of the antennae, second maxillae and maxillipeds. If the copepodid was capable of swimming at all, this stimulus should have been enough.

The temperature-dependent life span of S. edwardsii copepodids has important implications regarding the development of control strategies. At present, the copepodid is the only stage susceptible to chemical treatment. With so few approved chemical agents available to fish culturists, it is important to use them wisely. In treating fish with copepod infestations it would be useful to know when to begin treatment, what dosage to use and how often to administer it. Knowing that egg development, onset of hatching, swimming ability (infectivity) and survival are all temperature-dependent, one could devise a strategy whereby treatment could be applied less often but of longer duration at lower temperatures and more often but of shorter duration at higher temperatures.

CHAPTER 4: LARVAL DEVELOPMENT OF *SALMINCOLA EDWARDSII* ON BROOK TROUT (*SALVELINUS FONTINALIS*).

INTRODUCTION

Wilson (1911, 1915) gave us an early appreciation of the general pattern of development within the family Lernaeopodidae. Fasten (1912, 1913, 1914, 1916, 1918, 1919, 1921a), Savage (1935) and Dedie (1940) described various aspects of the biology and life history of *S. edwardsii* and Friend (1941) reported on the life history and ecology of *S. salmoneus*. However, it was not until Kabata and Cousens (1973) described the life cycle of *S. californiensis* that a nearly complete and comprehensive picture of the developmental stages of *Salmincola* became available.

Prior to Kabata and Cousens (1973), investigators had been unable to observe and describe early life cycle events as they occurred because of the lack of success in lab-rearing copepodids (Fasten 1913) or in holding and experimentally infecting host fish (Friend 1941). Modern experimental facilities and improved microscopy techniques allow us to do these things, facilitating the description of events from start to finish.

Kabata and Cousens (1973) used two methods to obtain their results: a) continuous observation, using a dissection microscope, of the development of the parasite on experimentally infected hosts, and b) sacrificing experimentally infected fish at set intervals to determine the stage of development of attached parasites over time. Following the latter method, with slight modifications, this chapter reports on the timing of development of the larval stages of *S. edwardsii* at 13°C ±1.5.

MATERIALS AND METHODS

The term "infection" shall refer to the experimental exposure of fish to infective copepodids and their subsequent attachment, whereas I have previously used the term "infestation" to describe the natural occurrence of the parasite on its host.

The infection and development experiments were carried out on the side of the fish facility reserved for infected fish (see details and schematic diagram in Chapter 2). Healthy brook trout (S. fontinalis) fingerlings (100-160 mm fork length) were placed in 5 and 10-liter glass aquaria in lots of 10 or 20 fish, respectively, and exposed to large doses of recently hatched copepodids (≤12 hours old) for a period of 12 hours using the fish infection protocol described in Chapter 2. Fish were selected such that all fish of the same lot were approximately the same size. Each aquarium was aerated during exposure periods and covered to prevent fish escape. Feeding was reduced for the duration of the experiment to prevent accumulation of wastes and resulting fungal growth.

Copepodids used to infect the trout were obtained from egg sacs incubated for that purpose. Mature female S. edwardsii with egg sacs at various stages of development were removed from infested brook trout and their egg sacs detached and placed in aerated, dechlorinated tapwater in 24-well plastic culture plates (Falcon Multiwell # 3047, well volume: 3 ml) with 1 pair per well. The plates were incubated at 8 C, under low light, and examined at 12-hour intervals for hatched copepodids to be used in experiments.

After exposure, the aquaria were flushed with water for 5 minutes to remove all unattached copepodids. The fish were kept in the same aquaria where they had been infected and the covered aquaria submerged in a holding tank such that water in the aquaria was freely exchanged. Water temperature throughout the experiments was maintained at 13 C ±1.5.

Three infected fingerlings were sacrificed at 12-hour intervals until day 6 and then at 24-hour intervals until day 20. The fish were dip-

netted from their aquarium and placed in a solution of MS-222 (tricaine methanesulfonate) at 200 ppm (an overdose). After fish weight and fork length were recorded, the fish were placed in a styrofoam holder, (described in Chapter 2), observed under a dissection microscope, and all copepods removed using fine forceps. Larval stages were removed with filaments intact and juvenile and young adult females were removed while still attached to fish tissues. In some cases, fins were removed with larval and/or juvenile stages attached. Site of attachment was noted to determine parasite distribution on the host. All stages were fixed in 10% formalin and stored in 5% formalin until life cycle stage could be determined. Life stage was determined with a compound microscope (Olympus, Model BH-2; 10X, 20X, and 40X objectives) and its length and sex (if detectable) recorded.

RESULTS

Means (± SD) of fork length and weight of experimentally infected fish are shown in Table 4.1. No mortalities occurred during the experiment.

The growth of S. edwardsii larvae from chalimus I to adult are illustrated by scatterplots in Fig. 4.1. Data for length (in microns) of chalimus I, II and III stages (sex indeterminable) are shown in Fig. 4.1a; of chalimus III and IV and adult males in Fig. 4.1b; and of chalimus III and IV and adult females in Fig. 4.1c. Sexes of early chalimus stages are indeterminable until the latter part of the chalimus III stage, hence the data in Fig. 4.1a include male and female measurements. Mean (± SD) length of each stage by sex is shown in Table 4.2.

The data were sorted by stage (chalimus and adult) for each day and the mean length (± SD) determined. The results are plotted in Fig. 4.2 (chalimus I, II, II; sex indeterminable), Fig. 4.3 (male chalimus III, IV, adult) and Fig. 4.4 (female chalimus III, IV, adult).

The common method of reporting results after an experimental infection by using the term "days post-infection" or "DPI" was inappropriate in this study. The long exposure period (12 hours) and the inherent variability in attachment times between copepodids made it impossible to determine the precise time of infection. Therefore, the term "days post-exposure" or "DPE" is used.

Chalimus I larvae were present .5-1.5 days post-exposure, chalimus II larvae, 1-2.5 DPE; chalimus III larvae (sex indeterminable), 2-4 DPE (see Fig. 4.2a, Fig. 4.2b and Fig. 4.2c, respectively). Male chalimus III larvae were observed 3-4 DPE; chalimus IV larvae, 2.5-5 DPE; and adults, 3-8 DPE (see Fig. 4.3a, Fig. 4.3b and Fig. 4.3c, respectively). Female chalimus III larvae were present 3-5.5 DPE; chalimus IV larvae, 4-20 DPE; and adults, 11 DPE and 13-20 DPE (see Fig. 4.4a, Fig. 4.4b and Fig. 4.4c, respectively).

Adult male S. edwardsii appeared as early as 3 days after fish had been exposed to copepodids and were present for 5 days (see Fig. 4.3c); no adult males were detected after day 8 of the experiment Therefore, the life span of the male copepod at 13°C from time of contact with the fish host varies between 3 and 8 days. The last day on which male chalimus IV larvae were detected was day 5 post-exposure (see Fig. 4.3b) and the last day on which adult males were observed was day 8 (see Fig. 4.3c), indicating that adult males may live for up to 3 days.

Adult female S. edwardsii were detected as early as 11 days post-exposure but not consistently until day 13-20 of the experiment (see Fig. 4.4c). It can be concluded that final attachment to the host by adult female copepods takes place approximately 2 weeks after initial host contact but may vary due to factors yet to be elucidated.

The total number of attached copepods declined over the course of the experiment. Fig. 4.5 shows the number of copepods recovered per day of the experiment with a smoothed line through the data using LOWESS (Cleveland 1979, 1981). There was a fairly rapid loss of copepods up to

day 9 post-exposure which then slowed for the duration of the experiment.

The distribution of S. edwardsii on experimentally infected fish is presented in Fig.4.6 (number of copepods as a function of time) and Fig. 4.7 (percentage of copepods as a function of time). The gills, opercula and pectoral fins were the major sites of attachment. Table 4.3 shows the location of attachment, number of copepods and percentage of the total at that location.

The adult female copepods from which the egg sacs were removed and incubated to provide copepodids for this and other experiments were obtained from naturally infested brook trout as previously described. Fish which were killed and the adult copepods removed were weighed and measured (fork length) and the number of copepods counted. Regression analysis showed a significant linear relationship ($P < 0.01$) between intensity of infection and fish weight, such that larger fish were more heavily infected than smaller fish (see Fig. 4.8).

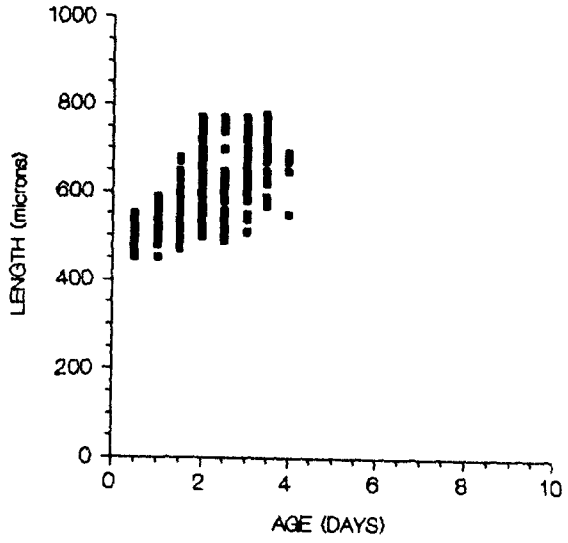
Table 4.1. Fork length and weight (mean ± SD, range in parentheses) of fish used in larval development study.

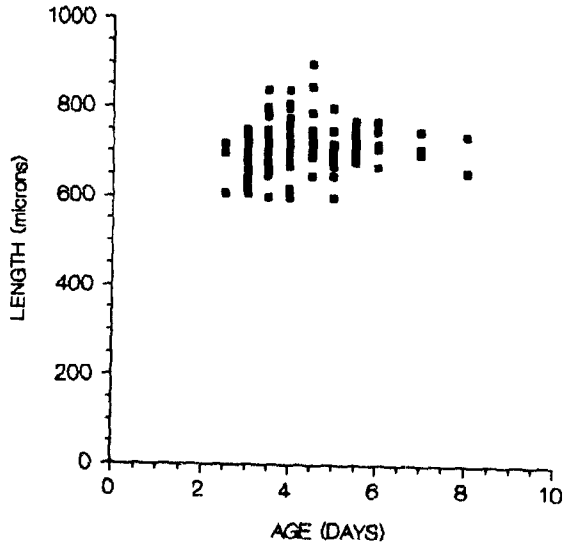
Length (mm)	Weight (g)	N
128.5 ± 13.07	20.4 ± 6.99	78
(102 - 158)	(10.1 - 44.0)	

Figure 4.1a. Scatterplot of chalimus I, II and III larvae length (in microns) as a function of age, sex indeterminable.

Figure 4.1b. Scatterplot of male chalimus III and IV and adult length (in microns) as a function of age.

Figure 4.1c. Scatterplot of female chalimus III and IV and adult length (in microns) as a function of age.


1000
800
600
400
200
0
LENGTH (microns)
0
2
4
6
8
10
AGE (DAYS)


1000
800
600
400
200
0
LENGTH (microns)
0
2
4
6
8
10
AGE (DAYS)

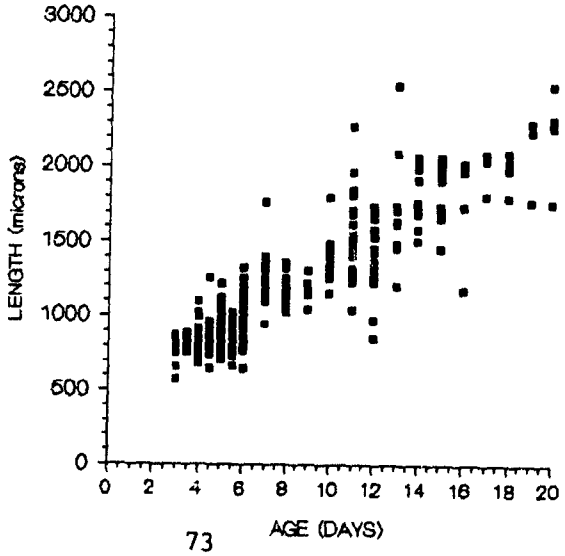

3000
2500
2000
1500
1000
500
0
LENGTH (microns)
0
2
4
6
8
10
12
14
16
18
20
AGE (DAYS)

TABLE 4.2. Length (mean ± SD, range in parentheses) and total number (N) of each larval and adult stage by sex.

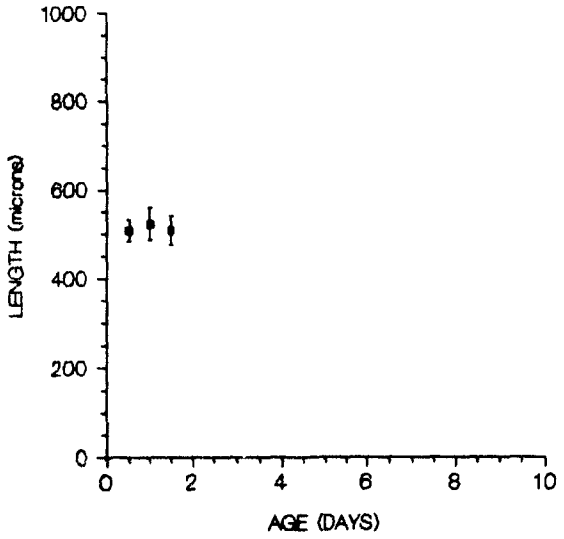
Stage	Sex	Length (microns)	N
Chalimus I	I	512.4 ± 30.4 (450 - 590)	167
Chalimus II	I	580.2 ± 64.1 (480 - 770)	154
Chalimus III	I	660 ± 65.7 (490 - 780)	99
Chalimus III	M	733 ± 57.3 (650 - 840)	10
Chalimus III	F	789.4 ± 64.5 (570 - 1000)	86
Chalimus IV	M	701.9 ± 51.3 (600 - 840)	105
Chalimus IV	F	1137.7 ± 310.2 (650 - 2000)	227
Adult	M	718.6 ± 47.7 (600 - 900)	65
Adult	F	2055.8 ± 210.7 (1590 - 2560)	32

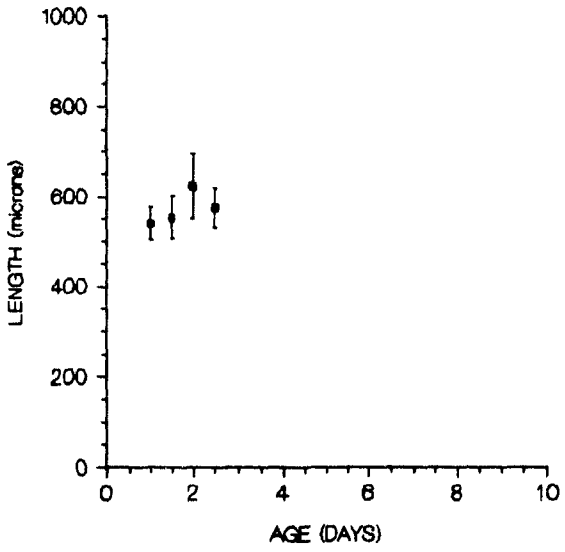
I - Sex Indeterminable, M - Male, F - Female

Figure 4.2a. Mean (± SD) length of chalimus I larvae (in microns) as a function of age, sex indeterminable. (Note: includes male and female data).

Figure 4.2b. Mean (± SD) length of chalimus II larvae (in microns) as a function of age, sex indeterminable. (Note: includes male and female data).

Figure 4.2c. Mean (± SD) length of early chalimus III larvae (in microns) as a function of age, sex indeterminable. (Note: includes male and female data).


1000
800
600
400
200
0
LENGTH (microns)
0
2
4
6
8
10
AGE (DAYS)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800
600
400
200
0
LENGTH (microns)
0
2
4
6
8
10
AGE (DAYS)

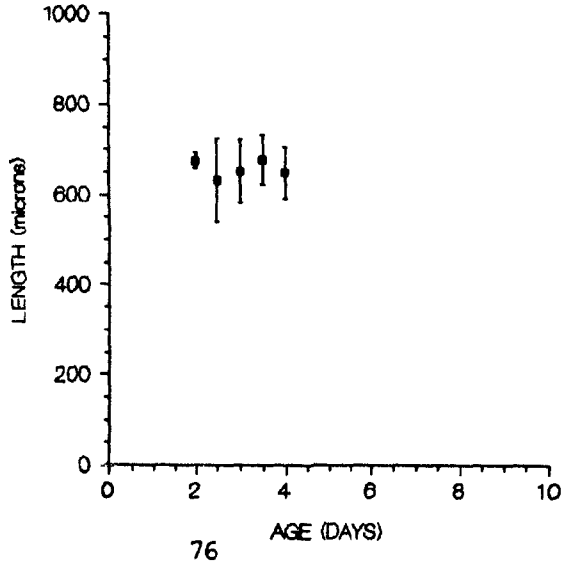
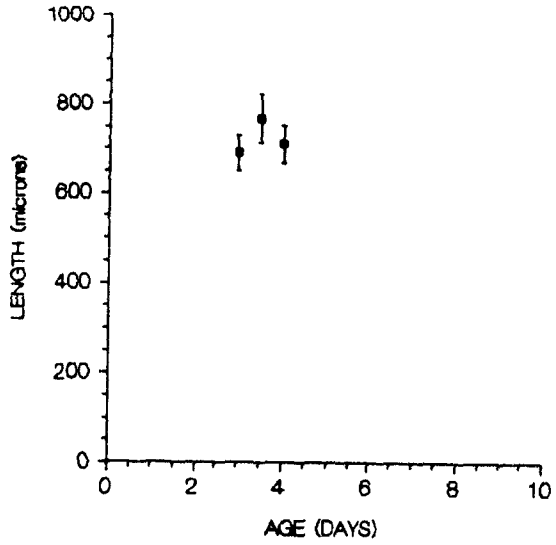
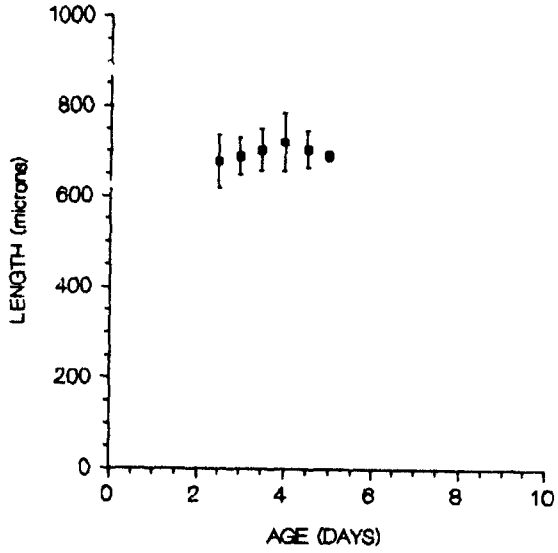

1000
800
600
400
200
0
LENGTH (microns)
0
2
4
6
8
10
AGE (DAYS)

Figure 4.3a. Mean (± SD) length of late chalimus III males (in microns) as a function of age.

Figure 4.3b. Mean (± SD) length of chalimus IV males (in microns) as a function of age.

Figure 4.3c. Mean (± SD) length of adult males (in microns) as a function of age.


1000
800
600
400
200
0
LENGTH (microns)
0
2
4
6
8
10
AGE (DAYS)


1000
800
600
400
200
0
LENGTH (microns)
0
2
4
6
8
10
AGE (DAYS)

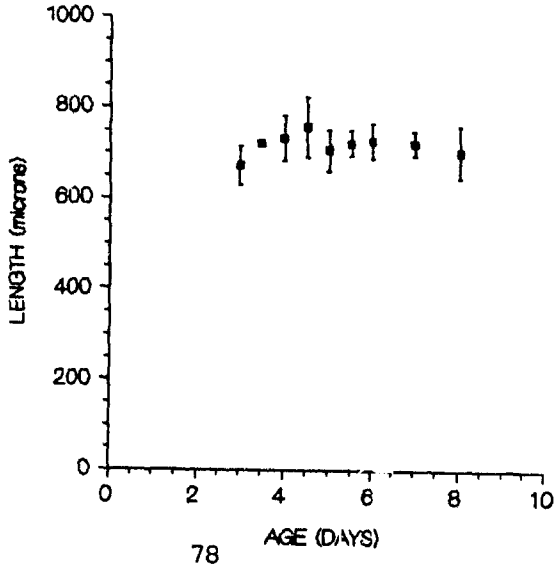
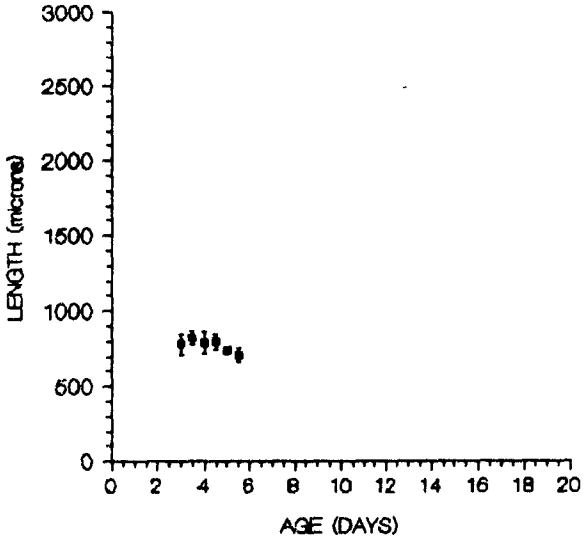
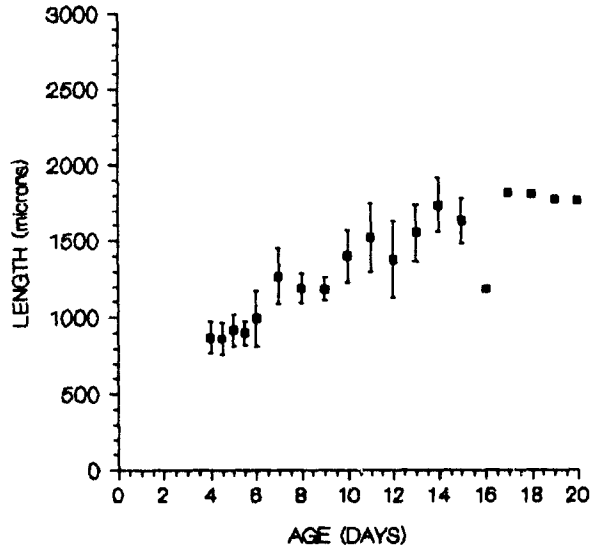

1000
800
600
400
200
0
LENGTH (microns)
0
2
4
6
8
10
AGE (DAYS)

Figure 4.4a. Mean (± SD) length of late chalimus III females (in microns) as a function of age.

Figure 4.4b. Mean (± SD) length of chalimus IV females (in microns) as a function of age.

Figure 4.4c. Mean (± SD) length of adult females (in microns) as a function of age.


LENGTH (microns)
3000
2500
2000
1500
1000
500
0
0 2 4 6 8 10 12 14 16 18 20
AGE (DAYS)


LENGTH (microns)
3000
2500
2000
1500
1000
500
0
0 2 4 6 8 10 12 14 16 18 20
AGE (DAYS)

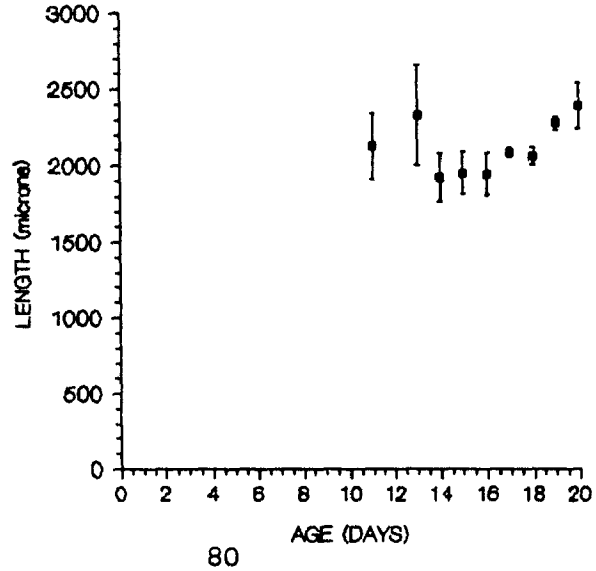

LENGTH (microns)
3000
2500
2000
1500
1000
500
0
0 2 4 6 8 10 12 14 16 18 20
AGE (DAYS)

Figure 4.5. Number of copepods removed per day of the experiment with smoothed line through the data. Smoothing method used is by LOWESS (Cleveland 1979, 1981).

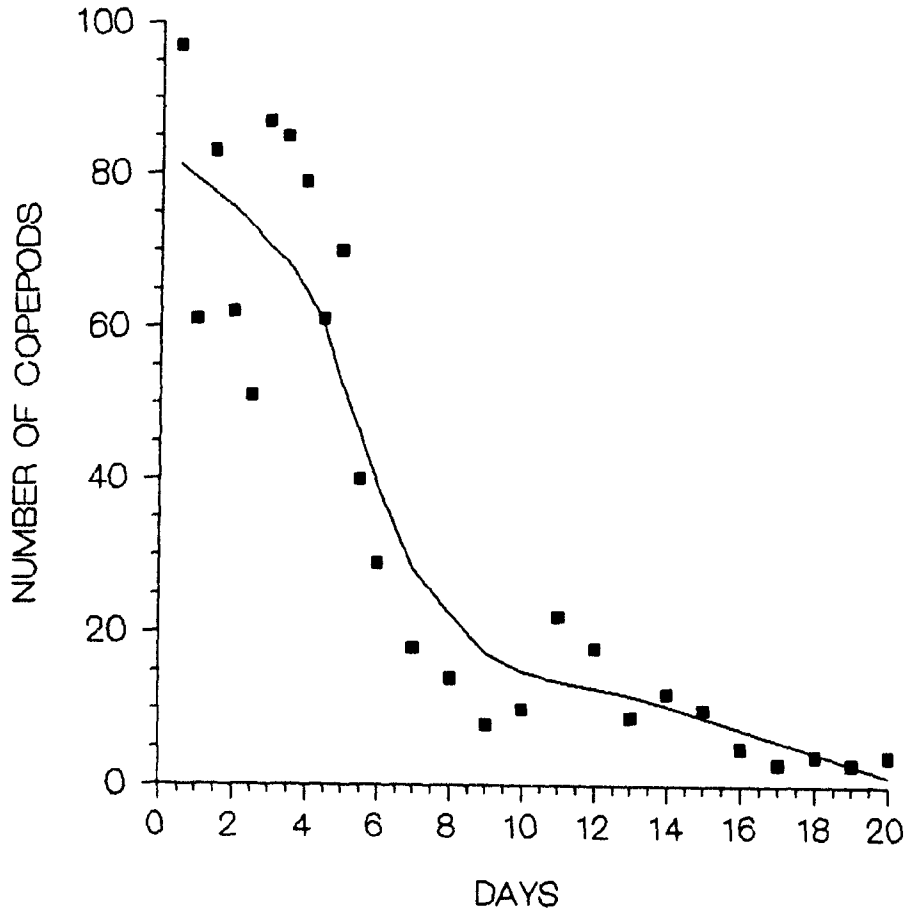

NUMBER OF COPEPODS
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
DAYS

Figure 4.6. Distribution of S. edwardsii (number of copepods) on experimentally infected fish as a function of time. Major sites are gills, opercula, pectoral fins, pelvic fins and other (includes mouth, dorsal fin, anal fin and caudal fin).

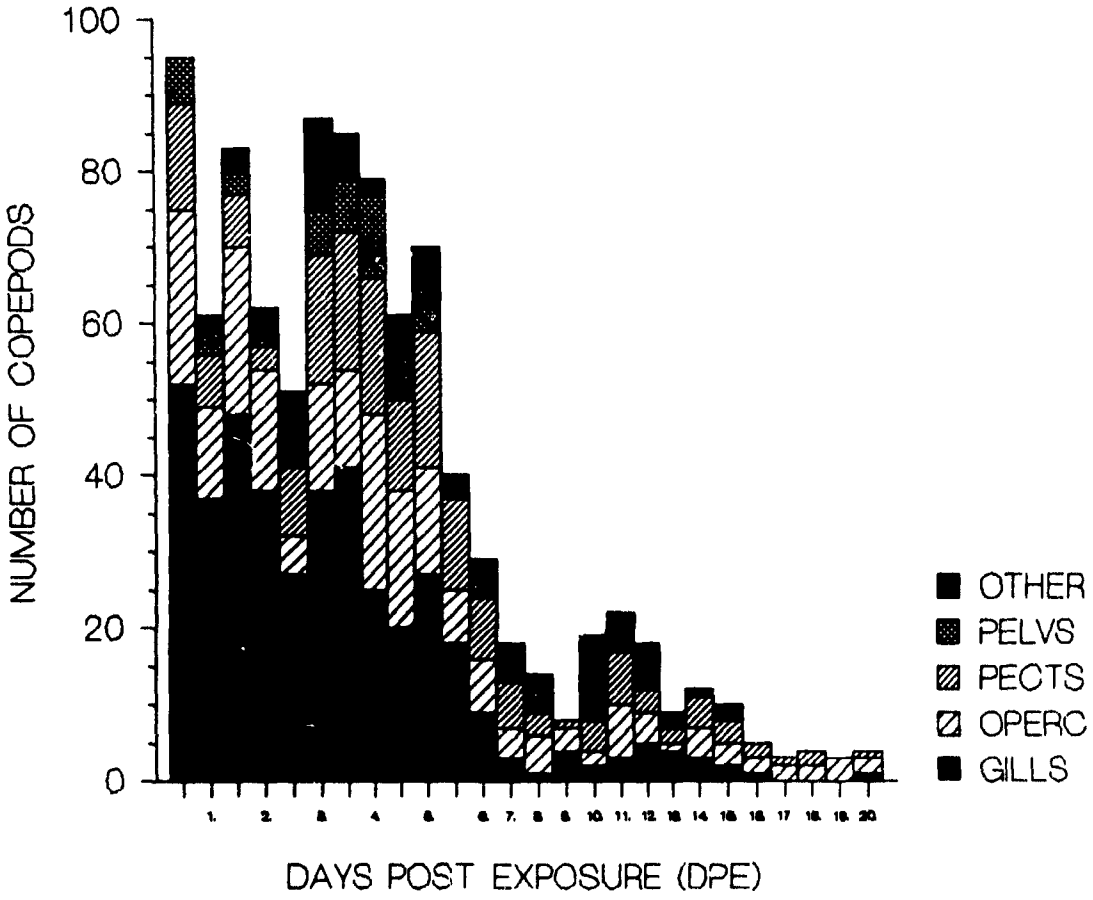

100
80
60
40
20
0
NUMBER OF COPEPODS
DAYS POST EXPOSURE (DPE)
OTHER
PELVS
PECTS
OPERC
GILLS

Figure 4.7. Distribution of S. edwardsii (percentage of copepods) on experimentally infected fish as a function of time. Major sites are gills, opercula, pectoral fins, pelvic fins and other (includes mouth, dorsal fin, anal fin and caudal fin).

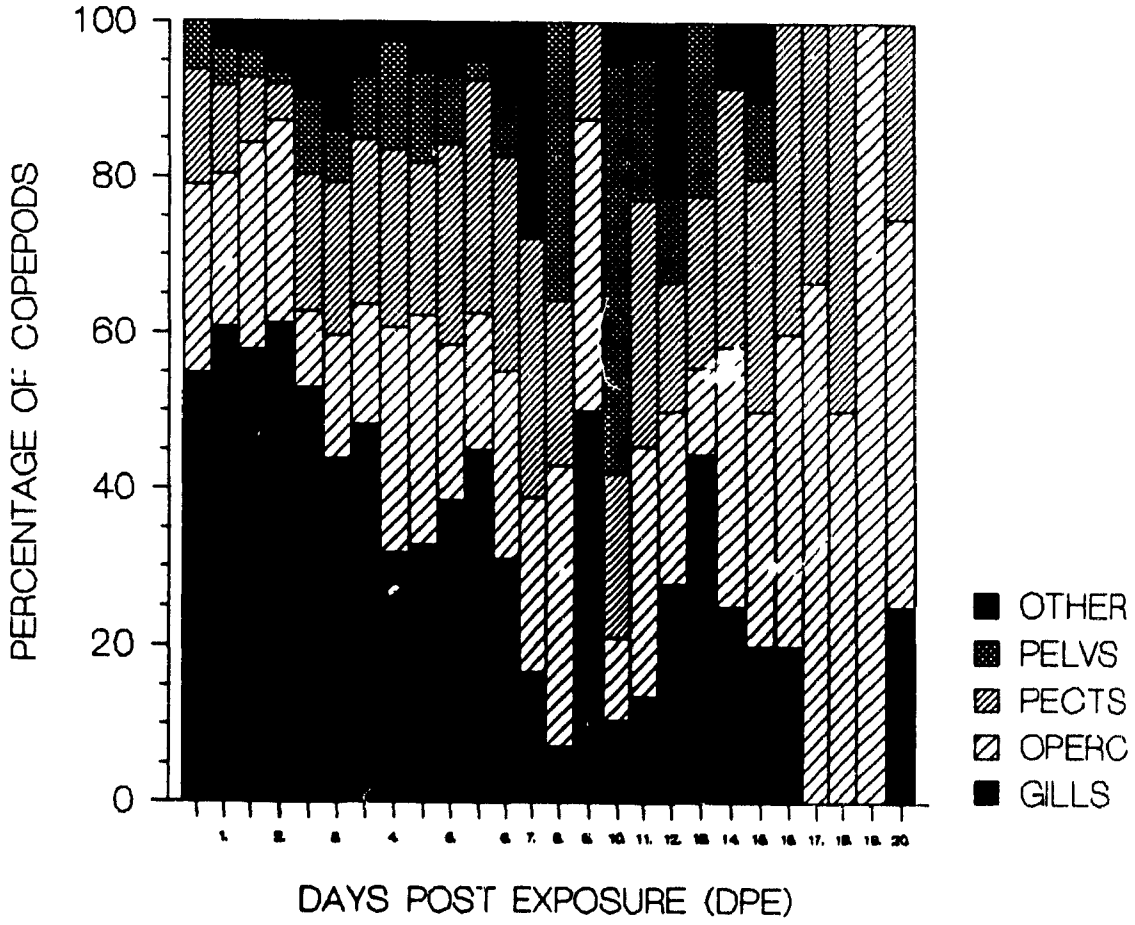

100
80
60
40
20
0
PERCENTAGE OF COPEPODS
DAYS POST EXPOSURE (DPE)
OTHER
PELVS
PECTS
OPERC
GILLS

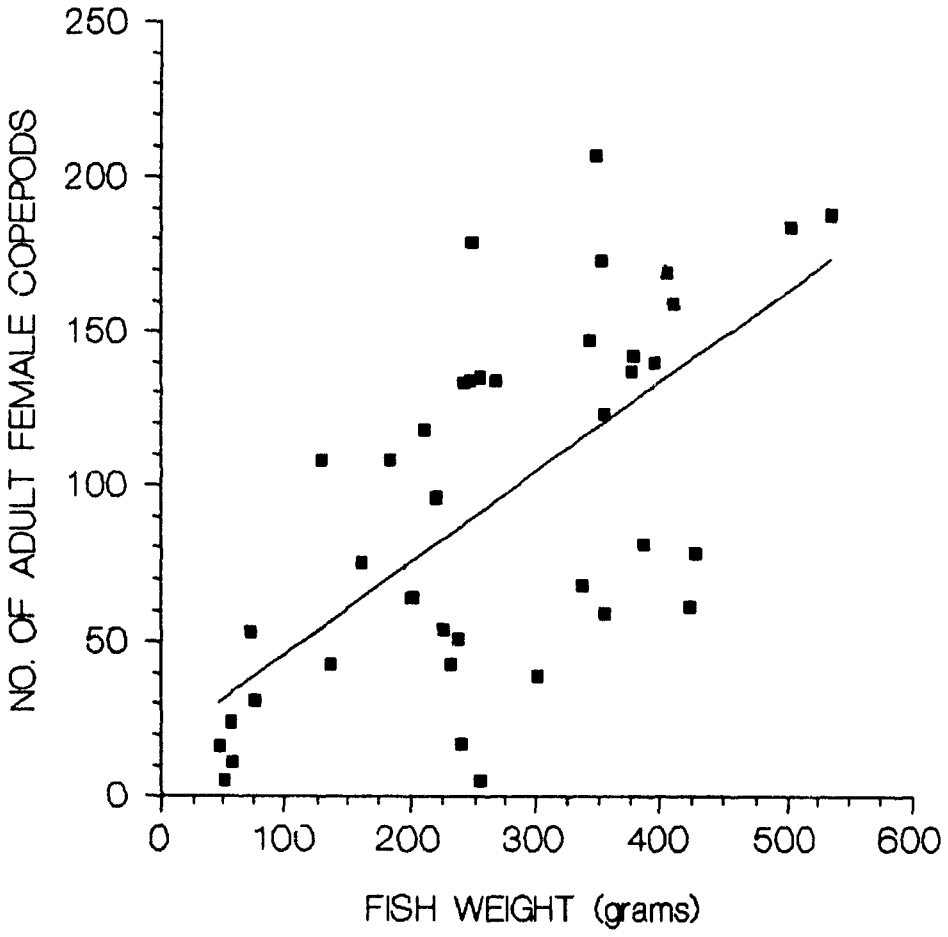
Table 4.3. Parasite distribution on experimentally infected fish showing location of attachment, number of copepods and percentage of the total.

Location	No. of Copepods	Percentage
Gills	409	43 %
Opercula	218	23 %
Pectoral Fins	181	19 %
Pelvic Fins	73	8 %
Other	64	7 %
TOTAL	**945**	

Other - includes mouth (29, 3.1%), dorsal fin (24, 2.5%), anal fin (10, 1.1%), caudal fin (1, 0.1%).

Figure 4.8. Relationship between the total number of adult female S. edwardsii copepods removed from infested brook trout (S. fontinalis) and fish weight. Mean (± SD) number per fish was 94.8 ± 58.16, range = 5 -207; mean (± SD) weight of fish was 267.77 ± 128.39 g., N = 40. (Median number of copepods was 88.5; median weight of fish was 251.75 g. (r^2 = 0.42, df = 39, P< 0.01).

Regression equation: Y = 16.68 + 0.292 W (W=fish weight in grams).


250
200
150
100
50
0
NO. OF ADULT FEMALE COPEPODS
0
100
200
300
400
500
600
FISH WEIGHT (grams)

DISCUSSION

Kabata and Cousens (1973) published the first complete description of larval development of S. californiensis on experimentally infected sockeye salmon (Oncorhynchus nerka) over 18 years ago. This study of larval development of S. edwardsii on brook trout (Salvelinus fontinalis) was undertaken for comparative purposes. The timing of development reported in this study is that for 13°C ±1.5.

Chalimus I larvae were present as early as .5 days post-exposure (DPE), chalimus II larvae at 1 DPE and chalimus III larvae at 2 DPE. Sexual differentiation was detectable at this point and chalimus IV males were observed at 2.5 DPE and adult males at 3 DPE. In contrast, chalimus IV females appeared at 4 DPE and adult females as early as 11 DPE. The duration of larval stages was difficult to assess due to the variability in timing. However, maximum duration of life cycle stages were as follows: chalimus I, 24 hours (.5 to 1.5 DPE); chalimus II, 36 hours (1 to 2.5 DPE); male chalimus III, 48 hours (2 to 4 DPE); female chalimus III, 60 hours (3 to 5.5 DPE); male chalimus IV, 60 hours (2.5 to 5 DPE), female chalimus IV, 7+ days (4 to 11+ DPE; adult males, 5 days (3 to 8 DPE). The longevity of the adult female stage could not be determined from this study.

The greatest number of adult males occurred between 4.5 and 5.5 DPE, amounting to 11, 25 and 12 on days 4.5, 5 and 5.5, respectively. Prior counts amounted to 2, 1 and 4 on days 3, 3.5 and 4 post-exposure, respectively, while later counts totaled 5, 3 and 2 on days 6, 7 and 8 post-exposure, respectively. These results indicate that the average male takes approximately 5 days to reach maturity, which is close to that for S. californiensis (4.6 days) at a similar temperature (12 C) (Kabata and Cousens 1973).

Growth of larval stages was progressive, although there was a fair degree of variability in size among individuals of the same stage and sex.

There appeared to be a pattern such that faster maturing individuals were larger than later maturing ones. For example, adult males aged 4-4.5 days were slightly larger than adult males aged 5-8 days and adult females aged 11 and 13 days were considerably larger than adult females aged 14-18 days. Males matured sooner than females (3-4 days vs. 11-14 days) and were much smaller (719 vs. 2056 microns, average). S. edwardsii are smaller in size than S. californiensis at all stages, most noticeably the adult male: 600-900 microns vs. 1060-1240 microns, respectively (Kabata and Cousens 1973).

Variability in larval size may be genetically determined but may also be related to site of attachment. More favourable sites which offer protection and a more nutritious food supply, such as the gills, may promote rapid development. Variability in timing of developmental stages may also be due to length of exposure period and subsequent attachment at a suitable location. The 12-hour exposure period in this study was used to ensure high levels of infection and probably contributed to the persistence of stages over a number of days. However, Kabata and Cousens (1973) reported variability in larval development due to the time spent in locating a suitable attachment site, possibly up to 24 hours.

Intensity of infection declined over time with a rapid loss of stages during the first 9 days followed by a slower rate during the balance of the experiment. Kabata and Cousens (1973) found a similar pattern in their study. The decrease can partly be explained by the disappearance of the males, none of which were detected after 8 days. Failure to complete moults and/or damage caused by the actions of the fish host could also account for some losses. The size of fish used may have been a factor as well. A relationship between fish weight (host size) and the number of parasites acquired by naturally infested fish was shown in the results of this study. Poulin et al. (1991b) reported similar results and indicated that abundance of S. edwardsii was most closely associated with surface area of the host.

The preferred location of attachment was in the gill region. The gills and opercula accounted for 66% of all attached stages, whereas the pectoral fins contained 19% and other fins and mouth only 15%. These results agree with other reports on the preference of S. edwardsii for the gill region of its host (Kabata 1969; Black 1982). However, the preferred location changed as the parasites aged. Initial preference for the gills shifted to the opercula and pectoral fins and may be due to space restrictions inhibiting copepod growth on the gills. The migration of attached larval copepods towards more suitable sites for permanent attachment is known to happen in Salmincola (Kabata and Cousens 1973; Kabata 1981; Poulin et al. 1991a).

CHAPTER 5: SUMMARY

Despite the long association of Salmincola species with commercially cultured salmonids, there are still no practical and cost-effective control therapies against the parasite after it has entered a fish culture facility. Due to the presence of S. edwardsii on some Quebec trout farms, this study was undertaken to learn more about the biology and early life history of this particular species and its association with brook trout.

An experimental protocol was developed to facilitate the study of the effects of temperature and photoperiod on the early life cycle events of hatching, swimming and copepodid survival. This methodology proved to be effective for obtaining consistent results and may be used in parallel studies of related species and genera of ectoparasitic crustaceans

Temperature was found to have a significant effect on copepodid swimming activity and survival, such that both decreased in duration as temperature increased. Copepodids remained active considerably longer than previously reported, up to 16 days at 8° and up to 5 days at 20 C. Although egg development was also found to be temperature-dependent, hatching duration and hatching success were not. Photoperiod had no measureable effect on the life histoy variables monitored.

The temperature-dependent relationship of copepodid swimming and survival time may have important application in the development of control therapies. Colder ambient water temperature retards egg development but prolongs copepodid infectivity whereas warmer temperatures promote egg development but reduce the duration of copepodid infectivity. The timing of chemical applications with the appropriate dosage for the most effective results can be developed through a series of empirical trials over a range of temperatures.

Larval development on the host was effectively monitored by the use of the experimental protocol adapted from Kabata and Cousens (1973). A

more accurate timing of events could be obtained by reducing the exposure time of the fish to copepodids. In any case, the dynamics of the early stages of the life cycle became more apparent and for the first time the life span of the male larvae was deduced; up to 3 days. It is interesting to note that the males mature prior to the females of the same generation, having only chalimus iv females to fertilize, yet figures in the literature depicting males and females mating show adult males on adult females. The ability of being able to mate with females of the same generation as well as those which remain unfertilized from a prior generation can reduce the detrimental effects of inbreeding

Permanent attachment of adult female copepods about 2 weeks after host contact corroborates the findings of Kabata and Cousens (1973) on S. californiensis at a similar temperature. However, the life span of the female could not be determined from this study.

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