THE UNIVERSITY OF CALGARY

The Life History of *Pteronarcys californica* (Plecoptera) in the Crowsnest River

by

Greg Townsend

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The undersigned certify that they read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The Life History of *Pteronarcys californica* (Plecoptera) in the Crowsnest River" submitted by Greg Townsend in partial fulfillment of the requirements for the degree of Master of Science.

Dr. G. Pritchard, Supervisor
Department of Biological Sciences

Dr. J. Post
Department of Biological Sciences

Dr. H. P. Arai
Department of Biological Sciences

Dr. W.A. Ross
Faculty of Environmental Design

3 Dec 1994

Date

ABSTRACT

The *Pteronarcys californica* (Plecoptera: Pteronarcyidae) population in the Crowsnest River has a merovoltine life history spanning four to seven years. Delayed egg hatch, probably due to some degree of diapause, constitutes approximately one year of the life cycle. Growth patterns of *P. californica* larvae are consistent with a complex life cycle that is characterized by extended larval recruitment leading to variation in the sizes of larvae of the same age and to cohort splitting. Larvae that hatch early in the year probably complete their development one year sooner than larvae that hatch in the middle of the year and probably take two fewer years to develop than larvae that hatch late in the year. Size-frequency, MULTIFAN, and a growth simulation model predict different times to complete larval development. These differences and the difficulty of accurately estimating generation time are due in part cohort splitting, continual recruitment, diapause, and differences between male and female growth patterns.

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CHAPTER 1

INTRODUCTION

"Life histories lie at the heart of biology; no other field brings you closer to the underlying simplicities that unite and explain the diversity of living things and the complexities of their life cycles" (Stearns 1992). A life history can be defined as "the quantitative and qualitative details of the variable events that are associated with an organism's life cycle (i.e. the sequence of morphological and physiological processes that link one generation to the next)" (Butler 1984). The important distinction between a life history and a life cycle is that a life cycle is the same for all members of a species, but a life history may be different between populations or between individuals. For example, the life cycle of all individuals of an aquatic insect species may include egg, larval and adult stages but the length of the life cycle may vary between individuals.

In 1978, at the Plenary Session of the 26th Annual Meeting of the North American Benthological Society, life history information and its importance to contemporary benthic science was formally addressed. The relevance of life history information in benthic research was examined via taxonomy, production, feeding, bioassay, environmental disturbances, and management (Rosenberg 1979), and one of the basic requirements suggested for future research was to collect life history data such as voltinism and determine how it changes under different temperature and food conditions. The importance of life history studies can be seen with a fish example. Fish have evolved diverse life history strategies. Some species are iteroparous, others are semelparous, and

time to sexual maturity and life cycle length can vary over many years. Within a species, different populations may have large variations in life history strategies, like differences in migration, growth, age at first reproduction, life span and fecundity (Wootton 1990). Because environmental changes will modify life history strategies, the effects of fishing on mortality rates and the adverse effects of pollution may impose new life history patterns on a fish population. The resulting life history strategy may have negative effects on its socioeconomic value. Therefore, many fish species have been the subject of life history studies. Given that insects are a primary food source for many fish species and that an insect's life history can be modified by the same environmental changes that affect fish, studies on their life histories may actually be of greater importance.

Stearns (1976) defined a life history strategy as a set of co-adapted traits designed, by natural selection, to solve particular ecological problems. Important life history traits, according to Stearns (1976), are both the mean and the variance in age at first reproduction, clutch size, size of young, number of clutches per lifetime, interbrood interval, the age distribution of reproductive effort, the interaction of reproductive effort with adult mortality, and the variation in these traits among an individual's progeny. Any combination of these traits can be considered a life history strategy. Thus an important measure of insect life history can be summarized by the time taken to complete growth from egg to adult, and this can be affected by factors such as water temperature, photoperiod, food, water quality, predation or competition.

Variation in environmental factors means that life histories are dynamic; they vary in time and space given different environmental conditions. However, the genome of an

organism will impose limits on life history variation. Every individual has inherited a set of genes that defines the total range of its ability to respond to change. Biochemical, physiological, behavioral and morphological mechanisms will provide a buffer, albeit limited, for the adverse effects of change. Therefore, physical factors such as photoperiod and temperature, work in concert with genetic constraints to determine how an organism will develop. Ultimately, the biological characteristics of an organism's life history such as birth rate, feeding habits, habitat selection, growth pattern and rate, social behavior, reproductive strategy, length of life, and mortality rate will be modified by genetic and environmental factors. Furthermore, life history strategies often include morphological and/or behavioral plasticity, thereby increasing an individual's ability to cope with unpredictable factors.

Obviously not all factors will have equal effects on an organism's life history, but variability in environmental temperature can be especially important for a small poikilotherm, like an insect. Diapause is a common strategy in insects and there are several environmental factors such as photoperiod, temperature, food and density which influence diapause and therefore growth rate by acting as a cue, a regulator, or both (Danks 1992). Diapause "routes the metabolic programme of the organism away from direct developmental pathways and into a ... clearly organized break in development" (Danks 1987) and will constrain the detrimental effects of some environmental factors by the synchronization of resistant stages with adverse conditions, synchronization of activity with favourable conditions, and suspension of activity close to periods that can prevent reproduction and/or growth (Danks 1992).

For insect eggs, temperature has the greatest influence on the duration of the incubation period (Harper and Hynes 1972; Butler 1984; Sweeney 1984; Leggott and Pritchard 1985; Mutch and Pritchard 1986; Butler and Burns 1989). For example, Brittain (1977) found that, as in many insects, the incubation period for *Taeniopteryx nebulosa* (Plecoptera) eggs decreased as temperature increased up to a point, then the incubation period increased. He also found that eggs kept in a 12 hr light: 12 hr dark regime hatched at the same time as eggs kept in total darkness at the same temperature. This suggests that the role of photoperiod in egg development may be minimal for some aquatic insects.

For aquatic insect larval growth, it has been shown that temperature, photoperiod and diet are particularly influential (Anderson and Cummins 1979; Sweeney and Vannote 1984; Danks 1992). For example, Sweeney *et al.* (1986) ran three experiments on the larvae of the winter stonefly *Soyedina carolinensis,* combining different diets at various fixed and fluctuating temperature regimes. They found a temperature at which growth was maximum and a temperature at which larval mortality was near 100% regardless of diet. They also found that diet significantly affected larval growth and mortality. However, only temperature had an effect on larval developmental time. Of the three factors - temperature, diet and photoperiod - temperature is likely most influential because of its direct effect on insect metabolism and its indirect effect through the food supply.

Although there has been a trend of increased numbers of studies examining how voltinism changes under different temperature and food conditions (Clifford 1982), not all insect Orders have been represented equally. For example, detailed life history

information is available for less than 5% of the species of Plecoptera (Stewart and Stark 1988).

Stoneflies are generally cold-adapted, rarely being found in water above 25°C (Hynes 1976). Although there is a relatively large variation among stonefly species in temperature at which growth is most rapid, the majority grow slowly during the summer. Some stonefly larvae enter diapause during high summer temperatures (Khoo 1964; Harper and Hynes 1970). However, there are others which are known to grow rapidly during high summer temperatures: *Acroneuria evoluta* (Perlidae) (Ernst and Stewart 1985), *Pictetiella expansa* (Perlodidae) (Baumann and Gaufin 1969; Baumann *et al.* 1977), and *Pteronarcys californica* (Pteronarcyidae) and *Claassenia sabulosa* (Perlidae) (Stewart and Stark 1988). These studies suggest that some species in the Group Systellognatha (Figure 1) have adapted to warmer temperatures. This is especially so in the Perlidae, which is the only stonefly Family which has adapted well to tropical environments (Illies 1965). Optimal temperatures for egg development in perlids (e.g. *Dinocras cephalotes* [Lillehammer 1987], *Perla burmeisteriana* [Marten 1991], *Agnetina capitata* [Moreira and Peckarsky 1994]) are high relative to other stoneflies.

There are four general categories of voltinism: multivoltine (more than one generation annually), univoltine (one generation annually), semivoltine (one generation every two years) and merovoltine (one generation every three or more years). North American stoneflies (of which few have been studied in detail) have univoltine, semivoltine and merovoltine life cycles. For example, *Isoperla signata* in the Little Rib River in central Wisconsin is univoltine (Jop and Szczytko 1984), *Hesperoperla pacifica* in Birch

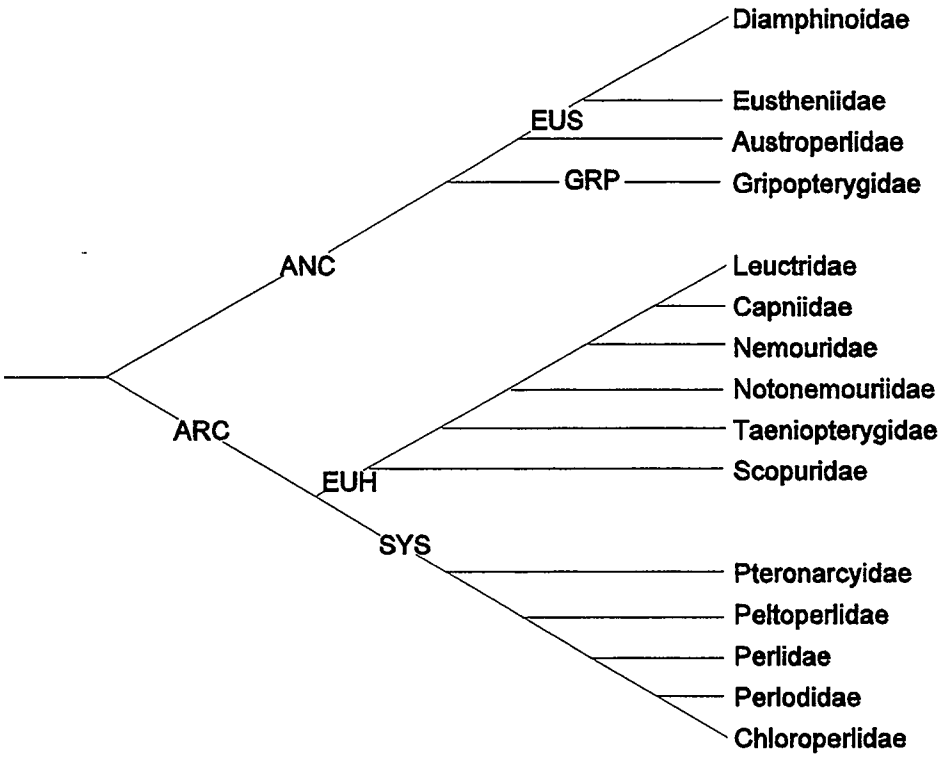

Figure 1: Phylogenetic relationships among Plecopteran families. Some species from the Perlidae, Perlodidae and Pteronarcyidae have adapted to warmer temperatures (see text). EUS, Eusthenioidea; GRP, Gripopterygoidea; EUH, Euholognatha; SYS, Systellognatha; ANC, Antarctoperlaria; ARC, Arctoperlaria. Adapted from Zwick (1980).

Creek, Idaho is semivoltine (Robinson *et al.* 1992), and *Agnetina capitata* in Cascadilla Creek, New York is merovoltine (Moreira and Peckarsky 1994). To date, no stoneflies have been found with a multivoltine life cycle. This lack of a multivoltinism is likely due to stoneflies living mainly in cold temperatures. Cold temperatures slow enzymatic processes and therefore development is slow.

Stonefly voltinism can be either determinate or indeterminate. Species with determinate voltinism remain univoltine, semivoltine or merovoltine regardless of changes in factors such as stream temperature, altitude, or latitude, whereas voltinism changes in response to changes in those factors in species with indeterminate voltinism (Ernst and Stewart 1985; Stewart and Stark 1988). *Hastaperla brevis* is an example of a stonefly with determinate voltinism; in a stream in the Ozarks of Oklahoma (Ernst and Stewart 1985) and in a southern Ontario stream (Harper 1973) it is univoltine. On the other hand, Lechleitner and Kondratieff (1983) found that in southwestern Virginia, *Pteronarcys dorsata* is univoltine whereas in Wisconsin it is semivoltine (Hilsenhoff and Narf 1972), and in Alberta (Barton 1980) and Saskatchewan (Dosdall and Lehmkuhl 1979) it is merovoltine. All stonefly life cycle types may or may not have a period of egg or larval diapause.

This research deals with the stonefly *Pteronarcys californica* (Plecoptera: Pteronarcyidae) from the Crowsnest River. *P. californica* is a large stonefly (6.5 cm body length, excluding caudal filaments, as an adult) found along the Rocky Mountains of Canada and the United States (Baumann and Gaufin 1969; Gaufin *et al.* 1972; Baumann *et al.* 1977; Merritt and Cummins 1984) and is considered a herbivore-detritivore (Fuller and

Stewart 1979). Based only on individuals with a head capsule width of more than 3.0 mm, Freilich (1991) estimated that *P. californica* had a three year life cycle in Pacific Creek, Wyoming, whereas Branham and Hathaway (1975) estimated a four year life cycle, based on body weight in Provo River, Utah.

Pteronarcys californica has been used in many studies such as the biochemical effects of specific toxic metals (Elder and Lords 1974), digestive enzyme activity (Martin *et al.* 1981), egg morphology and phylogeny (Stark and Szczytko 1982), the effect on nutrient availability to collector species (Short and Maslin 1977), salt and water balance (Colby 1972) and isolation and characterization of glycoproteins (Clubb *et al.* 1975). *P. californica* larvae play an important role in nutrient cycling, via detrital ingestion (Cummins *et al.* 1973), and in the Crowsnest River of Alberta are a substantial food source for trout and whitefish species (Townsend, pers. obs.).

An intriguing aspect of the Crowsnest River *P. californica* population is the color polymorphism shown in eggs and larvae (Townsend, pers. obs.). Within a population, polymorphism is formally recognized if two or more distinctly discontinuous phenotypes are maintained and comprise at least 1% of the population (Kennedy 1961; Vepsalainen 1978). On this basis the Crowsnest River population has three larval color morphs which I describe as stripe, black and diamond. Electrophoresis on larval color morphs (18 enzymes at 23 loci) demonstrated no difference between morphs and laboratory crosses provided eggs that hatched. However, the larvae died before attaining a size that would allow color patterns to be distinguished. There are no differences between head capsule widths and wing pad lengths among the morphs and they are neither age nor sex specific

and are found in the same habitats: all three morphs may be found under the same stone. However, there is an unequal distribution within the population regardless of sample date; 25.1% black, 70.4% stripe, and 4.5% diamond (n=3610). The color dimorphism observed in the eggs is either dark gray (typical) or orange (rare). Both egg color morphs hatched successfully in the laboratory. However, the larvae died before attaining a size that could reveal a possible relationship with the larval color morphs. At present there are no explanations for the presence or frequency of the color morphs.

Basic life history features of *P. californica* such as the effect different temperatures have on egg and larval growth rates have not previously been investigated. *P. californica's* relatively large size is an asset for many laboratory studies, but its long life cycle makes studies requiring laboratory rearing, such as genetic crosses or determination of egg and larval growth rates, difficult at best. Typically, these types of life cycle studies are reserved for organisms with shorter life cycles. Nevertheless, an objective of this thesis is to assess the importance of temperature in describing the life history of *P. californica* in the Crowsnest River. Egg and larval data collected from both field and laboratory studies are used to meet this objective. Another objective of this thesis is to compare estimates of the length of *P. californica's* larval life cycle in the Crowsnest River obtained from three estimation methods: 1] the widely used method based on visual inspection of the size-frequency distribution, 2] MULTIFAN, a software program designed to provide an objective interpretation of size-frequency data (Fournier *et al.* 1990), and 3] a growth simulation model based on larval growth rate data collected in the laboratory and two year's river water temperature data.

CHAPTER 2

METHODS

2.1 STUDY SITE

The study site is located on the Crowsnest River in the foothills of southwestern Alberta (49° 35'N), approximately 35 kilometres from the river's origin in Crowsnest Lake (Figure 2). This site was selected because *P. californica* abundance increased with distance from Crowsnest Lake (Townsend unpubl.), and at this point had reached a level at which large numbers of individuals could be obtained with minimal effort and without detrimental effects on the population. There is an elevational drop of 176 m from Crowsnest Lake to the study site. At the study site, the Crowsnest River has a drainage area of 676 km^2 (Environment Canada 1993). The only published data on Crowsnest River discharge rates near the study site, were collected from 1911 to 1930 from the Lundbreck gauging station. These trends show a variable discharge from year to year with an average discharge of 7.18 m^3/s (Figure 3A). The nearest active gauging station is at Frank, approximately 15 km upstream, with a drainage area of 404 km^2. At Frank, the mean yearly discharge for 1992 and 1993 was 3.20 m^3/s and 5.48 m^3/s respectively, and the peak discharge occurred between May and July (Figure 3B).

In mid-summer, the study site has a channel width of 12.5 to 15.0 m and the stream side vegetation is dominated by Alder (*Alnus tenuifolia*), Balsam Poplar (*Populus*

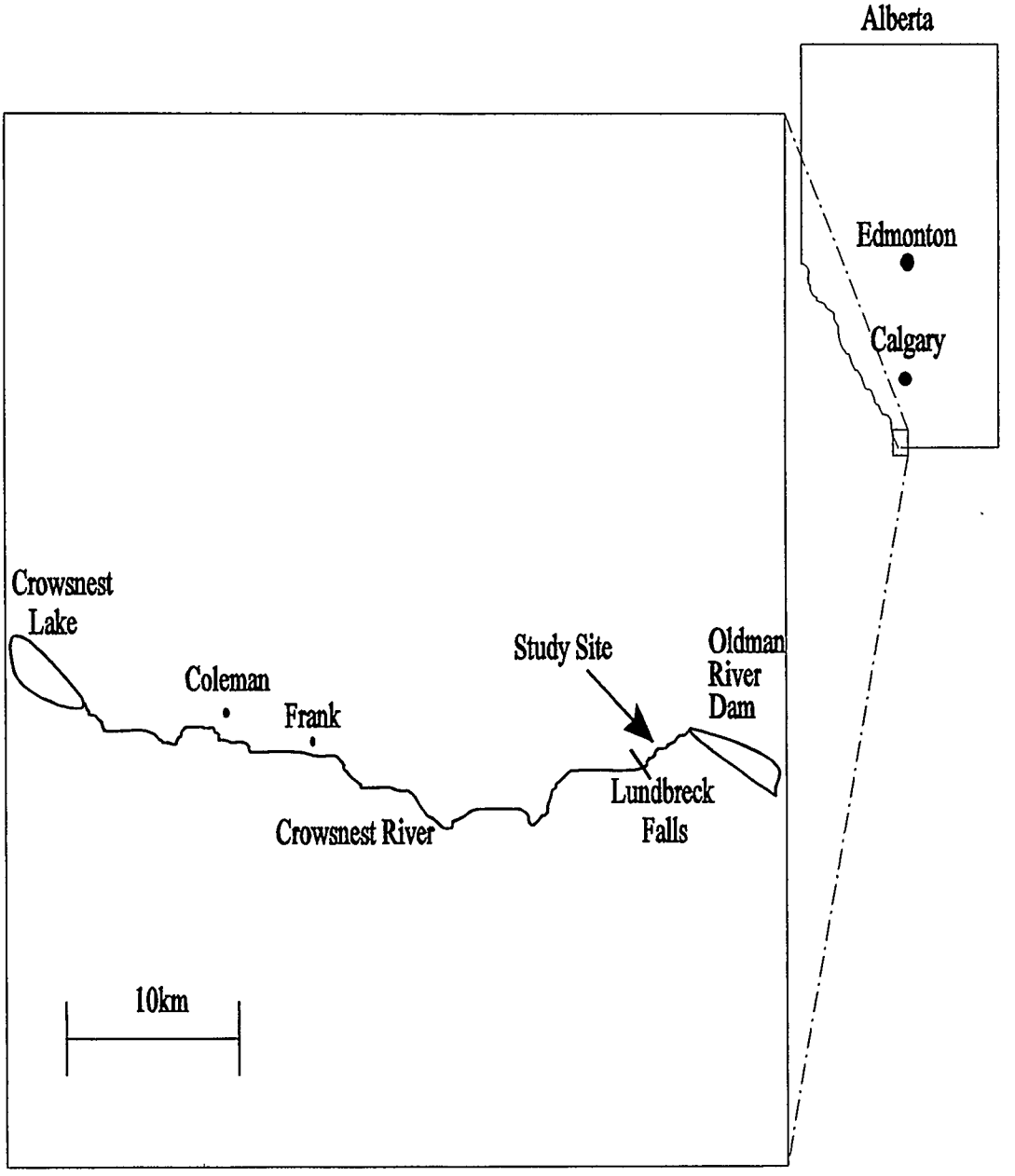

Figure 2: The study site is located between Lundbreck Falls and the Oldman River Dam, approximately 35 km from the Crowsnest River's point of origin.

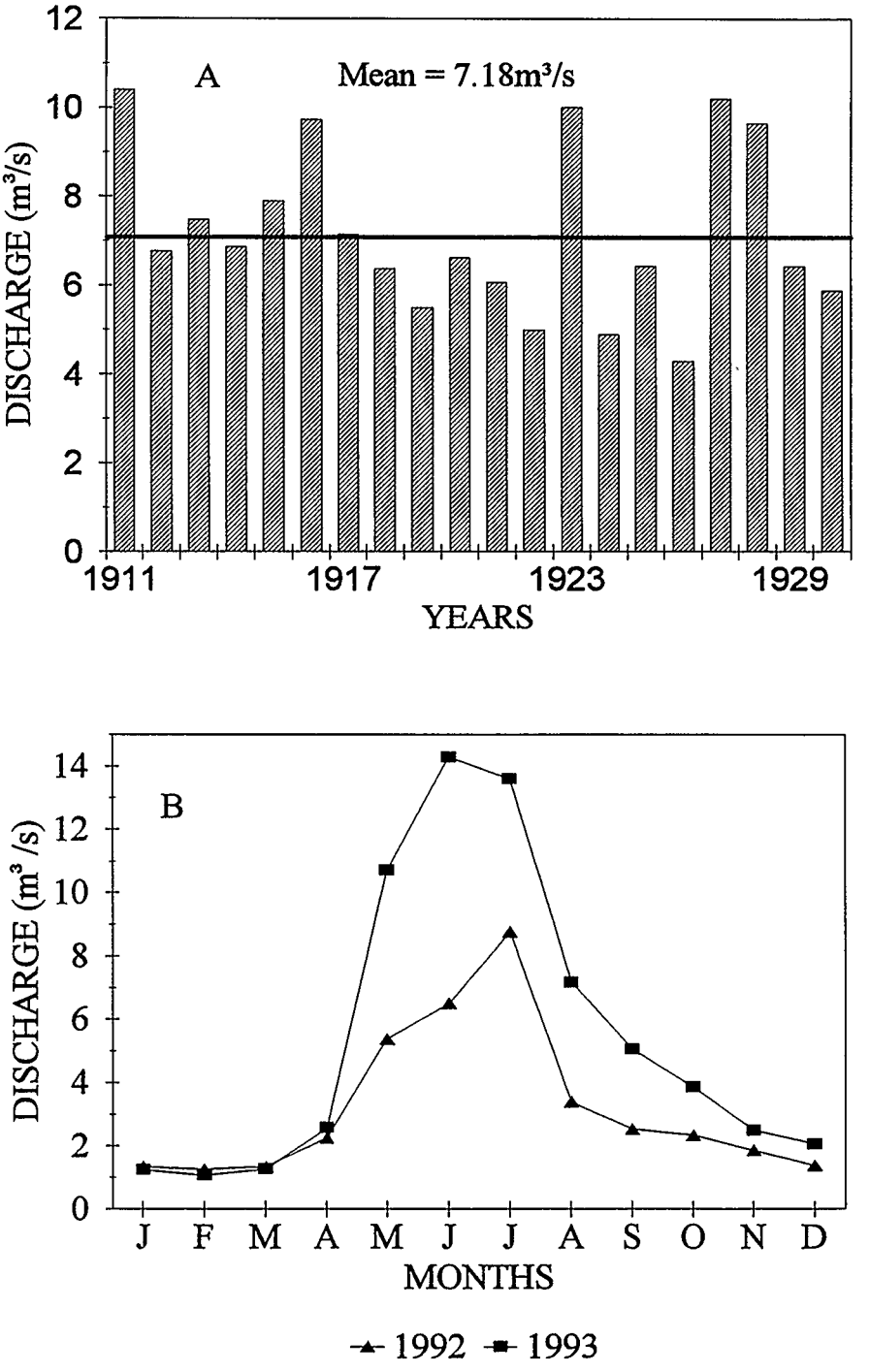

Figure 3: A) Crowsnest River yearly discharge rates collected from 1911 to 1930 from the Lundbreck gauging station, located near the study site. B) Mean monthly discharge rates collected from the Frank gauging station for 1992 and 1993.

balsamifera) and Wolf Willow (*Elaeagnus commutata*) (Figure 4). Water velocity, measured with a Pygmy Gurly meter and a cork technique in July 1990 and 1991, varied from 0.09 m/s in pools to 1.1 m/s in runs. Water depth, measured to the nearest cm at 2 m intervals across the stream, varied from 10 to 92 cm. Substrate particle size, measured at 1 m intervals along a 100 m stretch of the stream and grouped into six surface area categories (Figure 5) fell mainly in the 512 to 2048 cm^2 category (boulder). Substrate surface area was determined by multiplying perpendicular length and width measurements.

A continuously recording submersible Ryan Thermograph (Model-J) was anchored to the substrate at the study site from April 1992 until November 1993. The thermograph accuracy was checked with a mercury bulb thermometer before initial instream placement, whenever graph paper was changed, and after permanent removal from the stream, and readings were adjusted accordingly. Temperatures were read from the graph paper to the nearest 0.25°C at two hour intervals. Annual thermal ranges and degree-day (DD) accumulations above 0°C were determined from these records. Degree-days are a measure of the usable thermal energy available to an organism (i.e. above the temperature at which development is zero) which translate calendar time into physiological time by removing the effect of temperature variation on growth rate. For example, an organism might take five days to complete a particular phase of development at 10.0°C but require 10 days at 5.0°C. This time difference is simply a consequence of the difference in temperature and does not represent a physiological difference in the organism. Degree-days remove this latter implication; in both cases in this example 50 DD above 0°C are required to complete the phase of development. There was one relatively warm year

Figure 4: An upstream view of the Crowsnest River at the study site.

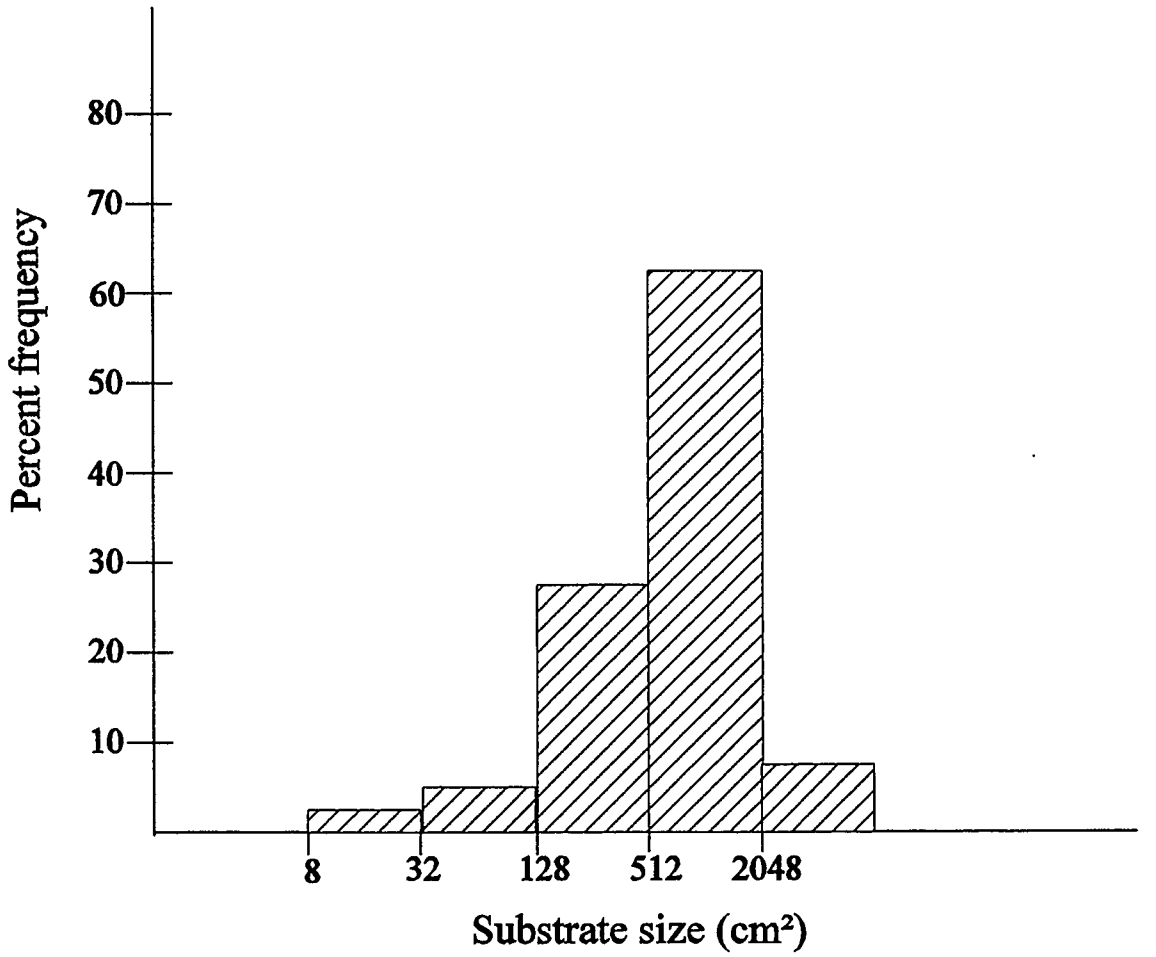

Figure 5: Percentage of substrate particle size (cm^2) at the study site, measured at 1 m intervals along a 100 m stretch of the Crowsnest River.

(1992) and one cool year (1993) during the period of this study (Figure 6). In 1992 the high and mean temperatures as well as monthly and total accumulated DD above 0.0°C were greater than those for 1993 (DD accumulated May - November 1992 = 2112, May - November 1993 = 1814). The lowest temperature was recorded in November of each year just prior to ice cover, and continued throughout the period of ice cover.

2.2 EGGS

2.2.1 Egg collection

Eggs were collected directly from the Crowsnest River population during adult emergence on May 6, 1992 and May 25, 1993. At the River, females were monitored from emergence until oviposition, and egg masses were removed from mated females by holding their abdomens over a container of river water. If the egg mass was not released, it was gently touched on the water surface. Once released into the water the egg mass would break apart. Eggs were either transported to the laboratory at river temperature or were placed in mesh enclosures anchored in the river.

2.2.2 Laboratory experiments

Single egg masses collected on May 6, 1992 from each of eight mated females were mixed and 50 eggs were placed in each of seven 50 ml plastic containers with river

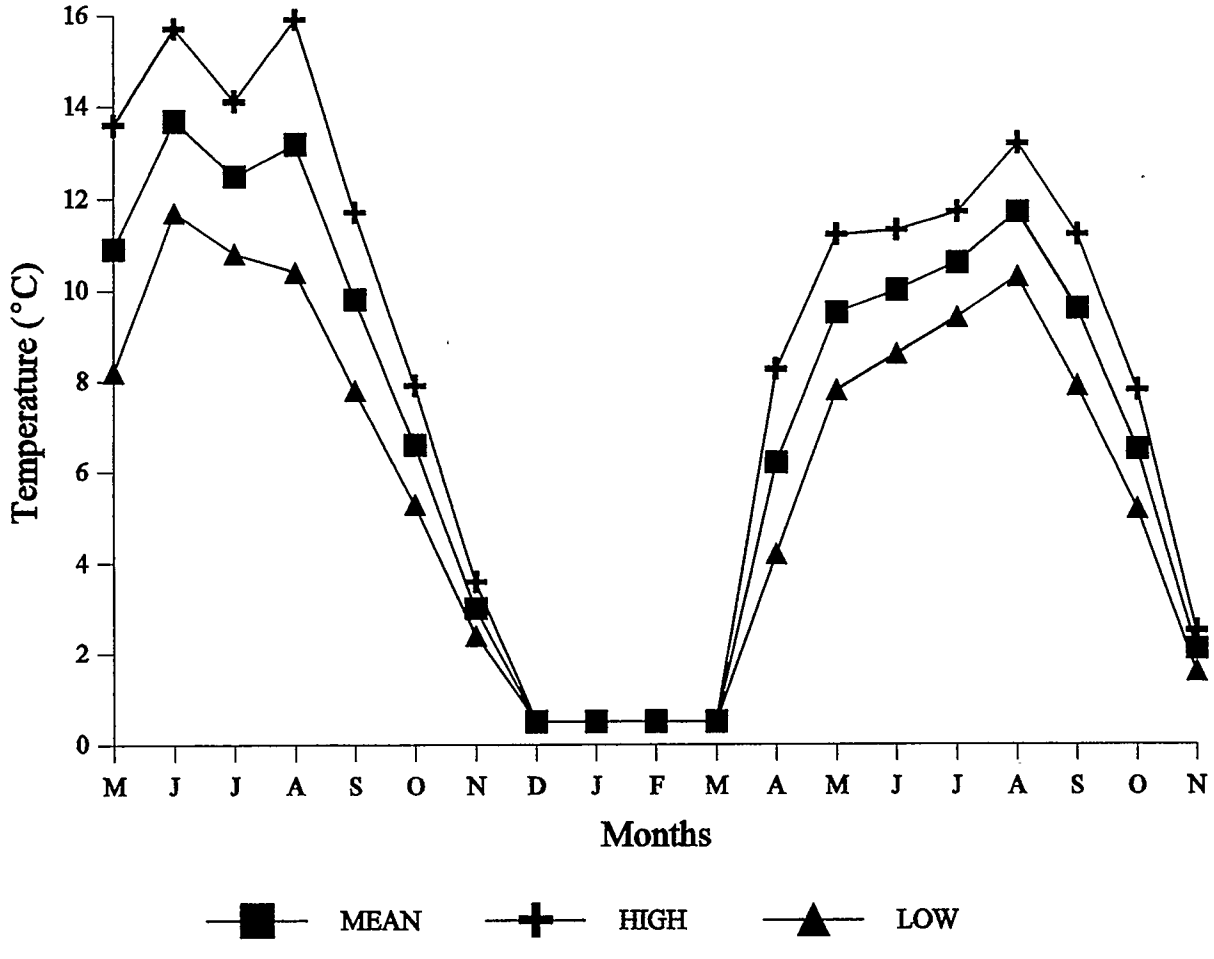

Figure 6: Water temperature in the Crowsnest River, from May 1992 to November 1993. "Mean" values are the overall mean temperature/month. "High" values are the monthly means of the daily maximum temperatures. "Low" values are the monthly means of the daily minimum temperatures.

water. Preliminary data from the Crowsnest River indicated that temperatures during the ice free period are usually between 5.0 and 20.0°C. Therefore, eggs were maintained at a nominal fixed-temperature of either 5.0°C, 7.5°C, 10.0°C, 12.5°C, 15.0°C, 17.5°C or 20.0°C in a PRECISION water bath. Eggs were maintained at a fixed-photoperiod of 16 hours light and 8 hours dark (16L:8D) to simulate the daylight conditions associated with summer.

Eggs were gradually warmed or cooled when placed in fixed-temperature treatments. Eggs in all treatments were examined daily, and the number of hatched eggs and water temperature were recorded. Dechlorinated tap water was used to replenish any water loss. Hatching success (%), the number of days (D) and the number of degree-days (DD) above developmental zero required for 1st, 12.5%, 25% and 50% egg hatch were calculated for each treatment.

2.2.3 Field experiments

To examine if eggs exposed to stream conditions show different hatching patterns than those exposed to fixed temperatures in the laboratory, approximately 75 eggs were placed in each of 24 enclosures on May 25, 1993. These enclosures were stainless steel mesh (0.56 mm) tubes, 35 mm in diameter, with plastic caps. Locations within the stream were chosen to ensure a wide range of water depths (15 to 85 cm), velocities (0.09 to 1.0 m/s), and substrate size (8.0 to 5000 cm^2). The enclosures were tethered near the substrate to steel reinforcing-bar. The eggs were checked for hatching, at approximately

monthly intervals, whenever larval collections were made (see 2.3.1). Of the 24 in-stream enclosures, six were lost due to flow conditions and/or tampering.

Egg samples were removed twice during the summer of 1993 and returned to the laboratory to examine whether short-term exposure to stream conditions early in egg development affected hatching patterns. On July 5, 1993 eight river enclosures were removed, and eggs were divided equally and placed at fixed temperatures of 12.5°C, 15.0°C, 20.0°C and 22.5°C and a fixed-photoperiod of 16L:8D.

A cold treatment is often required to break diapause in insect eggs and to examine this effect, eggs were removed from two more river enclosures on August 15, 1993. Half of these eggs were maintained at 12.5°C while the other half were cooled to 1.0°C over an 18-day period, maintained at 1°C for 34 days, then returned to 12.5°C over 9 days.

To avoid the potential loss of eggs during spring break-up all remaining egg enclosures were removed from the stream on November 21, 1993, prior to ice cover. Eggs were returned to the laboratory, divided equally, and placed at a fixed-temperature of either 12.5°C, 15.0°C, or 20.0°C with a fixed-photoperiod of 16L:8D.

2.3 LARVAE

2.3.1 Larval collection

Larval collections were made monthly during the ice free period of 1992 and once or twice monthly during 1993. A modified kick net with an inner 1mm mesh bag and an

outer 0.2 mm mesh bag was used to collect a composite sample from a wide range of water depths (10 to 92 cm), velocities (0.09 to 1.1 m/s), and substrate sizes (8.0 to 5152 cm^2). Stones immediately upstream of the sampler were rolled and wiped, and the substrate below the stones was disturbed. The contents of the bags were either preserved in 70% ethanol or placed in river water and maintained near to river temperature, in an insulated container, for the trip to the laboratory.

2.3.2 Size structure in the field population - visual inspection of size-frequency distributions.

Larvae from the preserved samples were sorted under a dissecting microscope. Head capsule widths (across the eyes) and mesothoracic wing pad lengths were measured to 0.04 mm with the ocular scale of a dissecting microscope. Female larvae were differentiated from males by the presence of a genital groove on the ventral surface of the 8th abdominal segment (Figure 7B). This groove could not be determined accurately for larvae with a head capsule width <1.5 mm. Head capsule widths and wing pad lengths were plotted as percent size-frequency distributions for each sample date, and the life history was followed by visually tracing the movement of age classes in the size-frequency plots through time.

As age classes are defined by modes in the frequency distributions, it is important that the modes that are used accurately represent age classes. To do this the size-class interval used to construct the frequency distribution must be carefully chosen; an interval

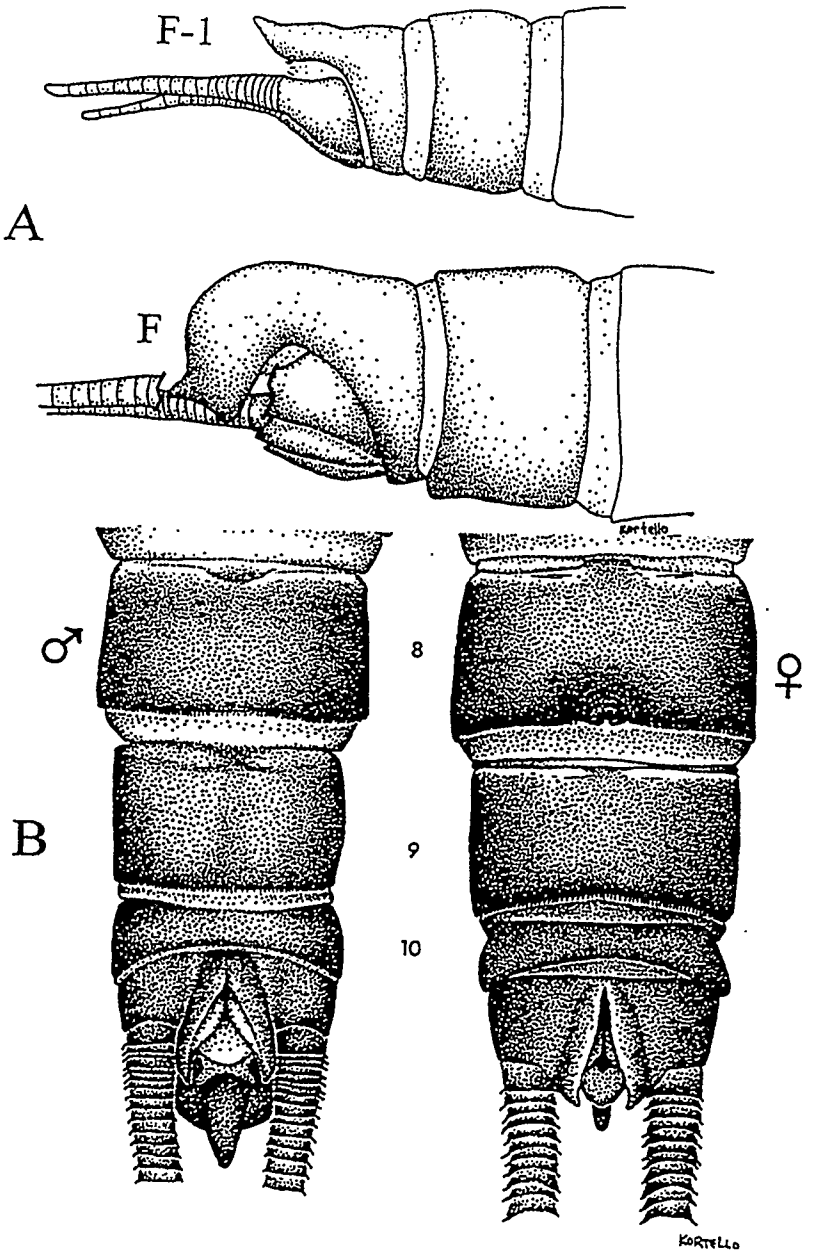

Figure 7: A) Lateral abdominal surface of penultimate (F-1) and final instar (F) male *P. californica* larvae showing the highly developed epiproct associated with the final instar. B) Ventral abdominal surface of male and female *P. californica* larvae. Female genital groove on the eighth abdominal segment used to separate females from males when head capsule widths are greater than approximately 1.5 mm.

that is too small will exaggerate the number age classes, and one that is too large will combine age classes. The June 1992 sample data are used to illustrate the choice of size-class interval (Figure 8). The smallest interval (0.05 mm) shows 25 modes, most produced by the absence of records for many size classes. However, each of these clearly does not represent an age class. Given variation in growth rates of individuals in a cohort, a range of sizes that follows a normal frequency distribution is expected within an age class, and the distributions of different age classes will overlap. Within a single age class, head width data from insects is likely not to form a continuous frequency distribution because moulting leads to step-wise changes in size and so some sizes may not be represented. Furthermore, small sample sizes may not properly represent all sizes present in the population, giving rise to many modes in what in reality is a unimodal distribution. A further complication is introduced by differences in growth rate between the sexes. Of the nine individuals in peak *a* in Figure 8, seven are males, and 10 of the 12 individuals in peak *b* are females. In addition, these larvae are in their final instar and will all emerge in the next year. Thus, the two modes centered on *a* and *b* that encompass seven of the original 25 modes, actually represent a single age class. If the same criteria are applied to the rest of the data, two more age groups are defined (Figure 8). The three modes can be followed as size interval is increased, until at an interval of 0.40 mm the distinction between the two oldest groups becomes blurred and it disappears when the interval is increased to 0.50 mm. At 0.30 mm, the three modes are still well defined and the male and female modes in the oldest group are more or less combined. Therefore, the size class interval of 0.30 mm was used for all head capsule width data and, in a separate analysis, a

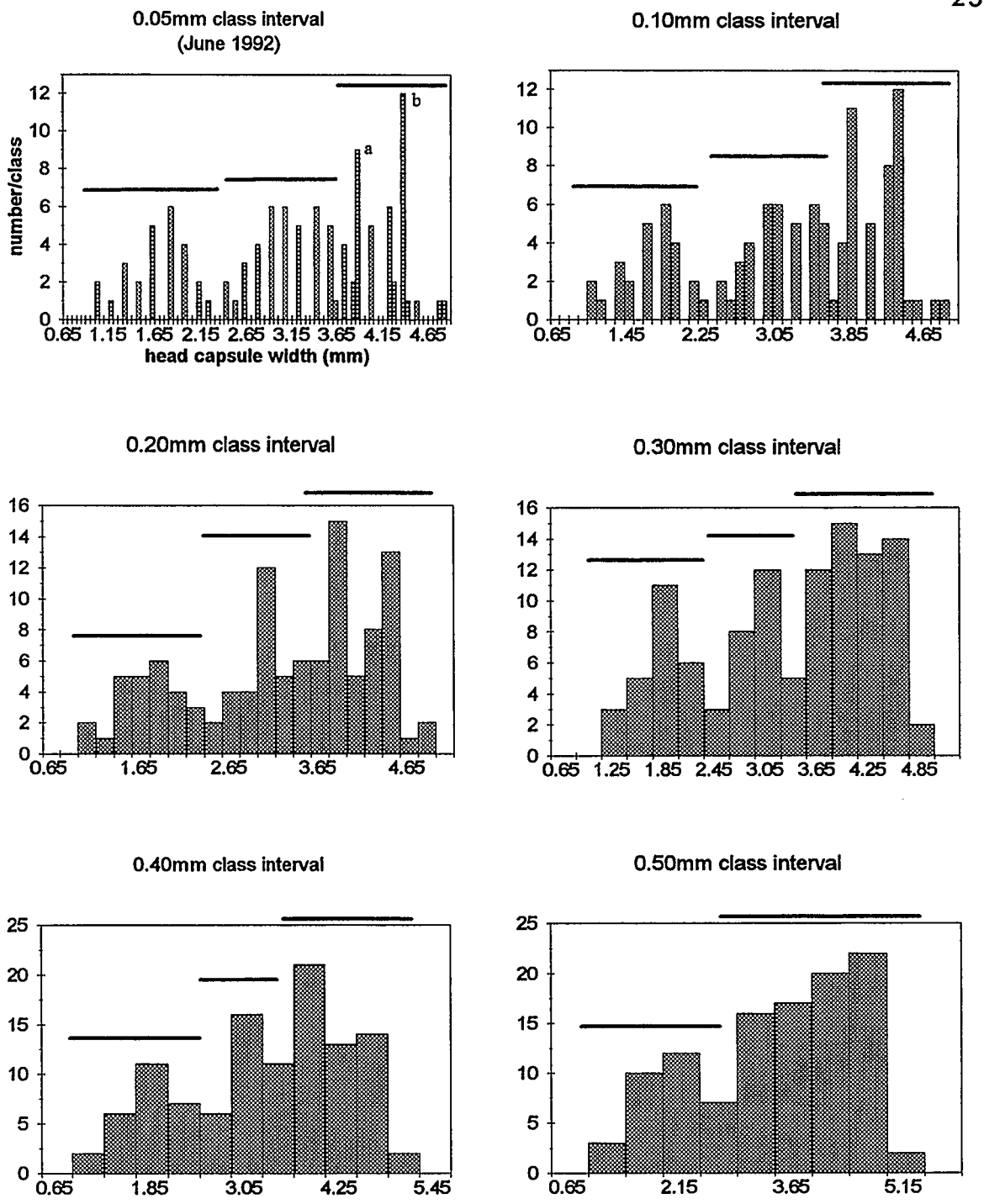

Figure 8: Three age classes, evident in the June 1992 larval head capsule width data, persist until the width of the size class interval exceeds 0.40 mm (see text).

class interval of 0.50 mm was used for wing pad length data. Both of these analyses divide the total size ranges into 18 classes, which falls within the range of 10 to 20 classes given by the "rule of thumb" proposed by Zar (1984) and Sokal and Rohlf (1981).

2.3.3 Size structure in the field population- MULTIFAN

A detailed description of the MULTIFAN model is given by Fournier *et al.* (1990) and the program is described in the MULTIFAN *3.2 User's Guide and Reference Manual* (Otter Research 1992). MULTIFAN was designed to simultaneously analyze multiple sets of fisheries length-frequency samples and it can incorporate specific structural hypotheses into fitted models. MULTIFAN uses a log-likelihood function (proposed by Wilks 1935) to compare, for the set of growth parameters tested, the expected probability that an individual picked at random will lie in a given size-class interval with the observed number of individuals in that interval. The model provides a structured and relatively objective means of evaluating alternative interpretations of the observed size-frequency distributions.

MULTIFAN has three major assumptions: 1] widths in each age-class are normally distributed about the mean width at that age; 2] growth follows the von Bertalanffy growth function (von Bertalanffy 1957); 3] standard deviations of the actual widths about the mean width at an age vary as a simple function (involving only 2 parameters) of mean width at that age. A chi-square test, based on a log-likelihood ratio test, is used to determine the best-fitting model hypothesis. This test answers the question of whether a

more complicated model should be accepted instead of a simpler model (fewer parameters), in the following manner. If there are two log-likelihood values, L_1 and L_2, for a simple and complicated model respectively, and if the more complicated model is a better fit, then $L_1<L_2$. The question now becomes, is the difference between the two log-likelihood values large enough to justify accepting the more complicated model. Under fairly general conditions, the difference has a distribution which is approximately equal to a χ^2 random variable with degrees of freedom equal to the number of parameters in the more complicated model. If the probability of observing this difference is less than 0.10 for the age-class parameter or 0.05 for all other parameters, then the more complicated model is chosen. Better estimates of the von Bertalanffy growth function have been obtained when the number of age-classes (modes) was overestimated rather than underestimated (Fournier, *et al.* 1990). Therefore, when determining the number of age-classes, a type 2 error (rejection of an extra age-class when it is actually present) is more serious than a type 1 error. To reduce the probability of a type 2 error, the 0.90 point of the χ^2 random variable is used for age-classes and 0.95 is used for all other parameters (Fournier, *et al.* 1990). The significance test compares fits, and no fit to the data is a candidate for best fit if adding one or more parameters to the model produces a model with a significantly better fit or removing one or more parameters from the model produces a model which does not significantly degrade the fit.

The best fit determined by the significance test is then tested to ensure the parameter estimate is located at the maximum of the log-likelihood function. This test estimates the covariance matrix of the parameter estimates for the selected best fit.

The basic set of parameters (which may be estimated or held constant), in terms of which the model can be expressed and the log-likelihood function calculated, include the proportions in each age-class, the mean length of the first age-group, the mean length of the last age-group, the von Bertalanffy growth parameter K, two parameters that determine the pattern of the standard deviation of length at an age, a parameter determining the overall variance of the sampling errors in the length-frequency data sets, and a parameter that determines the age-dependent selectivity of the sampling process (Fournier *et al*, 1990; 1991).

The simplest structural hypothesis assumes that the mean lengths for each age lie on a von Bertalanffy growth curve and that the standard deviations of length at each age are identical. More complex structural hypotheses can be tested to determine if they provide a statistically significant improvement in fit to the data. Some of the more complex hypotheses that can be tested assume that the following processes can occur:

1) Sampling bias for the first cohort. This could result from selectivity during the sampling process induced by the net mesh size or the sampling method. The sampling bias is assumed to apply only to the first cohort and to decrease linearly with age until the next recruitment pulse.

2) Age-dependent standard deviation in length-at-age. This hypothesis allows the standard deviation of length at each age to increase or decrease linearly with age.

3) Seasonally oscillating growth. Seasonal growth patterns are known to occur in some insect populations. This process was incorporated into the growth model by the addition of two parameters, the magnitude of the seasonal effect and the time of the year at which growth is slowest because of the seasonal effect.

4) Total yearly mortality for the final age class. For most aquatic insects it is typical for final instar larvae to leave yearly, as adults. The default in MULTIFAN is for the largest size class to accumulate. Therefore, an internal switch in the program must be altered to allow yearly removal of the largest sizes.

The models were systematically fitted incorporating all possible combinations of the applicable structural hypotheses and the log-likelihood ratio tests were used to identify the most parsimonious model structure. Fitting and testing procedures were done automatically by MULTIFAN, although some user-specified input was needed to ensure that the model exhibited stable behavior (see Fournier *et al.* 1990 for a detailed explanation). The user-specified input can be basic (minimum subjectivity) - identification of the month and size (width of mode) of individuals entering the population - or complicated (increasing subjectivity) - placing constraints on the analysis such as bounds on the width of a mode and proportions of individuals in each mode for all modes identified by visual examination.

Head capsule width size frequency data were used in the MULTIFAN analysis. Final instar females are larger than final instar males, so size classes composed of only females were removed from the size frequency data. Otherwise, they would have been mistaken for a mode (independent age class) by MULTIFAN. To ensure stable results, the smallest individuals should not occur prior to the sample (month) representing the start of recruitment. Therefore, the smallest individuals were removed in the three months preceding July 1992. For the analysis, Fournier (pers. comm.) recommended a class width of < 1.0 standard deviation of an evident sample mode. The size of an age class interval was tested by increasing the class width until the merging of classes reduced the significance of the fit. The initial data were in class widths of 0.05 mm. Neither doubling (0.10 mm) nor tripling the width (0.15 mm) reduced the significance of the fit. However, increasing the width to 0.20 mm did reduce the significance of the fit. Therefore, 0.15 mm was used as the size of the age class interval for the MULTIFAN analysis.

2.3.4 Laboratory rearing

Twelve larvae, with head widths ranging from 0.42 to 5.00 mm, were reared individually in 300 ml containers of stationary stream water with substrate provided by a stone, at nominal fixed-temperatures of 5.0°C, 7.5°C, 10.0°C, 12.5°C, 15.0°C, 17.5°C or 20.0°C and a fixed-photoperiod of 16L:8D. Containers were aerated at temperatures greater than 10.0°C. Larvae were fed Alder and Aspen leaves, from the Crowsnest River and Bragg Creek, that were conditioned for at least three weeks in river water under full spectrum light. Larval food was replenished and water was changed twice weekly for temperatures greater than 10.0°C and once weekly for temperatures 10.0°C and lower. Food was available in excess for all larvae in all treatments. Temperature was recorded and larvae were checked for moulting every day. Head capsule width and wing pad length were measured to 0.04 mm for moulted larvae. The time period between moults was recorded for each larva until it died, emerged, or the experiment ended. Dead and emerged larvae were replaced until some larvae of most sizes had moulted in each treatment.

For each treatment, mean specific growth rates *(G)* were calculated for each instar with the function (Travis 1980):

$$G_{(n)} = \frac{L_{(n+1)} - L_{(n)}}{T_{(n)}} * \frac{2}{L_{(n+1)} + L_{(n)}}$$

where $G_{(n)}$ is the mean specific growth rate of instar n, $L_{(n)}$ is the mean head capsule width

of instar n and $L_{(n+1)}$ that of the next instar, and $T_{(n)}$ is the mean developmental period of instar n. Multiplication by 100 gives the mean percentage growth rate per instar *(%G)*.

One growth rate datum point was collected from each larva that moulted twice. Because growth rates from larvae that moulted three or more times are more closely related than growth rates from different similar-sized larvae, these data points were considered nested within each treatment. Therefore, the effect of fixed-temperature and of individuals nested within fixed-temperatures on larval growth rate were tested with a Mixed Model ANCOVA using larval size as the covariate. The null hypothesis was that mean specific growth rates are the same for all treatments tested.

2.3.5 River temperature growth simulation model

At fixed-temperatures the growth curve reflects enzyme kinematics and is non-linear with the growth rate increasing to a fixed-temperature thermal maximum then dropping. In order to approximate this growth curve when using variable river temperatures a two stage growth function was used (Figure 9). The first stage of this model is described by a linear equation expressing the effect of fixed-temperature on larval growth rates, and is of the form;

$$Gr = a + bTemp$$

where *Gr* is the mean specific growth rate (mm/mm/day), *a* is the intercept, *b* is the slope, and *Temp* is river temperature in°C. The second stage is a constant growth rate for all river temperatures which exceed the fixed-temperature thermal maximum. The second

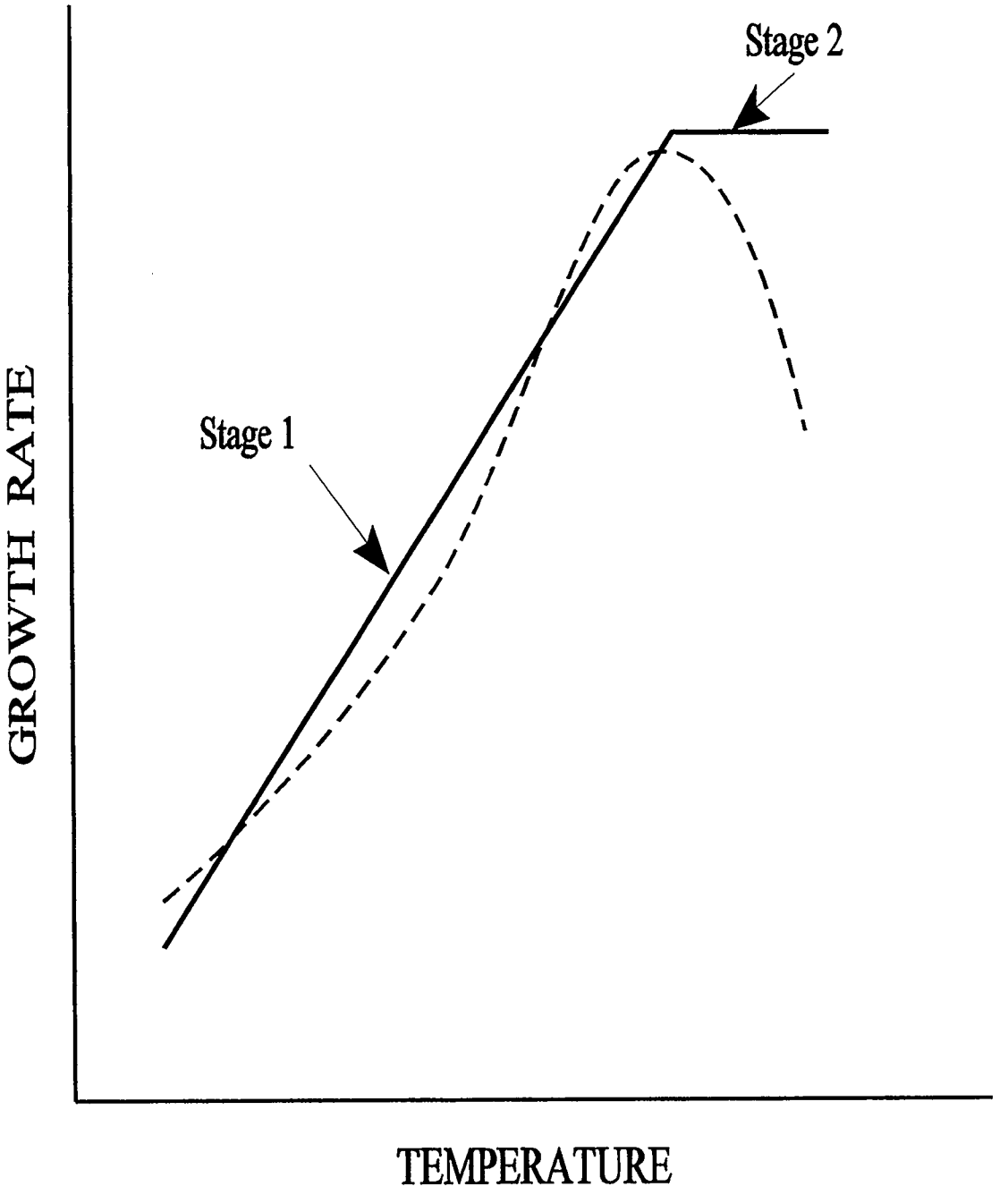

Figure 9: A two stage growth model where in the first stage growth rates increase linearly with increasing temperature and in the second stage the growth rate is constant. The dashed line is a generalized growth curve reflecting the effect of temperature on growth rate based on enzyme kinematics.

stage reflects the assumption that very short time periods when river temperatures are above the fixed-temperature thermal maximum would not have a negative effect on larval growth rates.

To incorporate the effect of larval size on the growth rate, an equation of the form

$$Gr = a + bTemp + cSize$$

was used, where *c* is the size constant and *Size* is the head capsule width (mm). Fixed-temperature mean specific growth rates and river temperature data were used in the 2-stage growth function to predict the time required to complete larval growth in the river. A simulation was started in each month, April through November, with first instar larvae (0.42 mm head capsule width) and was run until a head capsule width of 4.00 mm was reached. In the laboratory treatments, larvae with head capsule widths >4.00 mm did not moult. For the two years of river temperature data there was a warmer year and a cooler year (Figure 6). The simulation was run with all warm years, all cool years, and alternating warm/cool years, starting first with a warm year then with a cool year, until growth was complete. The results were then compared with the time required to complete larval growth predicted from the analysis of the field data.

CHAPTER 3

RESULTS

3.1 Treatment temperatures

Because temperature in each fixed-temperature treatment varied from day to day, and because individual eggs and larvae spent different periods of time in a fixed-temperature treatment, each egg or larva experienced a slightly different temperature regime. Table 1 shows the ranges of mean temperatures at which individuals were reared and the largest standard error associated with these means.

3.2 EGGS

3.2.1 Fixed-temperature experiments

The percent egg hatch and the number of days required for egg hatch at seven fixed-temperatures are shown in Table 2. Eggs at 5.0°C and 7.5°C were still hatching at the end of the experiment and so the number of days required for specific percentages of viable egg hatch could not be calculated. Within the range of 10 to 20°C, highest hatching success and fewest days required for egg hatch occurred at 15.0°C - 17.5°C.

Because eggs were still hatching at temperatures of 5.0°C and 7.5°C, the

Table 1: Ranges of mean fixed-temperatures (℃) at which individuals were reared and the maximum standard error associated with these means.

Treatment	1	2	3	4	5	6	7	8	9	10
Nominal temperature (℃)	1.0	5.0	7.5	7.5	10.0	12.5	15.0	17.5	20.0	22.5
Mean temperatures (℃)	0.81 to 1.10	4.77 to 5.13	7.47 to 7.51 16L:8D	7.50 to 7.71 8L:16D	9.89 to 10.03	12.38 to 12.63	14.88 to 15.09	17.41 to 17.56	19.69 to 20.11	22.50 to 22.81
±SE	0.500	0.727	0.270	0.593	0.316	0.742	0.492	0.399	0.514	0.396

Table 2: Hatching success and the number of days required for specific percentages of egg hatch at fixed nominal temperatures of 5.0°C to 20.0°C at 2.5°C intervals. There were 350 eggs in each treatment with the exception of 7.5°C which had 150 eggs.

Nominal temperature (°C)	Overall % hatch	Number of days to			
		1st hatch	12.5% hatch	25% hatch	50% hatch
5.0	0.9*	757	----	----	----
7.5	6.7*	526	----	----	----
10.0	42.9	229	234	237	262
12.5	40.0	181	184	192	206
15.0	50.0	149	168	173	182
17.5	72.9	152	168	176	198
20.0	25.1	168	187	202	233

* at the end of the experiment, eggs were still hatching at these temperatures

temperature representing developmental zero (i.e. the low temperature threshold for development) was derived from the data for first hatch. A linear equation was fitted to the increasing portion of the curve of mean rate of development (1/incubation period in days) against temperature (°C), i.e. from 5.0°C to 15.0°C:

$$RD = -0.0018 + 0.000576(TEMP)$$

where *RD* is the rate of egg development (1/days), and *TEMP* is the treatment temperature (°C) (r^2=0.9699). From this equation the developmental zero was estimated to be 3.125°C (Figure 10).

The number of degree-days (DD) above 3.125°C required for egg hatching are shown in Table 3. As temperature increased the numbers of DD required for egg hatch also increased.

3.2.2 Field and Laboratory composite data

None of the eggs placed in the River enclosures on May 25, 1993 hatched before 17 October 1993. Between October 17 and November 21, when eggs were removed from the river, 1.4 % of these eggs hatched. As of November 21, 1993, 1565 DD above the developmental zero (3.125°C) had accumulated in the river.

For eggs that remained in the River enclosures for 41 days prior to four fixed-temperature treatments, the percent egg hatch and the number of days required for various percentages of the viable eggs to hatch are shown in Table 4. Compressor failure terminated the 22.5°C treatment on day 261 and so only first hatch data could be used.

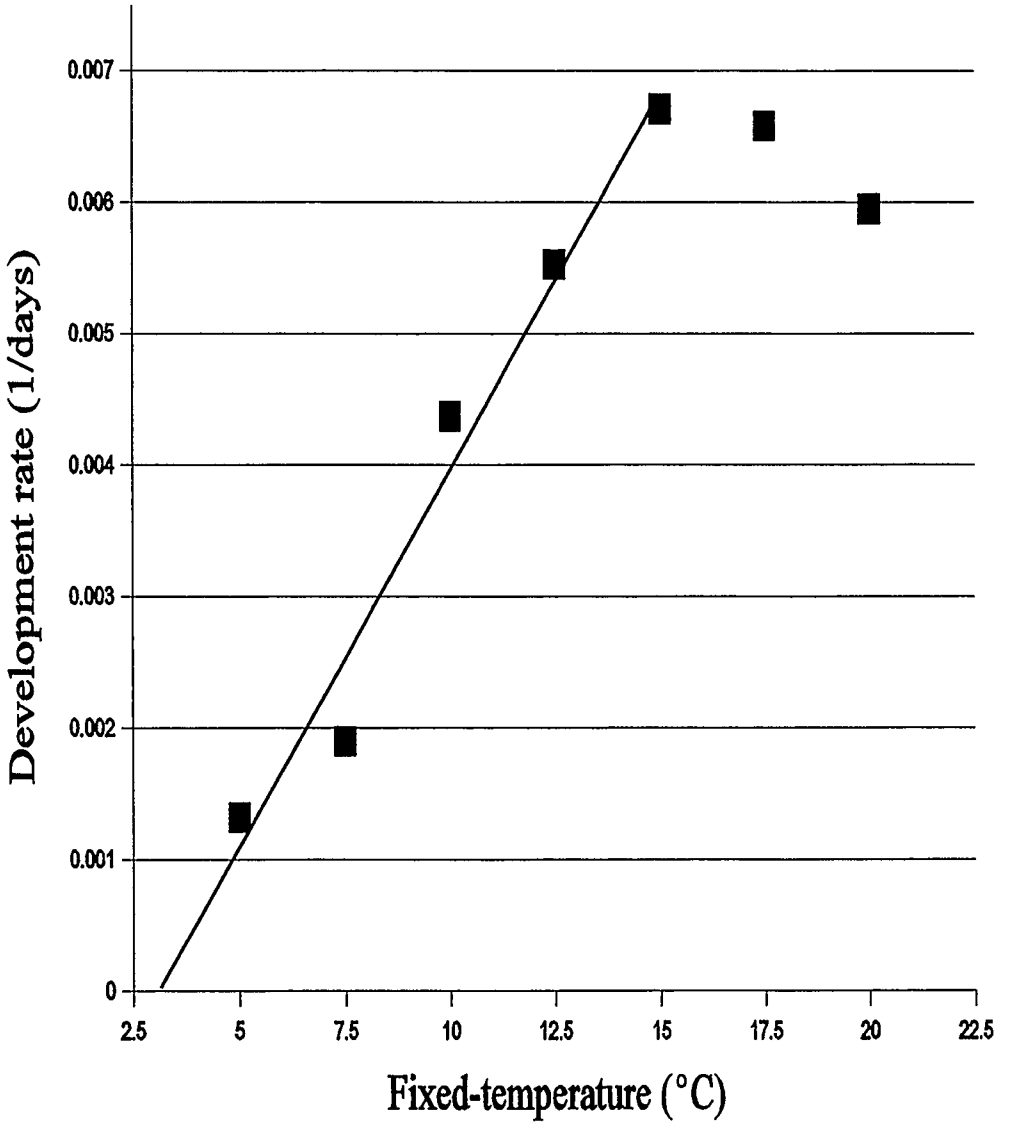

Figure 10: Developmental zero (3.125°C) for egg hatching estimated from the data for first hatch. A linear equation was fitted to the increasing portion of the curve of rate of development (1/days) against fixed-temperature (°C) (see text).

Table 3: The number of DD above 3.125°C required for specific percentages of egg hatch at fixed nominal temperatures of 5.0°C to 20.0°C at 2.5°C intervals.

Nominal temperature (°C)	Number of DD to			
	1st hatch	12.5% hatch	25% hatch	50% hatch
5.0	1419	----	----	----
7.5	2301	----	----	----
10.0	1574	1609	1629	1801
12.5	1697	1725	1800	1931
15.0	1769	1995	2054	2161
17.5	2185	2415	2530	2846
20.0	2835	3156	3408	3932

Table 4: Hatching success and total number of days required for specific percentages of egg hatch after 41 days in the river and subsequent exposure to four fixed-temperature treatments.

Nominal temperature (°C)	n	Overall % hatch	Number of days to			
			1st hatch	12.5% hatch	25% hatch	50% hatch
12.5	73	43.8	187	194	203	239
15.0	88	74.1	160	175	183	259
20.0	69	29.0	179	189	200	225
22.5	90	4.5①	200	----	----	----

① compressor failure terminated this treatment at day 261

Highest percent hatch occurred at 15.0°C, but there was no consistent difference in the minimum number of days for egg hatch between temperature treatments.

The number of DD required for various hatching percentages for eggs that remained in the River enclosures for 41 days prior to the fixed-temperature treatments are shown in Table 5. The fewest number of DD to egg hatch occurred at 12.5°C.

For eggs which were removed from the River after 83 days, the effects of a "cold" temperature treatment versus a "warm" temperature treatment on the percent hatch and the total number of days for egg hatch, are shown in Table 6. Hatching success was greater for the "cold" treatment but the "warm" treatment shows fewer days to hatch.

The effect of a cold treatment on the numbers of DD required for egg hatch is shown in Table 7. The cold treatment led to more DD for egg hatch.

For eggs that remained in the River enclosures for 180 days prior to four fixed-temperature treatments, the hatching success and the number of days for various percentages of eggs to hatch are shown in Table 8. Hatching success was greatest at 15.0°C. The fewest number of days for egg hatch, occurred at 20.0°C. However, there was a relatively small difference between 15.0 and 20.0°C. Similarly, there was little difference in physiological time spent in the various treatments (Table 9).

Table 5: Total number of DD above 3.125°C required for specific percentages of egg hatch after 432 DD in the river and subsequent exposure to four fixed-temperature treatments.

Nominal temperature (°C)	Number of DD to			
	1st hatch	12.5% hatch	25% hatch	50% hatch
12.5	1801	1866	1951	2288
15.0	1845	2023	2118	3020
20.0	2761	2930	3115	3537
22.5	3513	----	----	----

Table 6: Hatching success and total number of days required for specific percentages of egg hatch in "warm" and "cold" temperature treatments after spending 83 days within the River. Eggs in the warm treatment were maintained at 12.5°C after removal from the River. Eggs in the cold treatment were cooled to 1.0°C, where they remained for 34 days, then returned and maintained at 12.5°C.

Treatment	n	Overall % hatch	Number of days to			
			1st hatch	12.5% hatch	25% hatch	50% hatch
Warm	82	50.0	202	218	242	259
Cold	101	83.2	299	321	327	344

Table 7: The total number of DD above 3.125°C required for specific percentages of egg hatch in "warm" and "cold" treatments. Eggs were removed from the River egg enclosures on August 15, 1993 after accumulating 875 DD. Eggs in the warm treatment were maintained at 12.5°C after removal from the River. Eggs in the cold treatment were cooled to 1.0°C, where they remained for 34 days, then returned and maintained at 12.5°C.

Treatment	Number of DD to			
	1st hatch	12.5% hatch	25% hatch	50% hatch
Warm	1991	2141	2356	2525
Cold	2485	2691	2747	2906

Table 8: Hatching success and total number of days required for specific percentages of egg hatch after 180 days in the river and subsequent exposure to four fixed-temperature treatments.

Nominal temperature (°C)	n	Overall % hatch	Number of days to			
			1st hatch	12.5% hatch	25% hatch	50% hatch
12.5	227	45.8	203	205	207	218
15.0	171	69.0	189	192	196	199
20.0	189	56.9	188	191	194	199
22.5	200	12.0①	194	----	----	----

① compressor failure terminated this treatment at day 261

Table 9: Total number of DD required for specific percentages of egg hatch after 1565 DD in the river and subsequent exposure to four fixed-temperature treatments.

Nominal temperature (°C)	Number of DD to			
	1st hatch	12.5% hatch	25% hatch	50% hatch
12.5	1781	1800	1819	1922
15.0	1672	1708	1755	1790
20.0	1700	1751	1801	1886
22.5	1836	----	----	----

3.3 LARVAE

3.3.1 Size structure in the field population

Size structure in the field population from April 1992 to November 1993 is shown in Figures 11 and 12. I was unable to identify first instar larvae (head capsule width ≈ 0.40 mm) until November 1992. When I obtained them from eggs hatched in the laboratory it was clear that first instar larvae differ markedly from later instars; they are unpigmented and have fewer antennal and cercal segments (Figure 13). Now it became apparent that there was continual recruitment of first instar larvae over most of the ice free period, with a pulse of recruitment from the end of April to the end of July, 1993 (Table 10). Although first instar larvae were not identified in the 1992 samples, the sample from July still indicates a recent pulse of recruitment (Figure 11).

The abrupt increase in wing pad length at about 4-5 mm head capsule width (Figure 14), and the presence of a highly developed epiproct in males and very distinct genitalia scarring in females (Figure 7) indicate final instar larvae. Adult emergence started on May 6, 1992 and May 25, 1993 and lasted for approximately 7 to 10 days. The disappearance of final instar larvae in June emphasizes adult emergence from the population (Figure 12). Final instar larvae reappeared in August (Figure 12).

The single yearly adult emergence and wide range of larval sizes after emergence, eliminate the possibility of a multi- or a uni-voltine life cycle. If larvae take two years to develop then all individuals present after emergence would be beginning their second year

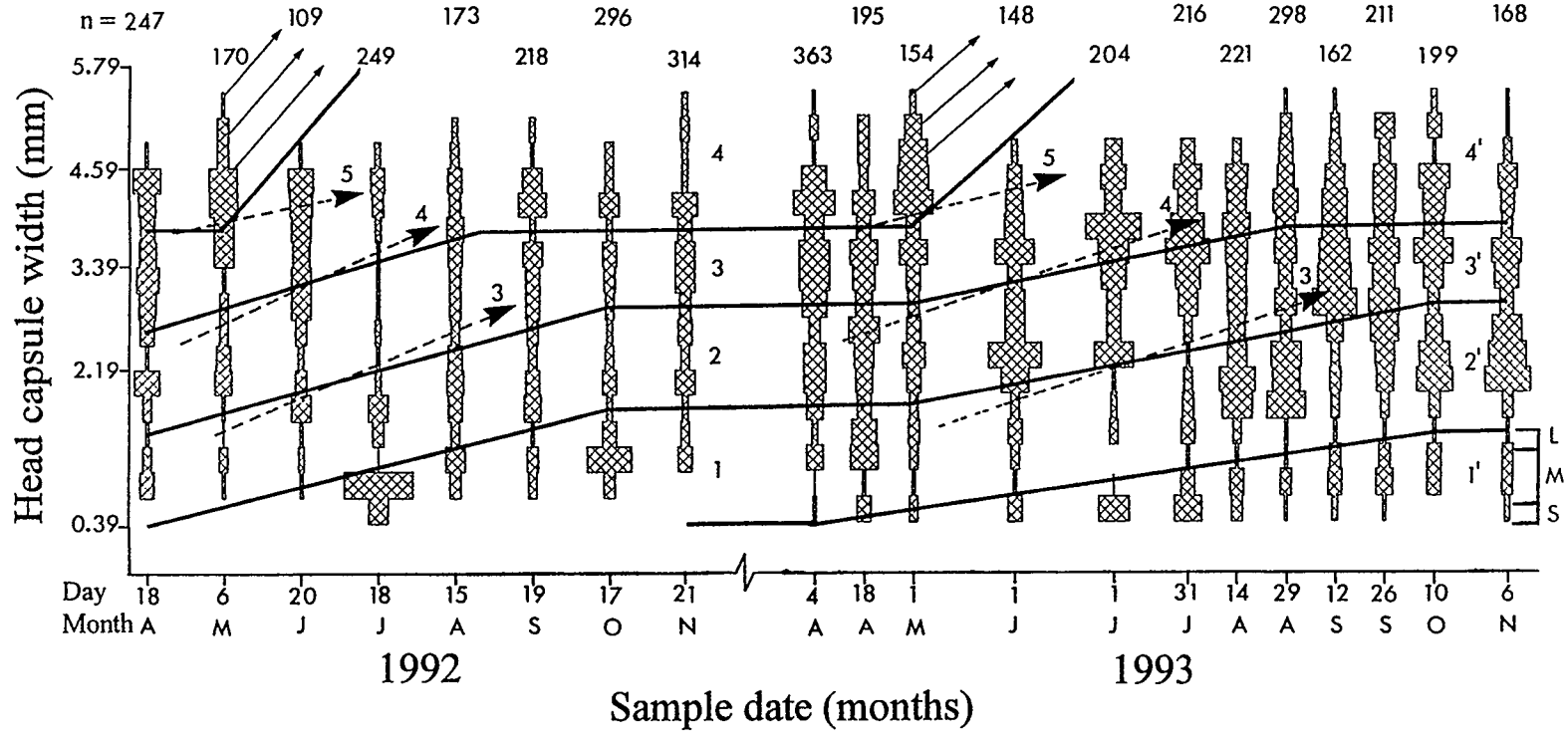

Figure 11: Percent frequency distributions of head capsule widths in 0.30 mm intervals of *P. californica* larvae collected from the Crowsnest River from April 1992 to November 1993. Solid lines represent estimated age classes (cohort) boundaries. Dashed arrows 3, 4 and 5 represent a cohort split of age classes 2, 3 and 4. Larvae of a split cohort will complete their development in either 3 (dashed arrows 3 and 4) or 5 (dashed arrow 5) years. Sample size (*n*) is indicated above the histogram. Size categories in age class 1' at the end of 1993 represent larvae hatched early in the year (*L*), in the middle of the year (*M*) and late in the year (*S*). Solid arrows indicate adult emergence.

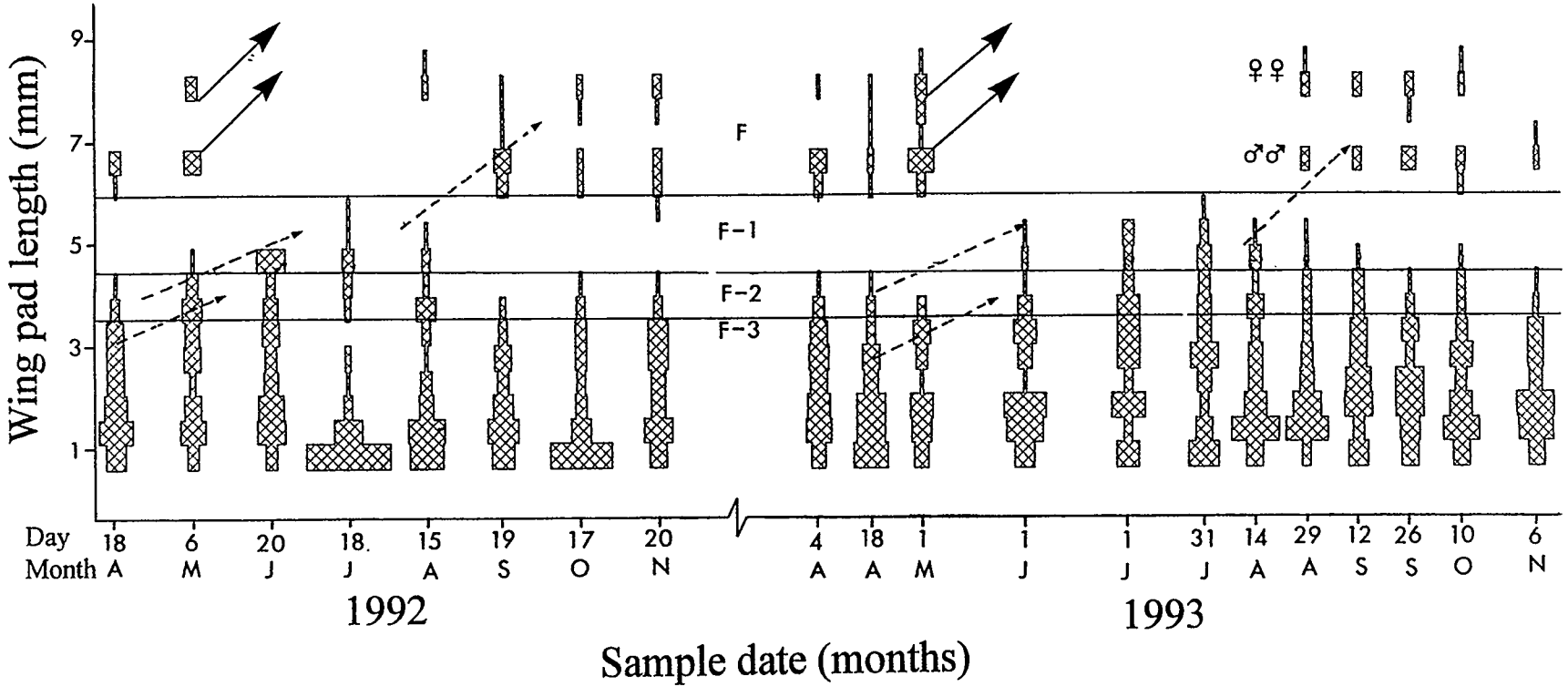

Figure 12: Percent frequency distribution of wing pad lengths in 0.50 mm intervals of *P. californica* larvae collected from the Crowsnest River from April 1992 to November 1993. Larvae with wing pad lengths > 5.75 mm are final instar (F) larvae. Solid lines represent boundaries between four larval instars. Dashed arrows represent larvae moulting into the next instar. Solid arrows represent adult emergence. (F = final instar larvae; F-1= the instar prior to F; F-2 = two instars prior to F; F-3 = three instars prior to F)

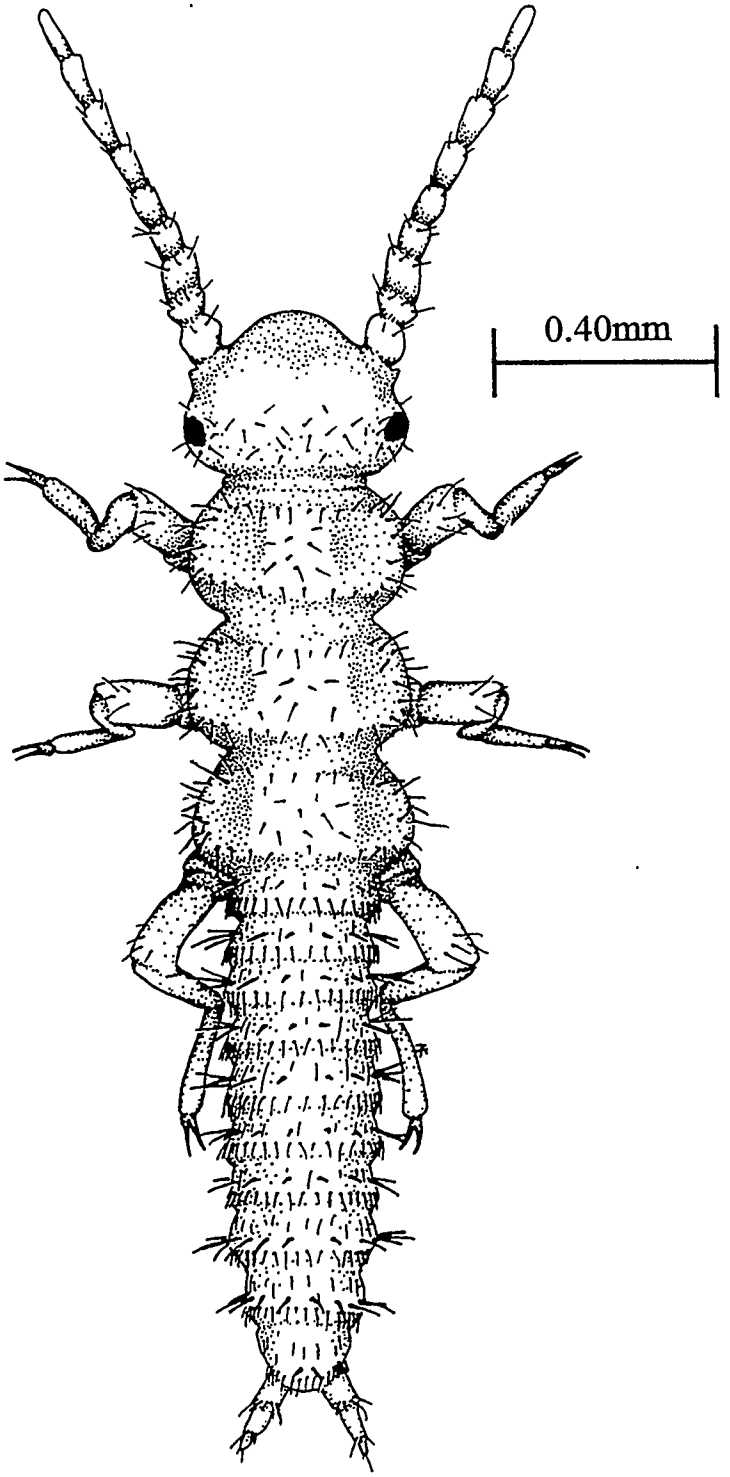

Figure 13: *P. californica* first instar larva.

Table 10: Percentage and number of 1st instar larvae from samples collected in 1993, with pre-emergence samples adjusted (Adj %) by removal of final instar larvae so that they were more comparable with post-emergence samples.

Sample Date	Apr 4	Apr 18	May 1	Jun 1	Jul 1	Jul 31	Aug 14	Aug 29	Sep 12	Sep 26	Oct 10	Nov 6
% 1st instar	0.8	3.6	3.3	4.7	4.9	4.2	1.8	1.0	2.5	0.9	0.0	1.8
(Adj %)	(1.0)	(4.2)	(5.4)									
Number	3	7	5	7	10	9	4	3	4	2	0	3

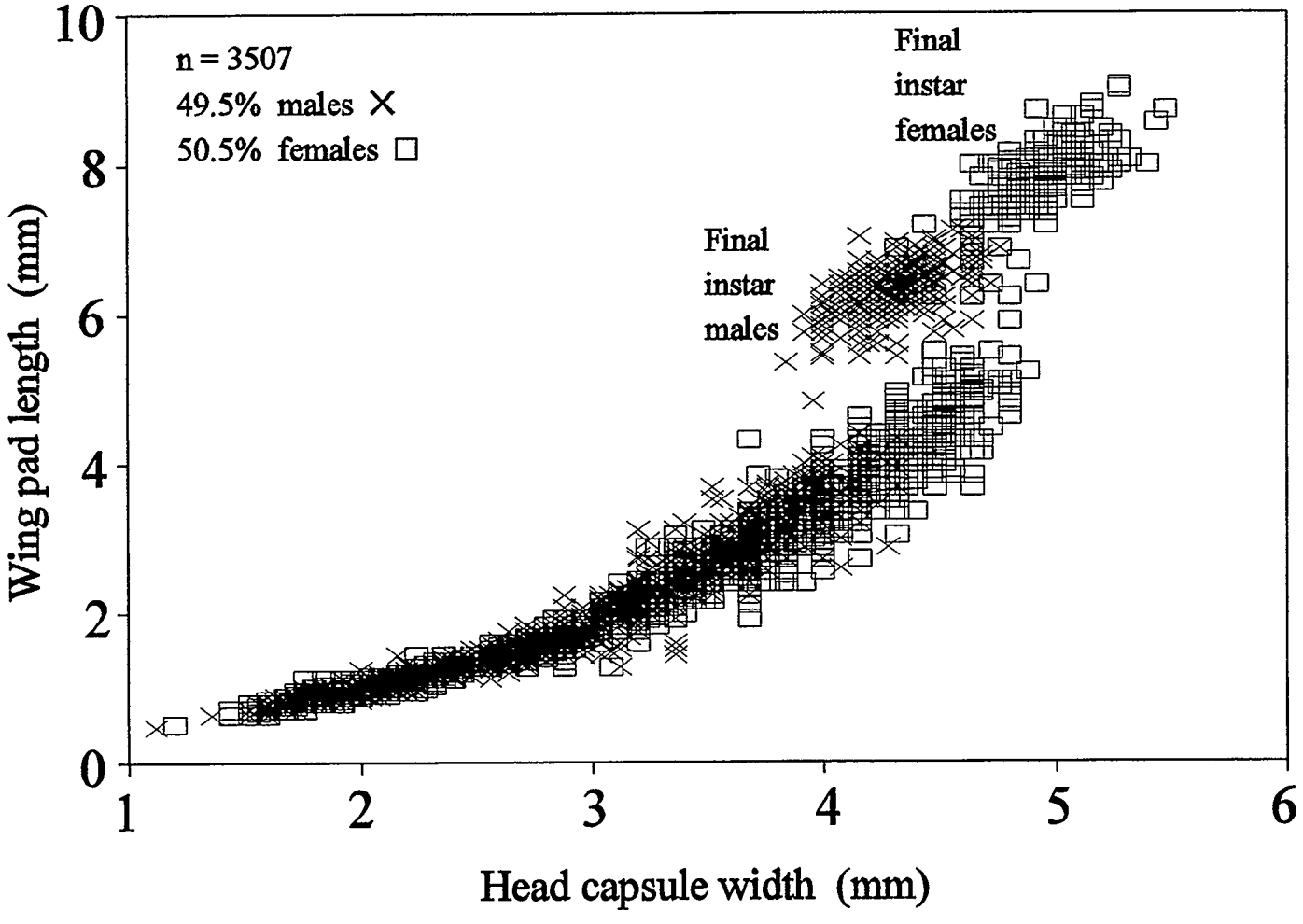

Figure 14: Wing pad length versus head capsule width for male and female *P. californica* larvae with head capsule widths greater than approximately 1.5 mm, collected from the Crowsnest River from April 1992 to November 1993.

(Figure 15). This size range (0.69 to 4.89 mm) suggests much variation in individual growth rates but even with continual recruitment this size range is unlikely given laboratory larval growth rate data. If larvae take three years to develop there should be two distinct modes in June (Figure 16): individuals entering their second year (Y2) and individuals entering their third year (Y3). However, there appear to be three discrete modes in June 1992 before recruitment occurred (or had been recognized) suggesting a four-year larval life (Figure 17). Although there are only two years of sample data, the pulse of recruitment in July 1992 can be traced through the samples (Figure 11) for 16 months, when larvae are less than half grown (cohort 2' in November 1993). If this size group is equivalent in age to the same size group in April 1992, it would emerge in May 1996 almost four years after hatching. Furthermore, four size classes are distinct in many of the samples, although there is blurring of size classes (especially with head capsule widths greater than approximately 2.5 mm) during the warmest time of the year.

Continual recruitment, evident in this *P. californica* population, leads to three possible developmental pathways. Because of continual recruitment, larval age classes are relatively wide modes moving through time (Figure 11). Larvae that are recruited in April and May will be larger at the start of the following year than recruits from October and November. Thus, larvae can be divided into three size categories at the end of the first year: small (S1) [hatched late in the year], medium (M1) [hatched in the middle of the year], and large (L1) [hatched early in the year]. Because recruitment is not distributed equally throughout the year, there are fewer S1 and L1 larvae than M1 larvae. Early in their second or third year, the extended growth advantage of L1 larvae may enable them

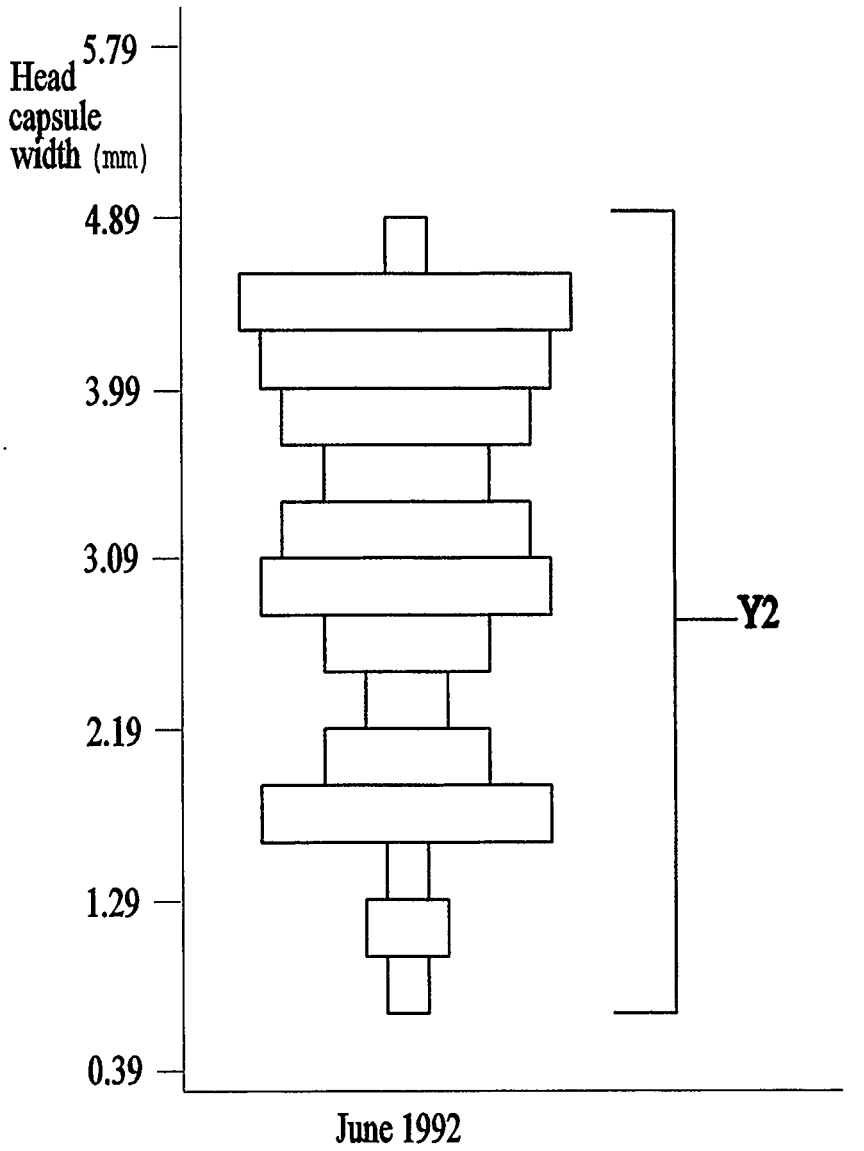

Figure 15: Percent frequency distribution of larval head capsule widths for June 1992. If larvae take two years to develop then all larvae would be entering their second year (Y2).

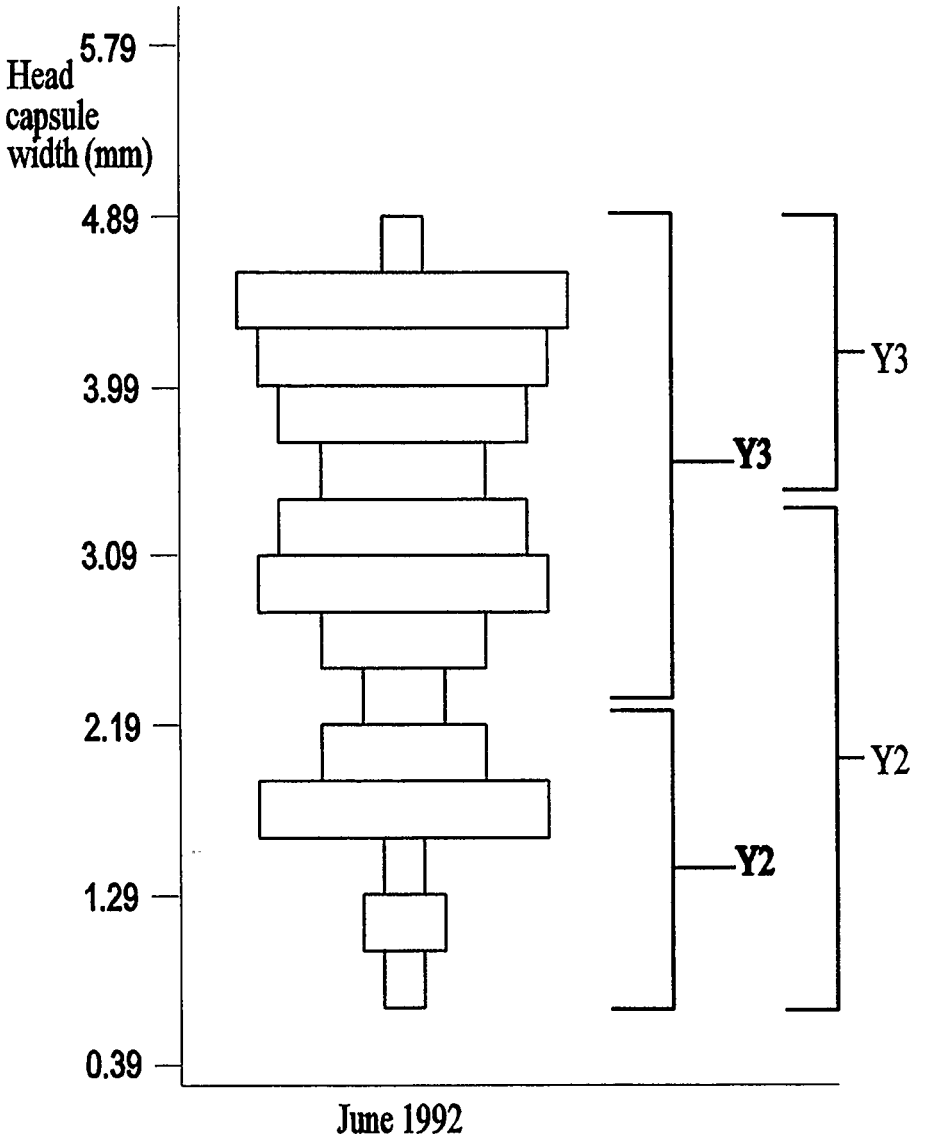

Figure 16: Percent frequency distribution of larval head capsule widths for June 1992. Y2 and Y3 represent modes of larvae entering the second and third year of their life, respectively, if larvae take three years to complete development.

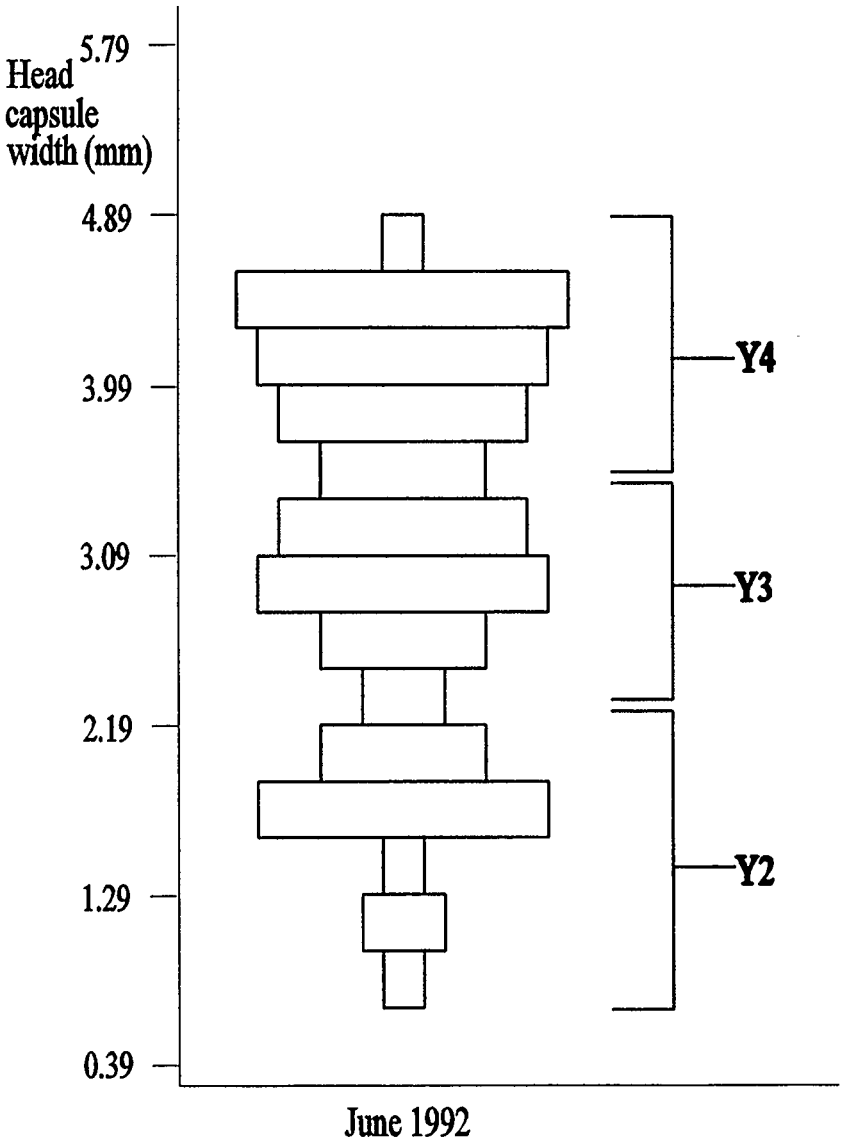

Figure 17: Percent frequency distribution of larval head capsule widths for June 1992. Y2, Y3 and Y4 represent modes of larvae entering the second, third and fourth year of their life, respectively, if larvae take four years to complete development.

to achieve the size of S3 or S4 larvae respectively (dashed arrows "3" and "4"). These larvae would then take three years to develop instead of four, and represent a cohort split. The M1 larvae would continue to a four year life cycle. Late in the fourth year, S1 larvae that did not achieve emergence size (dashed arrow "5") would have their life extended for another year, representing another cohort split. Therefore, hatching very early in the year may lead to a three year larval life, while hatching very late may delay emergence by two years (a five year larval life). However, the majority of larvae appear to take four years to complete development.

3.3.2 MULTIFAN data analysis

MULTIFAN can analyse data with one of two search types - Unstructured and Survey Sample. One difference between the search types is that the Survey Sample analysis assumes the samples are produced by a biased sample of age classes (age dependent selectivity), whereas the Unstructured Sample assumes all age classes have equal opportunity to be sampled. In order to test if one analysis is more powerful than the other, sample data, identical in every respect, were run through both types of analysis. Both analyses agreed on the number of age classes that gave the best fit. Because the 1992 samples were biased such that the smallest individuals were not identified and the Survey Sample required fewer parameters for the same fit (23 compared to 216), the Survey Sample was used for every subsequent analysis.

Starting with minimum user input (the most objective analysis) models were

systematically fitted incorporating all possible combinations of the structural hypotheses that apply to the known biology of *P. californica*. Starting with the mandatory condition that a pulse of recruitment is defined (I used July 1992, which according to Fournier [pers. comm.] is a large enough pulse to ensure reliable results), user input (subjectivity) was increased in the following order;

1) include bounds on the width of the mode

2) As in 1) and include the proportion of individuals in that mode

3) As in 2) and make final instar larvae in May 1992 a well defined mode

4) As in 3) and include bounds on the width of that mode

5) As in 4) and include the proportion of individuals in that mode

6) As in 5) and make the largest individuals in June 1992 a well defined mode

7) As in 6) and include bounds on the width and proportion of individuals in that mode

Of the parameters that can be tested, yearly mortality for the final age class, seasonal growth and first instar size bias are most applicable because of adult emergence, reduced winter growth and the absence of the smallest larvae in 1992. Therefore, these were the first parameters added to the analysis. After the addition of each additional parameter, the rate at which the von Bertalanffy growth curve approaches the asymptote (K) was allowed to vary. After all parameters had been added the best fit was estimated.

As user input increased, the estimated number of age classes decreased from 14 to eight and then increased (Table 11, Figures 18 and 19). The first user input reduced the

Table 11: The best MULTIFAN fit to the data as user input is increased. Age classes - the number of modes in the data; K - the rate at which the von Bertalanffy growth curve approaches the asymptote; L_∞ - the head capsule width at the asymptote; 1st width - the head capsule width of the smallest age class; Last width - the head capsule width of the last age class; Age of 1st age class - the estimated age of the individuals in the first age class.

Increasing user input	Age classes (years)	K (1/year)	L_∞ (mm)	1st width (mm)	Last width (mm)	Age of smallest age class (years)
1	14	0.103	5.98	0.79	4.62	1.37
2	10	0.224	4.90	0.76	4.39	0.74
3	10	0.231	4.92	0.76	4.39	0.72
4	10	0.231	4.91	0.76	4.39	0.72
5	10	0.231	4.91	0.76	4.39	0.72
6	8	0.391	4.56	0.82	4.30	0.50
7	9	0.269	4.84	0.69	4.36	0.58
8	10	0.243	4.86	0.74	4.40	0.68

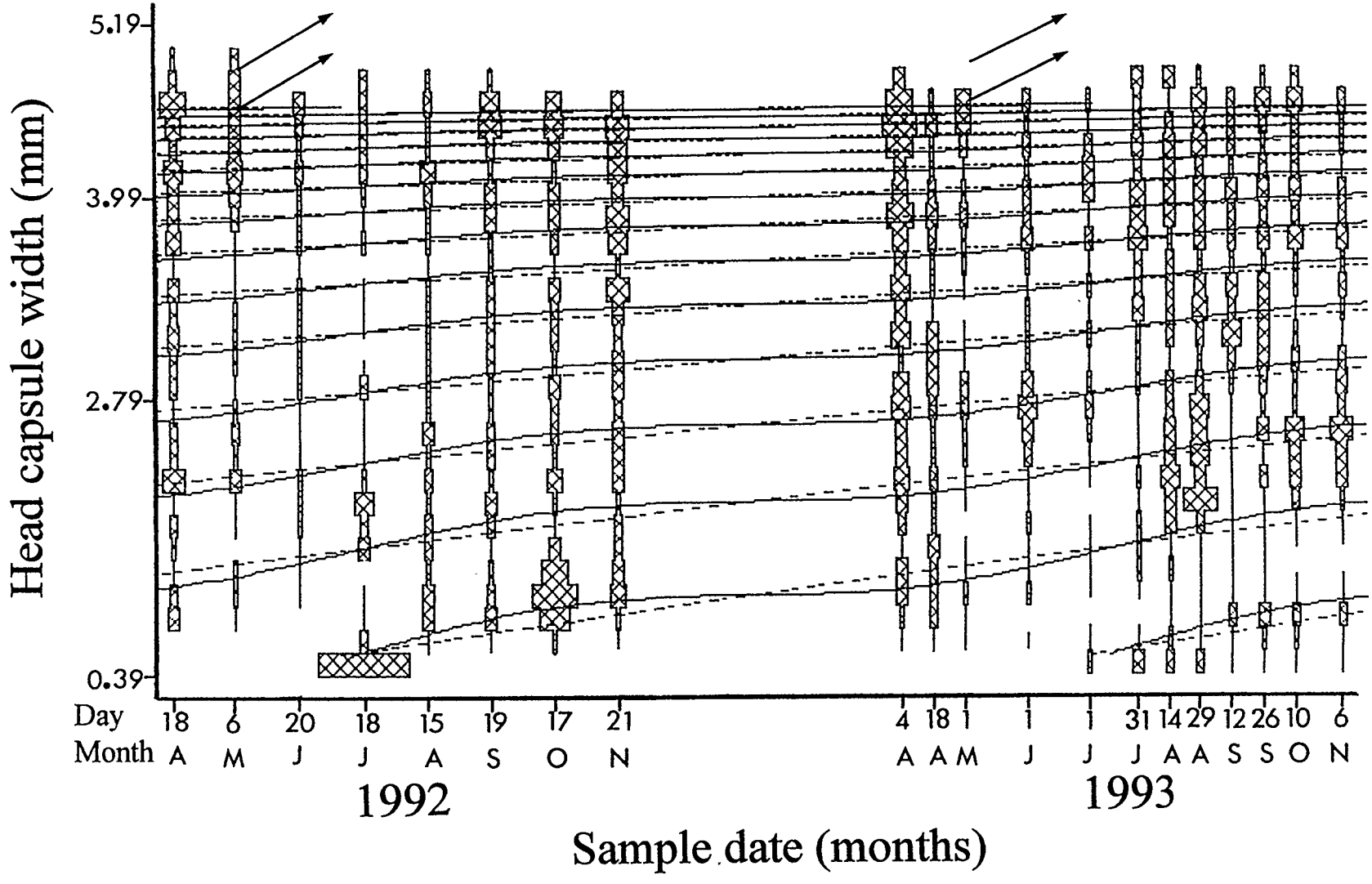

Figure 18: MULTIFAN best fit estimate of fourteen age classes based on minimum user input (most objective).

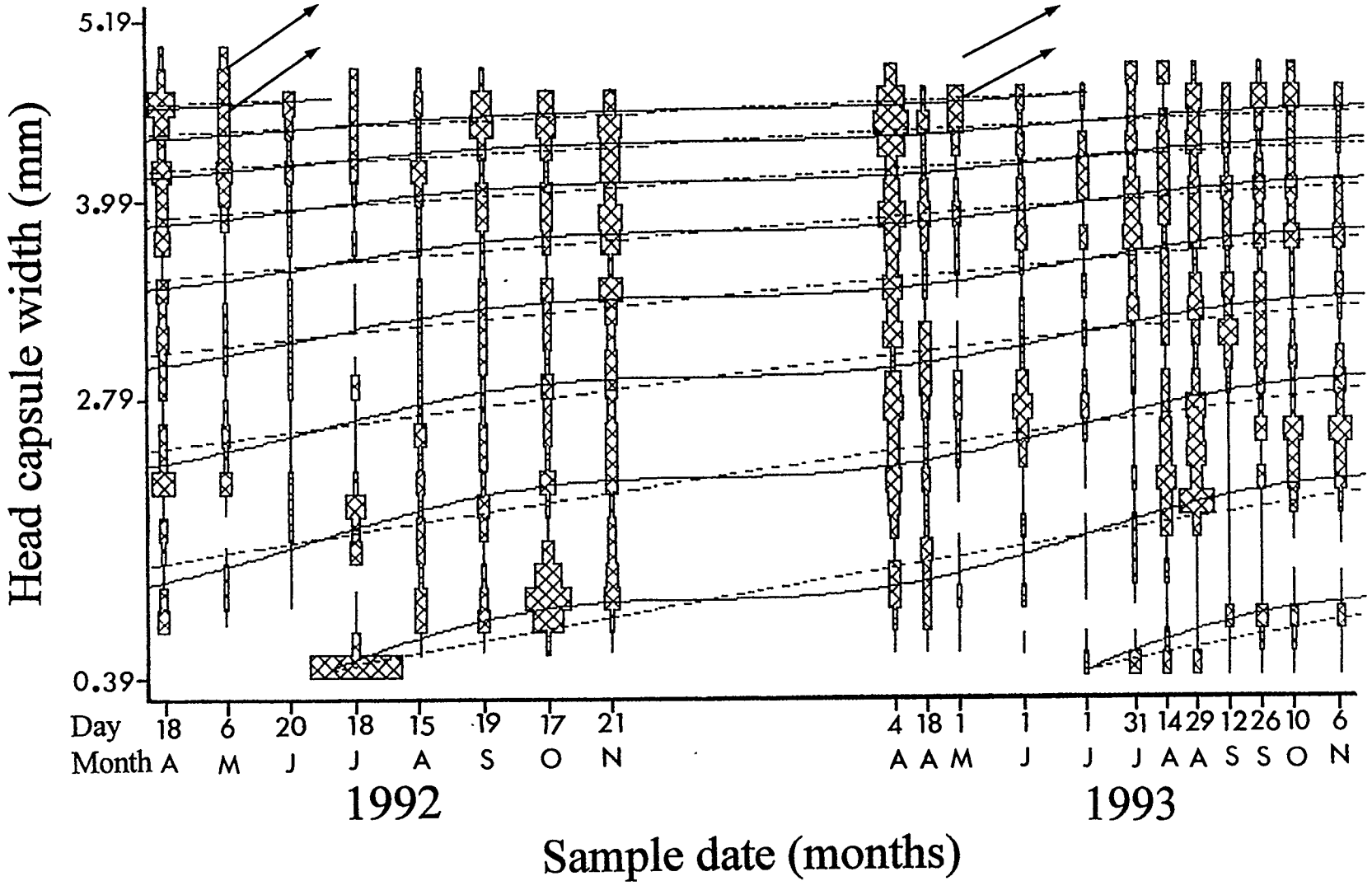

Figure 19: MULTIFAN best fit estimate of eight age classes based on complete definition of two recognizable modes in the data.

estimated number of age classes by four years. Increasing user input had little effect on the results until two modes were completely defined. A further increase in user input added estimated age classes.

3.3.3 Laboratory rearing

Growth rate changed with size at fixed-temperatures in a non-linear fashion (Figure 20). Therefore, the natural logarithm of size (lnsize) was used in the analyses. The residual plots for the effects of lnsize, temperature, and individuals nested within temperatures, on growth rate showed that the data were normally distributed. Therefore, no further data transformation was necessary. The initial test of the hypothesis that mean specific growth rates are equal for all treatments, tested with the Mixed Model Analysis of Co-variance (SAS), indicated no significant lnsize by temperature interaction (Error DF=1; Denominator DF=17; F value=2.891; P>F=0.1073; α=0.05). Therefore, the interaction was removed and the ANCOVA run again. For this test there was a significant lnsize effect and a significant temperature (temp) effect but there was no significant difference between growth rates of individuals which yielded one data point and individuals from which more than one data point was obtained (Table 12).

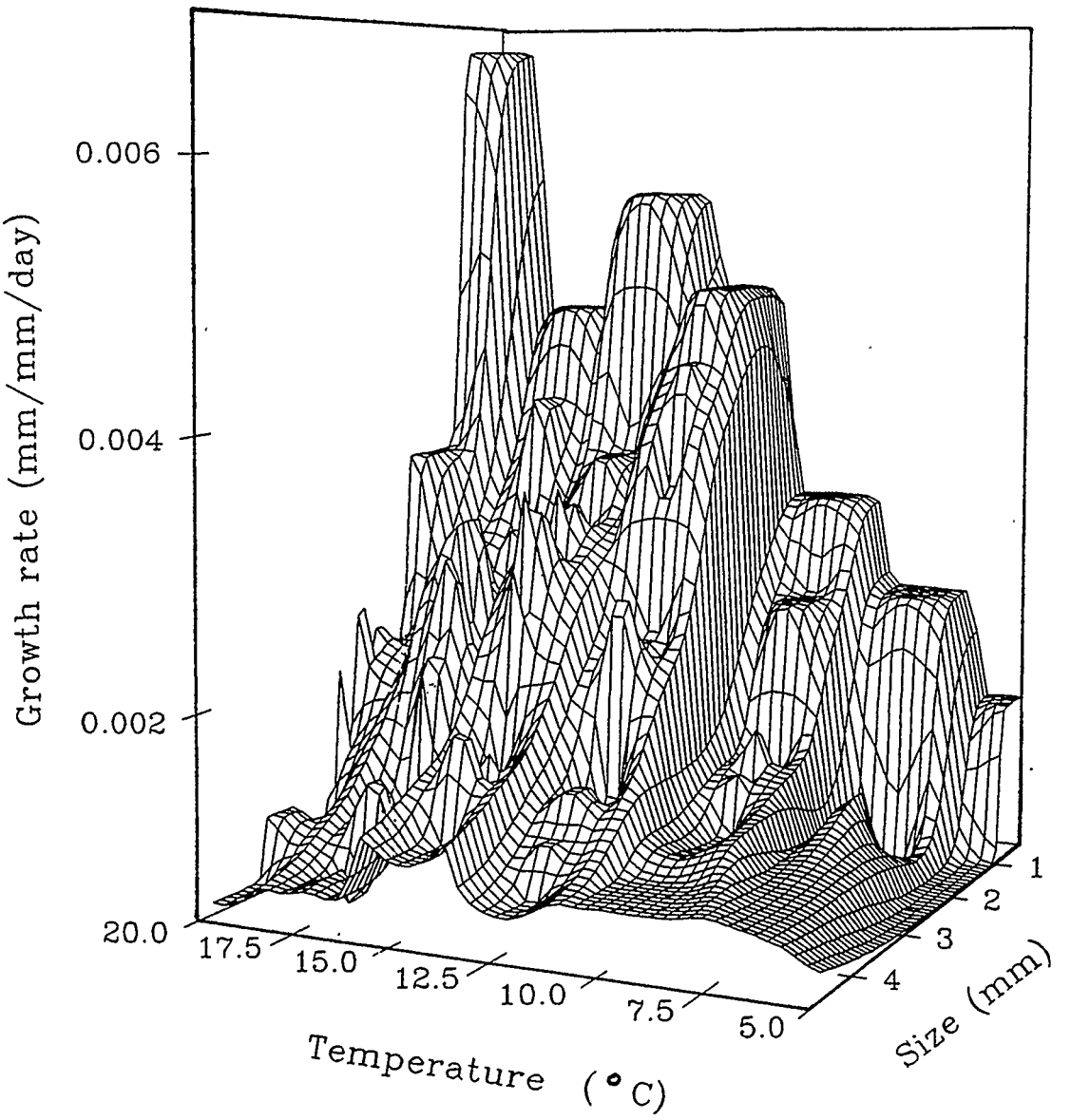

Figure 20: Non-linear change in *P. californica* larval growth rates with size at fixed-temperatures and a decrease in growth rates with temperature above 15.0°C with the exception of first instar larvae at 20°C.

Table 12: ANCOVA of the effects of temperature (5.0°C to 20.0°C at 2.5°C intervals), lnsize (0.42mm to 4.68mm), and individuals nested within temperature, with lnsize as the co-variate, on P. *californica* larval growth rate.

Source of variation	DF	MS	F value	P > F
temp	6	3.456×10^{-6}	5.932	0.0001*
ind	62	6.047×10^{-7}	1.201	0.3436
lnsize	1	4.493×10^{-6}	8.925	0.0079*

* a significant effect at α=0.05

3.3.4 River temperature growth simulation model

Except for first instar larvae at 20.0°C, larval growth rates (mm/mm/day) decreased above 15.0°C (Figure 20). Therefore, 15°C was used as the temperature separating the two stages of the growth model (see METHODS). The first stage of the 2-stage model was defined by the linear equation describing the effect of fixed-temperatures (5.0°C, 7.5°C, 10.0°C, 12.5°C and 15.0°C) and lnsize on growth rates:

$$Gr = 0.000096 + 0.00027Temp - 0.001648lnSize$$

with an r^2 of 0.6528 (SAS).

Because the linear equation for the growth rate simulation, includes a size component, developmental zero varies with size. Larvae with a head capsule width of 0.42 mm have a developmental zero of -5.65°C, and larvae with a head capsule width of 4.00 mm have a developmental zero of 8.11°C.

The effect of yearly temperature regimes on larval growth to a head capsule width of 4.00 mm (penultimate instar), with first instar larvae entering the system in April, is illustrated in Figure 21. The shortest period (5.2 years) occurred with all warm years (1992 temperatures) and the longest period (6.4 years) with all cool years (1993 temperatures). The final instar will increase the 5.2 years to a minimum larval life of six years and the 6.4 years to a minimum larval life of seven years. Starting a simulation of alternating river temperature sequences with either a warm or cool year resulted in only a two month difference in the time required to attain a larval head capsule width of 4.00mm.

The effect of hatch date on larval growth, with first instar larvae entering the system at the beginning of each ice free month in alternating cool/warm years, is illustrated

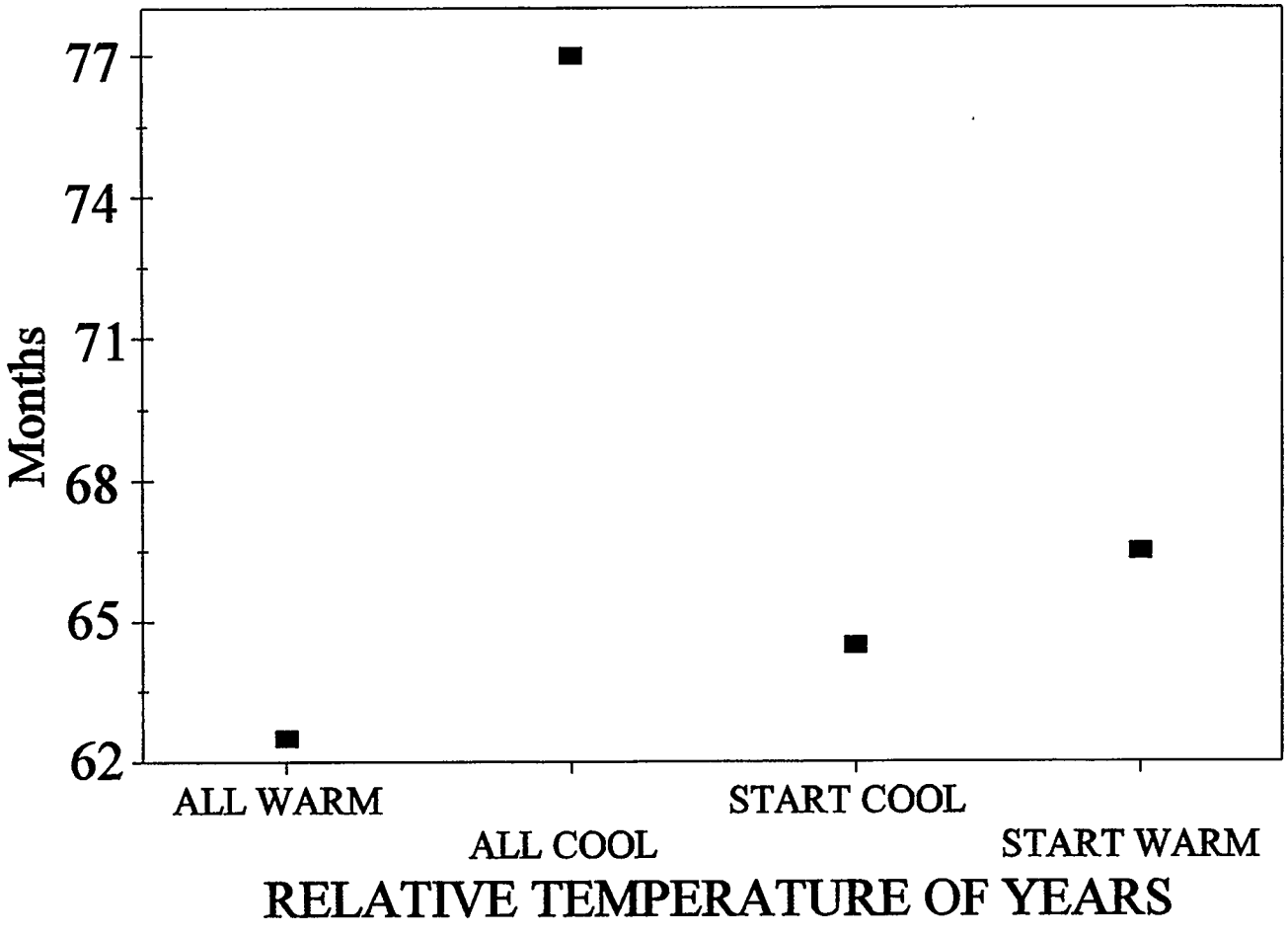

Figure 21: Growth simulation model estimate of the number of months required for larvae to attain a head capsule width of 4.00 mm. with all warm (1992 temperatures) years, all cool (1993 temperatures) years and with alternating year sequences starting with a cool and a warm year.

in Figure 22. There is a seven month difference in the time required to complete growth between larvae that enter the system in April - May and those that enter in June - November, because the second hatch-group require an extra winter when no growth occurs. In the simulation, larvae recruited in April and May reach the penultimate instar in August and September five years later; given river temperatures at this time these larvae would not emerge until May seven years after egg hatch. Larvae recruited in June through November reach the penultimate instar in May through October six years later and would not emerge until May eight years later.

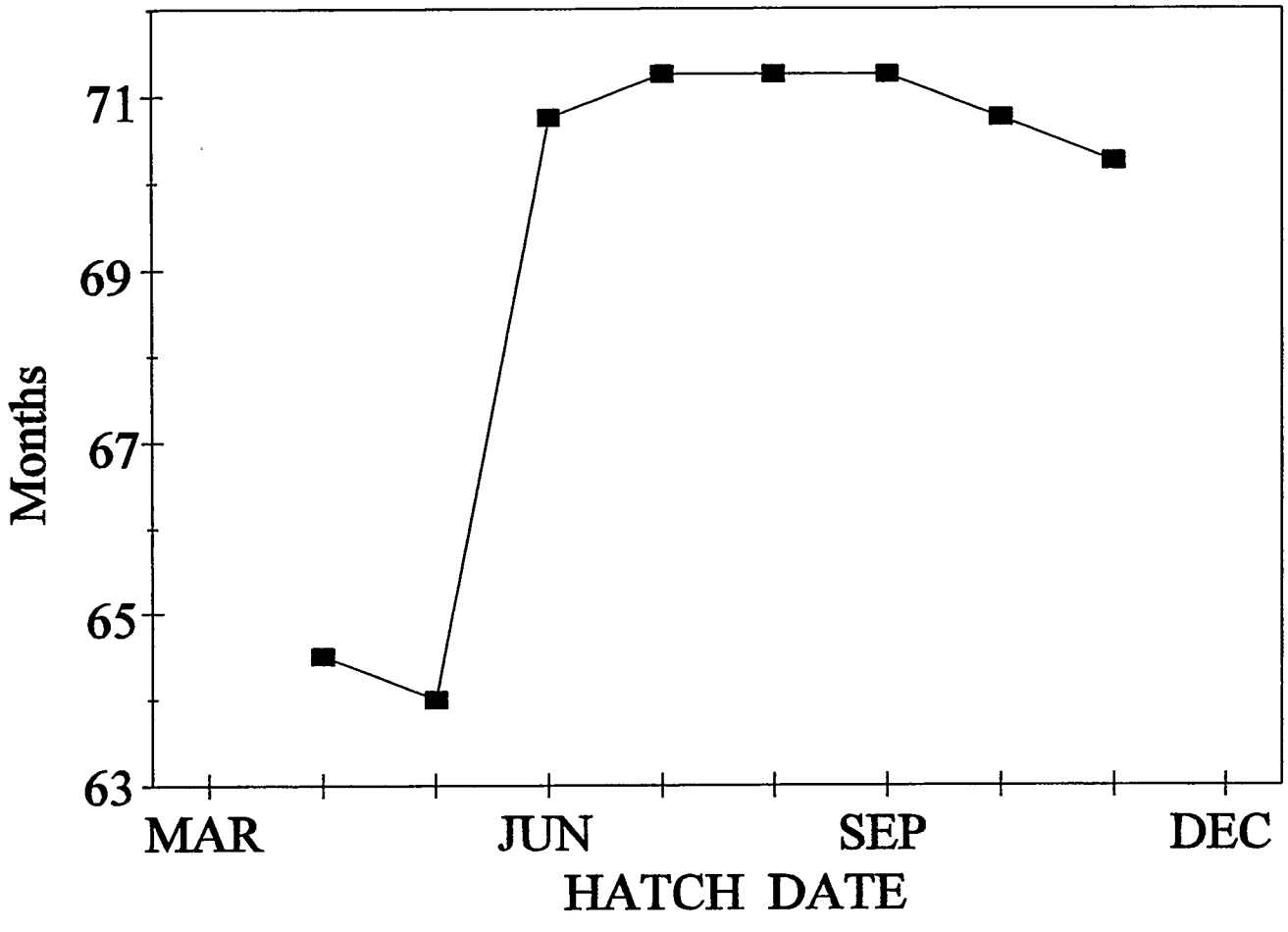

Figure 22: Growth simulation model estimate of the number of months required for larvae to attain a head capsule width of 4.00 mm. First instar larvae enter the system at the beginning of each ice free month in alternating cool (1993 temperatures)/warm (1992 temperatures) years.

CHAPTER 4

DISCUSSION

Insects in cool temperate rivers have life histories that are constrained by seasonal patterns of temperature and diet. Avoidance of unfavourable conditions by sensitive stages and synchrony between reproduction and favourable conditions requires that development be regulated. This is especially so if a large size must be attained before metamorphosis so that the life cycle takes several years to complete. But there are different routes to synchronization, and insect life history strategies are many and varied (Tauber *et al.* 1986) even within a single population. A principal component of life history regulation in insects is diapause (Danks 1987).

One indication of diapause is delayed development. Eggs of many stoneflies develop in 15-25 days at 15°C in the laboratory (Mutch and Pritchard 1986), whereas *Agnetina capitata* (Moreira and Peckarsky 1994) takes 64 days, and *Amphinemura banksi* (Mutch and Pritchard 1986) and *Dinocras cephalotes* (Lillehammer 1987) take >100 days. *P. californica* falls into the last category, requiring a minimum of 182 days to hatch in the laboratory, with no observable development for approximately 80 days, at any treatment temperature.

In the field, the pulse of larval recruitment of *P. californica* between April and August follows 11 to 15 months after oviposition. Eggs in the river enclosures showed

little development until late August - early September when eye spots and rudimentary limbs could be identified. This implies a summer diapause, which begins soon after oviposition and is terminated in late summer or early fall. The prompt start of egg hatch after removal from instream enclosures in November, regardless of treatment, suggests egg development was nearly complete. However, although samples were not collected during the periods of ice cover, it is unlikely eggs hatched during winter in the river because temperatures were then too low for the non-diapause component of egg development. Indeed, few first instar larvae were collected immediately before or after ice cover. This implies that diapause was holding back egg development when river temperatures were high and low metabolism was holding back egg development when river temperatures were low (during the period of ice cover). Other stoneflies such as *Hydroperla crosbyi* (Oberndorfer and Stewart 1977), *Perlesta placida* (Snellen and Stewart 1979a), *Zealeuctra claasseni* and *Zealeuctra hitei* (Snellen and Stewart 1979b) also have eggs with summer diapause.

Snellen and Stewart (1979a) suggest that summer diapause allows survival through a period when high river temperatures may be lethal to young larvae, or when streams become intermittent or dry. Further delay of egg hatch, until after ice melt, may also be a valuable life history strategy because during periods of ice cover, reduced light and water temperature may adversely affect early instar larvae by limiting food availability. Therefore, it would be beneficial to delay hatching until conditions are favourable for growth.

Another possible indication of diapause is an increasing number of degree-days

(DD) required to complete a particular phase of development with increasing temperature. The number of DD is a measure of the thermal energy necessary to drive metabolic processes. Therefore, the temperature at which the fewest day-degrees are used should represent the temperature to which the organism's metabolism is best suited. Normally, the number of DD will decrease with increasing temperature up to a temperature at which metabolic processes are impaired. However, Mutch and Pritchard (1986) noted that in several stoneflies the number of DD required for egg development actually *increased* with increasing temperature within the favorable range. They then argued this might imply a period of egg diapause, because diapause development is completed more rapidly at lower temperatures than those that are optimal for normal development. Unfortunately, *P. californica* egg development took so long below 10°C that mean incubation times could not be obtained over the full range of favorable temperature. However, above 10°C there is a trend of increasing DD with increasing temperature, suggesting diapause. Certainly there is not a negative relationship as would be expected for non-diapause development.

As well as delayed hatch, eggs of *P. californica* showed extended hatching. In the laboratory, *P. californica* eggs hatched over periods of 126 to 322 days. In the field, eggs oviposited in May and kept in field enclosures began hatching in late October/early November, and first instar larvae were collected from the river from April through November. Thus eggs laid in May apparently hatch over at least an 11 month period, from 5 to 16 months after oviposition. Because eggs are laid over a period of no more than three weeks, continual recruitment of larvae must be the result of variation in egg development time. The placement of diapause at the beginning of the egg period can

contribute to variation in overall egg development time. In the dragonfly *Lestes sponsa*, all eggs attain an advanced stage of development in summer within about two weeks of oviposition and prior to the onset of diapause (Corbet 1956). Diapause is broken in the spring with a temperature cue, creating a synchronous egg hatch in April. Unlike *Lestes sponsa*, where eggs are in an advanced stage of development when diapause begins, *P. californica* eggs are in an early stage of development at the onset of diapause. Therefore, the time period between diapause termination and egg hatch will allow differences in individual developmental rates to be manifested. Further variation in individual development time may occur if the cues for diapause termination do not affect all individuals equally. For example, diapause was terminated in eggs of the stonefly *Perlesta placida* over a 5 to 17 month period (Snellen and Stewart 1979a). Continual recruitment may be a "bet-hedging" strategy (Cohen 1967; Stearns 1976; Butler 1984), although it is difficult to see why this should be necessary in the Crowsnest River which is a permanent and relatively stable environment. Perhaps this strategy evolved when stream conditions were less predictable.

For aquatic insects with a one year larval life an accurate estimate of life cycle length is relatively simple, because after the adults emerge there will be no larvae in the population until the next generation of eggs hatch (Figure 23). However, for insects that require more than one year to complete development larvae will remain in the population after the adults have emerged, thereby complicating the estimate of life cycle length. Unlike organisms such as fish where permanent calcareous structures such as ear otoliths, bones and scales may record an annual pattern of alternating slow and fast growth

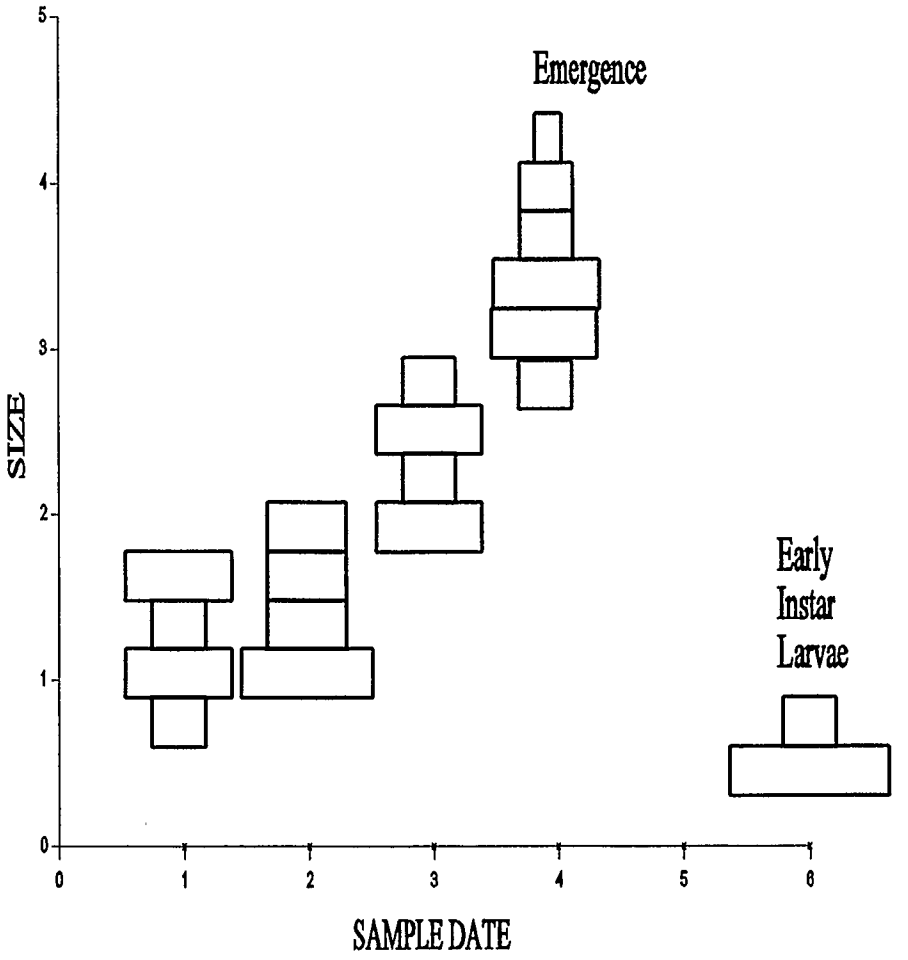

Figure 23: Illustration of the size distribution for an imaginary aquatic insect with a one year life cycle, showing the absence of larvae after emergence and the reappearance of early instar larvae after eggs hatch.

seasons, an insect has no such structures. Insect exoskeletons are moulted for a discrete increase in size and no permanent structures remain to aid in age estimation.

The very different results from the three estimates of time required to complete the larval stage of *P. californica* in the Crowsnest River (visual inspection: three to five years; growth simulation model: seven to eight years; and MULTIFAN: eight to fourteen years) delineate the difficulty associated with estimations of life cycle lengths greater than one year. Both MULTIFAN and visual inspection are based on a widely accepted method for estimating ages of insects. This method works best when recruitment to the population is a discrete event occurring at one time each year, samples contain all sizes in the population, and samples have been collected at regular intervals. For each sampling date, morphological measurements are plotted as a size-frequency distribution, then a visual and/or statistical examination estimates the number of year-classes and hence the length of the life cycle. For example, if eggs hatch as a pulse and all individuals grow at the same rate, each cohort has a 1 year growth advantage over the next cohort. Cohorts should then be represented as modes in the size-frequency distribution. Therefore, if there are two distinct modes, then there is a two-year life cycle, three distinct modes, then a three-year life cycle, and so on. So ideally, the size-frequency data can be used to estimate the growth of a cohort by following the shift in position of the mode through time. However, the method will not give an accurate estimate of the life cycle length if the modes as defined by the researcher are not equivalent to year-classes in the population. As described in Section 2.3.2, the width of the class interval used to construct the frequency distribution can have serious effects on the estimate, and so it is important that the interval

chosen neither obscure nor multiply the number of true modes present. For the visual inspection method, I used biological information to define the modes and then chose the size-class interval (0.3 mm) that produced these modes and no others. MULTIFAN uses a statistical procedure and came up with an interval (0.15 mm) half the size. This may be one reason for the difference in their estimates.

In the MULTIFAN output, the first age class is reasonably well-defined but as age classes are added, the size range within each age class decreases (Figure 18). This is contrary to expectations. Variance in larval size is least when eggs hatch. However, individual growth rates can vary because of factors such as exposure to different environmental conditions and genetic differences (including sexual differences). Female stoneflies, as with most aquatic insects, are invariably larger than males of the same age class (Butler 1984). Poole (1981) found this to be true for *P. californica* in the Warm and Provo Rivers (Utah) and it is also true for the larger age classes in the Crowsnest River population (Figure 14). Therefore, variation in larval size should increase within a cohort through time.

The size variation within an age class is particularly clear in final instar larvae, present in the population from the end of August until the end of May and which will emerge as a single unit in May. However, MULTIFAN puts seven modes into this single class, and even when the analysis was directed to treat all individuals with a head capsule width greater than 4.00 mm as a single mode (user input categories 6 and 7) three age classes were still estimated in the final instar (Figure 19). When these three age classes are combined the MULTIFAN estimate for the length of larval life is reduced to six years.

Thus, although MULTIFAN accurately estimates the growth of age classes based on length-frequency distribution in fish populations (Fournier *et al.* 1990; Terceiro *et al.* 1992; Labelle *et al.* 1993), a shrimp population (Fournier *et al.* 1991), and a squid population (Welch and Morris 1992), it is unable to incorporate some important *P. californica* life history information.

On the other hand, the visual method, although less "objective", allows easier incorporation of biological data. For example, the appearance of first instar larvae, and the morphological characteristics of final instar larvae, their appearance and emergence allows identification of first and final year classes. The problem then becomes distinguishing the number of age classes between the first and the final age class. At many times of the year there appear to be two distinct size groups present between the first and final age classes. However, there is considerable blurring of these age classes and cohort splitting may provide an explanation. Cohort splitting is common in aquatic insects with long life cycles living in temperate lakes, pools and rivers (Corbet 1957; Pritchard 1976, 1978; Carter 1980; Mutch and Pritchard 1984; Ernst and Stewart 1985; Moreira and Peckarsky 1994) and may be the result of 1) inherited differences, 2) occupation of different sets of environmental conditions in space, or 3) subjection to different environmental conditions due to temporal separation (Pritchard 1976). A major factor contributing to cohort splitting in stoneflies with long life cycles is asynchronous hatching which leads to temporal separation of members of the same cohort (Ernst and Stewart 1985; Stewart and Stark 1988; Moreira and Peckarsky 1994).

In theory the growth rate simulation model should give the best estimate of growth

rates in the field. However, in this study it probably overestimates because laboratory conditions did not simulate river conditions. In particular, although the diet used for laboratory rearing was sufficient for larval growth it almost certainly did not compare with food in the river. Other factors, either alone or combined, such as photoperiod, fluctuating water temperatures, water quality, and oxygen levels may also contribute to faster growth in the river. Because growth rate is driven primarily by temperature, it is not surprising that the growth simulation model predicted that a sequence of warm years leads to shorter life cycles than a sequence of cold years (Figure 21). More interestingly, the growth simulation model predicts that there is a critical hatch date between May 1 and June 1, and eggs hatching after this date add another year to larval life (Figure 22). Therefore, larvae recruited late in the year, require an extra year to complete their development. This supports the proposition that continual recruitment contributes to cohort splitting in *P. californica* by showing that the time required to complete larval growth can be affected by the month in which eggs hatch.

So how many years are required for *P. californica* to complete larval development in the Crowsnest River? According to all the information at hand, more than two but less than 14 years. Based on biological information, the first and final age classes can be identified and there are still ages in between; therefore, more than two years are required. When most biological information is added to the MULTIFAN analysis its estimate is reduced to six years. Because the growth simulation result is likely an overestimate, fewer than seven years would a reasonable prediction. The visual inspection method, taking cohort splitting into account, estimates three to five years. Therefore, an estimate of three

to six years to complete larval development encompasses the best predictions of all three methods. Little more can be said at this stage.

The life history of *P. californica* in the Crowsnest River involving delayed egg hatch, extended larval recruitment, and sexual size dimorphism, leads to variation in the sizes of larvae of the same age and to cohort splitting. Thus, it is difficult to separate genuine cohorts from the considerable mix and overlap of age classes. Faster growing individuals in a younger cohort may catch slower growing individuals in an older cohort, continual recruitment may lead to several or no distinct cohorts within a single year, slower growth of older individuals may result in older cohorts becoming indistinguishable, and males and females may have different growth patterns. It is these nuances in the life history that make the estimation of the life cycle length very difficult. Individual-based modelling techniques (DeAngelis and Gross 1992), that allow the incorporation of variation within populations, seem to hold most promise for further refinement.

Irrespective of the methods used to determine life cycle length in *P. californica*, the life cycle is very long, allowing considerable size variation to develop within a cohort. However, this variation largely disappears by the end of larval life and adult emergence is completed in one 7-10 day period per year. This suggests that specific environmental cues are being used to regulate this synchronous emergence. A synchronized emergence is especially important for insects with long life cycles, because years of individual variation must be channelled into a short emergence period to ensure mates are encountered. A photoperiodic reaction, as the basis for seasonal regulation, has been proposed by Corbet (1957) and modified by Norling (1984) for north temperate dragonflies (Odonata). A

similar system may control synchronous emergence in stoneflies. In the laboratory, penultimate (F-1) *P. californica* larvae did not moult into final instar (F) larvae, suggesting that a change in photoperiod and/or temperature may be required. In the field population, F-1 larvae (wing pad length ≈ 4.5 to 5.75 mm) are present from June through August and F larvae (wing pad length > 5.75 mm) appear in late August (Figure 12). The conspicuous absence of F-1 larvae from September through May suggests that entry to this instar has been delayed by a diapause in the F-2 larvae. In May, F-3 larvae begin moulting to F-2, then early in the summer, a long-day photoperiodic diapause (like that suggested by Norling (1984) for temperate-zone dragonflies) would prevent F-2 larvae from moulting into F-1. This diapause could accumulate larvae in the F-2 instar, and represent the first step toward emergence synchrony. The long-day diapause need only last until colder fall temperatures hold back development. Warm May temperatures allow F-2 larvae to moult into F-1, and the warmer June through mid-August temperatures will move those F-1 larvae into the final instar. Low temperatures from September through April will prevent emergence, but allow accumulation of the whole cohort of final instar larvae and so represent another synchronization period. When temperature rises above a threshold in spring, the whole cohort should emerge synchronously. The start of emergence for *P. californica* varies yearly, from early-May to late-June, supporting the view that the initiation of emergence is influenced more by temperature than photoperiod. Therefore, an early emergence date should be preceded by a short (warm) winter and a late emergence date proceeded by a long (cold) winter. This is supported by Crowsnest River temperatures and *P. californica* emergence dates for 1992 and 1993.

In summary, the *P. californica* population in the Crowsnest River has a long and complex life history, spanning at least four and perhaps as many as seven years. Delayed egg hatch, probably due to an egg diapause, accounts for approximately one year of the life cycle. Extended larval recruitment leads to variation in the sizes of larvae of the same age because they are exposed to different environments. This leads to cohort splitting with faster-growing larvae of a cohort completing development along with slower-growing larvae of previous years' cohorts. Although this long life cycle encourages individual variation, adult emergence is highly synchronous. Such synchrony may be achieved by a summer diapause in F-2 larvae followed by slow growth of F-2 and F larvae during the next two successive winters. This suite of life history tactics is apparently typical of aquatic insects with long life histories.

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