displayed partial cross-resistance to **2**. We assume that this antibacterial activity of **2** is caused mainly by a mechanism different from that of inhibition of bacterial DNA-dependent RNA polymerase.

Protozoa have been used as preliminary test organisms for screening cytotoxic antitumor agents, because these eukaryotic microorganisms and mammalian cells resemble each other morphologically and metabolically. Ansathiazin as well as **1** had inhibitory activities against *Tetrahymena pyriformis* W (table 4). This suggests that **2** may have antitumor activity. In fact, like **1**, **2** had strong cytocidal activity against murine leukemic cells (data not shown).

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Growth and the average duration of larval life in the southern hemisphere lamprey, *Geotria australis* Gray

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Summary. The average duration of larval life in the anadromous lamprey, *Geotria australis* (the sole representative of the Geotriidae) is estimated as 4¼ years. Compared with other lampreys, the ammocoetes of *G. australis* have a slow growth rate, increase in length during the year preceding metamorphosis and typically enter metamorphosis at a small mean length (< 100 mm) and weight (< 1.2 g).

Key words. Lampreys; *Geotria australis;* ammocoetes; larval life; growth; metamorphosis.

The extant lampreys, which have an antitropical distribution, are separated into three families[1]. All holarctic species are placed in the Petromyzontidae, while those of the southern hemisphere are divided into either the Geotriidae or Mordaciidae. Larval lampreys (ammocoetes), which are sometimes the most abundant vertebrate in temperate rivers and streams[2,3], are burrowing and relatively sedentary animals. They feed by filtering algae, detritus and microorganisms from the water overlying the substrate surface[4–8].

Since ammocoetes do not possess bony structures suitable for the examination of annual growth rings, estimates of growth and the duration of larval life have had to be derived from the analysis of length-frequency data[7,9]. These have shown that ammocoetes grow slowly and that the protracted larval phase in both the Petromyzontidae and Mordaciidae typically ranges from 3¼ to 6¼ years[7]. There have been no comparable studies on *Geotria australis*, the sole representative of the Geotriidae[1], although a tentative estimate of 3¼ years was given for the duration of larval life in this species in a review of ammocoete ecology[7].

The present study was undertaken to examine the trends exhibited by the modes in length-frequency curves for samples of larval *G. australis* collected over several years from south-western Australian streams. The results, when compared with the pattern of growth exhibited by groups of tagged ammocoetes, provide data on larval growth and a more accurate estimate of the average duration of larval life than that given by Potter[7]. The data are compared with those obtained for representatives of the two other lamprey families.

Materials and methods. Larval and metamorphosing lampreys were collected with an electric fish shocker in Southwestern Australia from a number of sites in Carey Brook (34°24'S, 115°50'E), which is a tributary of the Donnelly River, and Dombakup Brook (34°35'S, 116°04'E) and Big Hill Brook (34°32'S, 116°15'E), which enter the Warren River. Since lampreys show a tendency to occur in slightly different habitats just prior to and during metamorphosis[7], electrofishing was extended to areas surrounding those where large numbers of larvae were found. Sampling was carried out monthly between January 1977 and July 1982, whenever prevailing turbidity and stream flow permitted. All animals were measured to the nearest 1 mm. In addition, groups of 65 and 85 larvae from Carey Brook, with lengths of 50–70 and 65–80 mm respectively, were tagged in March 1980 with a small s.c. injection of cadmium dye[10,11] so that their growth could be followed in subsequent months. Those tagged animals which were recaptured at intervals were lightly anaesthetized with benzocaine[12], measured, allowed to recover and then returned to the site. All length data were smoothed by a moving average of 5 mm.

Results and discussion. The smallest group of larval *G. australis*, which came from Carey Brook, ranged in length from 10–13 mm. Although the precise time of spawning in *G. australis* is not known, the age of these very young larvae can be estimated by assuming that embryological development and early growth in this species is similar to that of holarctic *Petromyzon marinus*. Since this latter species has a similar egg size at maturity[13,14] and begins burrowing at lengths of 7.5–9 mm after 17–33 days and reaches 9–10 mm after 33–40 days[15], the 10–13 mm larval *G. australis* were probably 1.5 months old. Since they had been collected in mid-December 1978, they were apparently the product of an early November spawning. The capture of larvae in January and February 1977 with modal lengths of 17–19 mm from single sites in the upper reaches of Dombakup Brook and Big Hill Brook (fig. 1) would be consistent with this proposed spawning time.

The small size, narrow length range and sharply defined peak in the length-frequency curves for samples of larval *G. australis* taken from a single site in both Dombakup and Big Hill Brook in January and February 1977, provide strong evidence that these samples contained only 0+ recruits resulting from the 1976 spawning season (fig. 1). Since the same age group clearly predominated at both sites throughout the following 13–16 months, their modes reliably trace the pattern of growth during the first 1.5 years of larval life (fig. 1). The modal values increased from 17 mm in January and 19 mm in February 1977 to 52 mm in Dombakup in April 1978 and to 48 mm in Big Hill in February 1978. Unfortunately it was not possible to trace the 1976 year class beyond February 1978 at the site in Big Hill Brook because this part of the stream dried up in early March 1978. In the case of the Dombakup Brook site, the samples after April 1978 contained a predominance of larger animals which had entered following changes in water levels. The fact that such animals had not entered this site earlier is presumably attributable to the absence of a particular pattern of discharge which causes larvae to be deposited at the site.

The modal length of ca 50 mm for the I+ age class in frequency curves for two different tributaries in February/April (fig. 1), corresponds closely with those designated as representing this age class in three sites over four years in Carey Brook (fig. 2a–d). A comparison between the well-defined and remarkably consistent modes in the latter four curves with the trends shown by modes in preceding and following months provided strong evidence that they represent four different larval age classes. Although the distribution of the presumptive age class II+ at Site 3 was slightly bimodal in 1979 (fig. 2b), the validity of its designation as II+ is supported by the fact that the mid-point between its closely apposed peaks occurs at the same length as the mode representing this age class in the curve for the same site in 1981 (fig. 2d). Since both the distribution and single pronounced mode for the metamorphosing individuals always lay to the right of the III+ larval age class, these modes are assumed to represent predominantly the IV+ age class. The respective modes for the 0+ to III+ larval age classes ranged from 20–25, 42–49, 56–63 and 71–83 mm, with the modal lengths for the older larval age classes at the upstream site (3) being slightly greater than the comparable modes for downstream sites (1 and 2). Likewise, the mode for the metamorphosing animals (IV+) at site 3 (93 mm) was greater than at sites 1 and 2 (82 mm). The greater modal lengths for both the older larvae and metamorphosing animals from the upstream than the two downstream sites clearly reflect a faster growth rate. Moreover, since both the distribution and mode of the metamorphosing animals at site 3 lay to the right of those for the metamorphosing and equivalent age class animals at sites 1 and 2, the onset of transformation appears more dependent on age than size. The differences between the modes in the curves of the three Carey Brook sites (fig. 2a–d) also emphasize the importance of using samples from single and relatively stable sites for determining age classes from this type of data for a species such as *G. australis,* which has a slow rate of larval growth and a protracted larval phase. The view that site 3 in Carey Brook was stable is also supported by the continual recapture of tagged larvae over many months (fig. 3a, b).

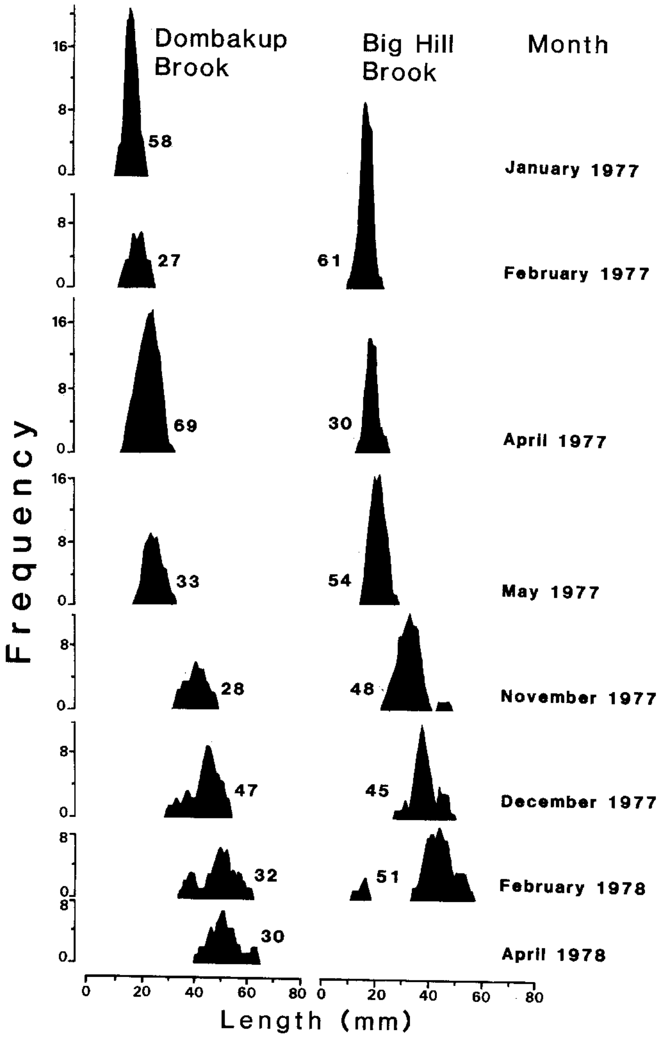

Figure 1. Length-frequency curves (with sample sizes) for larvae of the 1976 year class of *G. australis* from single sites in Dombakup Brook and Big Hill Brook.

Since spawning apparently occurs in early November and metamorphosis is initiated in late January/early February[16], the presence of four larval modes, together with a fifth representing metamorphosing individuals, indicates that the average duration of larval life is 4¼ years. It must be recognized, however, that some ammocoetes probably enter metamorphosis at 3¼ or 5¼ years of age, or possibly even older. While the III+ and metamorphosing IV+ age classes have particularly well-defined and sharp modal lengths in the curves shown in figure 2a–d, the marked overlap of their distributions accounts for the reason why these two age classes frequently could not be clearly distinguished from each other in the months prior to the onset of metamorphosis. The pattern of growth in older ammocoetes was therefore traced by following increases in the mean length of two groups of larvae tagged in March 1980 at site 3, Carey Brook (N.B. The size distribution of the whole sample was similar to that shown in figure 2b for the same site in April 1979). The mean length of the first group, consisting of larvae measuring 50–70 mm when tagged, increased from 64 mm to 77 mm by February 1981, at which time only one of the largest recaptured animals had entered metamorphosis (fig. 3a). During the following year, the mean length of this group increased to 92 mm, and two of the remaining tagged animals recaptured in March 1982 had entered metamorphosis (fig. 3a). The mean length of the second group, consisting of larvae measuring 65–80 mm when tagged, increased from 74 mm to 88 mm by February 1981 (fig. 3b), the latter value including the lengths of those tagged animals which had entered metamorphosis. The mean of the remaining untransformed larvae increased from 85 mm in February 1981 to 92 mm in July and 96 mm by December 1981 (fig. 3b). The single individual recaptured in 1982 was 99 mm and had entered metamorphosis (fig. 3b).

The changes in length of the tagged larvae support designating the modes for larvae from Carey Brook as representing separate year classes (fig. 2a–d), a view based on the correspondence between the annual growth increments of the two groups and the differences between the successive modal values of the older size classes in Carey Brook (fig. 2a–d). It is also evident from the data for the second group of tagged animals that metamorphosis occurred among the largest larvae, which, from the size of the largest ammocoetes tagged in March 1980, probably had an average age of 4¼ years by February 1981.

The longer lengths of the tagged metamorphosing animals in February 1981 than those of the largest tagged larvae 11 months earlier demonstrates that growth occurs during the last year of

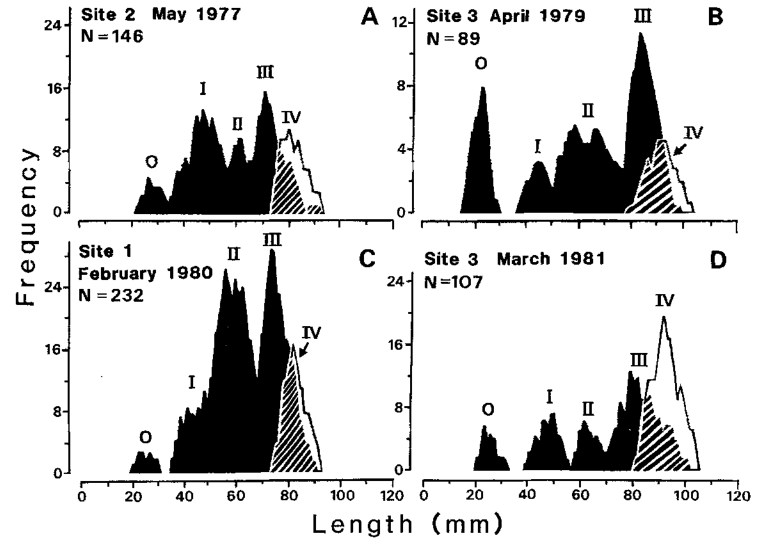

Figure 2. Length-frequency curves for larval (black) and metamorphosing (white) representatives of *G. australis* at two downstream sites (1 and 2) and an upstream site (3) in Carey Brook (N = sample size).

larval life, a finding entirely consistent with the fact that the modal lengths for metamorphosing animals collected from Carey Brook always lay to the right of those for the largest larvae (fig. 2a–d). This situation contrasts with the absence of an increase in length in the final year of larval life in several holarctic species and the southern hemisphere *Mordacia mordax*[2,9,11,17,18]. During this so-called arrested growth phase[9], the larvae of these species store a large amount of lipid, which subsequently acts as an important energy source during the ensuing lengthy non-trophic period of metamorphosis[6,20]. Since the relative amount of lipid also increases during the terminal part of larval life in *G. australis*[21], this species utilizes the food it assimilates in the months preceding metamorphosis for accumulating fat as well as increasing in length, albeit at a reduced rate. The ability of *G. australis* to perform both these functions has been related to the unusually small size at which it typically enters metamorphosis[21]. The small size is illustrated by the observation that, apart from a group caught in one Southwestern Australian brook having mean lengths of 102–106 mm in three successive years[16], all the populations of *G. australis* investigated in Australia, New Zealand and Chile entered metamorphosis at mean lengths and weights below 100 mm and 1.2 g[16,21–23]. Although *G. australis* grows throughout larval life, comparisons between length data for this species and those for representatives of *Mordacia, Ichthyomyzon, Petromyzon, Tetrapleurodon* and *Lampetra*[9,11,17–19,24] show that the growth rate of larval *G. australis* is relatively slow. For example, the ammocoetes of *G. australis* reach an average length of only 55 mm by the end of their second year of life, while comparable values for the other species range from above 55 to 135 mm. The difference in growth rate can also be demonstrated by comparing the modal length at the end of the third year of larval life (70–75 mm) in *G. australis* with that at the same age (95 mm) in *L. fluviatilis*[25], a species which enters metamorphosis at mean lengths 10 mm longer than *G. australis*[22].

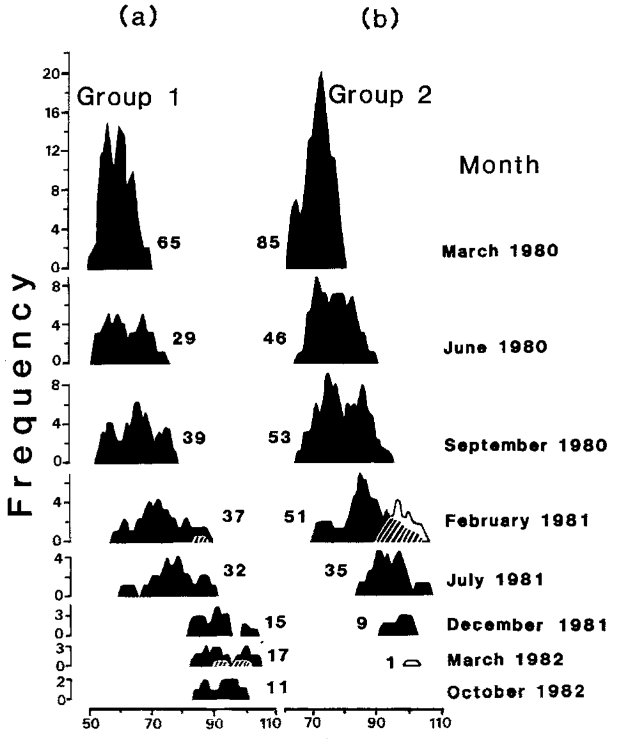

Figure 3. Length-frequency curves (with sample sizes) for larval (black) and metamorphosing (white) representatives of *G. australis* which were tagged in March 1980 as larvae measuring (a) 50–70 mm (Group 1) and (b) 65-80 mm (Group 2).

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Individual specific trails in the ant *Leptothorax affinis* (Formicidae: Myrmicinae)

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Summary. Leptothorax affinis lays chemical trails during nest emigration. Workers which carried colony members during nest movement refused trails of nest mates and searched for their own trails. The origin of the individual specific trail substance could not be localized.

Key words. Individual specific pheromone trails; *Leptothorax;* nest emigration; ant.

The myrmicine ants of the genus *Leptothorax,* which characteristically live in small colonies, possess two recruitment systems: tandem running for recruitment to food, and social carrying for nest emigrations (which are initiated by a brief phase of tandem running). As we demonstrated in marking experiments, individual *Leptothorax* workers do not follow narrowly defined trails but instead walk to their destinations over a broad area unless restricted by a narrow guiding structure like, e.g. a slender twig. On account of these observations we[2] at first did not look for the existence of chemical trails in *Leptothorax.* Chemical trails were demonstrated subsequently by Lane[3] in *Leptothorax unifasciatus.* We took up the problem of trail laying in *Leptothorax* again during recent investigations of territorial behavior in this genus. To our great surprise we discovered not only colonyspecific trials but also individualspecific trails in the tree-inhabiting *Leptothorax affinis.*

Our experimental colonies were maintained in artificial nests consisting of two small glass slides separated by a frame of cardboard. For our experiments we opened the old nest chamber and connected it to a new nest circa 90 cm away via a system of five 15-cm long wooden bridges linked together and supported by raised platforms. Soon the first scouts detected the new nest and – after an initial phase of tandem running – began to transport other workers and brood into it. Now we replaced the original marked bridge with a new unmarked one. The old bridge remained connected to the platform parallel to the former position but shifted aside by 4 cm. During the first half of the experiment, carrying workers which had already learned the route to the new nest became disoriented when encountering the new bridge. After initially palpating it with their antennae they either did not walk onto it at all or they turned around after walking on it for only a few cm. They kept walking around on the platform until they found the old bridge which they recognized instantly and crossed without hesitation even though it was lying in a different position. This indicates that the carrying workers used a chemical trail for orientation (15 tests with 6 colonies: 144 out of 158 workers refused). Carrying individuals which had frequently walked the distance between the old and the new nest, as well as noncarriers which had been walking back and forth, were only partially disoriented by the new bridge. Apparently they used additional cues for orientation.

When we exchanged the bridges of two different colonies we noted that carrying workers which refused new unmarked bridges refused bridges marked by the other colony as well. In three experiments 39 out of 44 workers refused the foreign bridge and instead searched for their own bridge which they crossed without hesitation after they had detected it in its new position ($p < 0.001$). Thus it can be concluded that carrying *Leptothorax affinis* workers prefer trails of their own colony to trails of other conspecific colonies.

Next we tested whether *Leptothorax affinis* produces individual specific trials, a behavior characteristic which was recently discovered in the ponerine ant *Pachycondyla tesserinoda*[4]. In order to investigate this question we caused small colony fragments consisting of only a few individuals and brood each to move to new nest sites. We allowed one marked worker of each colony fragment to mark a bridge during at least five successive runs. Other scouts were removed. Then we exchanged the marked bridges according to the method described earlier. We made sure that the trails tested were no older than those laid by the workers of the other colony fragments used for the tests. It turned out that the carrying workers used only those bridges that they themselves had marked for their emigration. Bridges marked by their nestmates were refused as were unmarked bridges (6 tests with 6 different colonies: 16 out of 17 workers refused to walk on bridges marked by their nestmates, $p < 0.001$). In another series of experiments we again offered bridges to carrying workers of small colony fragments. These bridges had been used by a large number of their colony members but not by themselves. These strongly marked bridges, too, were refused significantly as they did not contain the trails of the workers tested (6 tests: 10 out of 12 workers refused the strongly marked trails of their own colonies, $p = 0.019$).

The fact that workers follow mainly their own trails may explain our inability to localize the origin of the trail substance. During our search we tested three colonies on bridges marked with extracts of following body parts from some of their own workers: legs, sting glands and sting sclerites, recta and all gastral sclerites. For each test the different glands and body parts of ten workers were thoroughly ground in 0.04 ml water. The whole extracts were applied with brushes to the bridges. These bridges were offered to workers which carried nestmates.