GREAT LAKES FISHERY COMMISSION
Research Completion Report *

HORMONAL AND ENVIRONMENTAL CUES OF METAMORPHOSIS IN *PETROMYZON MARINUS*

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Hormonal and Environmental Cues of Metamorphosis in Petromyzon marinus

Progress Report for 1993

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1. Fall Condition Factor and the Incidence of Metamorphosis

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Objective: In an earlier study we suggested that presumptive metamorphic larvae in the fall were better able to survive adverse temperature regimes and food conditions over the winter and enter metamorphosis the following July. We did not explicitly examine this hypothesis in 1992 because larvae were not individually marked. The objective of this study was to investigate the influence of metamorphic status in the fall, as measured by condition factor (CF), and temperature on the incidence of metamorphosis of larval sea lampreys the following July.

Design: We used the experimental system developed at Scarborough College for a short-term experiment in 1992 (see Youson et al. 1993). Sixteen aquaria were available in two banks of eight and were supplied with aerated dechlorinated tap water. The tanks were covered with 2.5 cm of foam insulation on their sides and top to minimize temperature changes when the water was turned off overnight for feeding.

Larval sea lampreys ($\underline{N} \approx 600$) were collected from Brown Creek, a tributary to Lake Huron, in late September 1992 with assistance from the Sea Lamprey Control Centre in Sault Ste. Marie. The larvae were transported to our laboratory at Scarborough College and held in tanks with 5 cm of sand and dechlorinated tap water at temperatures of 10-14 °C until the experiment began.

Larvae having a total length $\geq$ 120 mm and a weight $\geq$ 3.0 g were randomly sorted into 10 tanks at a density of 11 larvae per tank (60 larvae•m^{-2}). Each tank contained nine presumptive nonmetamorphic larvae (CF < 1.50) and two presumptive metamorphic larvae (CF $\geq$ 1.50). CF was calculated as weight (g)/(length (mm))^3 x 10^6. The metamorphic larvae in each tank were individually marked with a subcutaneous injection of orange or green latex dye in the caudal sinus. We provided the same quantity of food (baker's yeast) to each tank as used in previous studies (Holmes et al.; Youson et al. 1993), but because larval density in the present study was less than half the density of previous studies (164 larvae•m^{-2}), each larva received approximately 1.8 g yeast•wk^{-1}.

Larvae were exposed to two temperature regimes, a constant 21 °C or seasonally adjusted temperatures, simulating the mean summer temperature and the ambient temperature regime,

respectively, in many southern Ontario streams that support larval sea lampreys. The ambient temperature was adjusted approximately weekly using a thermograph from Duffins Creek at Pickering, near our laboratory at Scarborough College, published by Environment Canada (1976). Photoperiod was held constant at 15 h light:9 h dark. Five replicate tanks were exposed to each temperature regime and a sixth uninsulated tank contained 24 replacement larvae (total length $\geq$ 120 mm but weight < 3.0 g) for each temperature to maintain density in the event of mortality. Replacement larvae were marked in the caudal sinus with yellow latex dye so they could be excluded from our data analysis.

The experiment began in early November 1992 and was terminated in early August 1993. During this time larvae remained undisturbed, except during cleaning and sand replacement, and two sampling periods in mid-March and mid-May. The March sampling was timed to occur just before the ambient temperature began to rise in the spring of 1993. During each sampling total lengths ($\pm$ 1 mm) and weights ($\pm$ 0.01 g) were measured and in August the stage of metamorphosis was determined using the descriptions in Youson and Potter (1979). Also, the larvae were examined for evidence of white eye deposits noted previously (Holmes et al. 1994), which we believe is symptomatic of starvation in larval sea lampreys.

Results: Presumptive metamorphic and nonmetamorphic larvae were the same length but the former were significantly heavier and as a consequence had significantly greater CFs at the beginning of the study in November 1992 (Fig. 1). There were no differences in the initial lengths, weights, or CFs of larvae between the temperature groups. Mean weekly instantaneous temperature in the constant temperature treatment varied between 19 and 21.5 °C while the ambient temperature declined from 7 °C in early November to 3 °C by early March (Fig. 2). Beginning at 22 weeks, the ambient temperature was increased at a rate of 0.4 °C•wk^{-1} for 3 weeks and then at a rate of 1.5 °C•wk^{-1} for 11 weeks until a maximum mean weekly temperature of 21 °C was attained in the first week of July 1993 (Fig. 2). Mortality was negligible during the study, consisting of one presumptive metamorphic larvae in the constant 21 °C water. Two nonmetamorphic larvae, one in each temperature regime, were observed with white deposits in the region of the presumptive eye in March, May, and August; we do not know if the same larvae were observed at consecutive samplings.

The overall incidence of metamorphosis among larval sea lampreys in August 1993 was 27% (30 of 110), with significantly more (ANOVA, $P = 0.0018$) animals metamorphosing in the ambient temperature (29/55, 53%) than in the constant 21 °C water (1/55, 2%). The incidence of metamorphosis in the ambient temperature regime varied between 2 and 9 animals•tanks^{-1}, with a mean ($\pm$ SD) of 6 larvae•tank^{-1} $\pm$ 3 (55%) compared to ~ 0 larvae•tank^{-1} (0.4 $\pm$ 0.5, 3.6%) in the constant temperature water. Metamorphosing animals in the ambient temperature regime ranged from stage 1 to stage 3 in their development. The metamorphosing animal in the constant temperature was at stage 3 in its' development.

Metamorphosing animals were not signficantly different from nonmetamorphosing larvae on the basis of length or weight in early August ($P > 0.05$). The mean size (mean $\pm$ 1 SE) of a metamorphosing animal at stages 2 or 3 ($N = 20$) was 127 $\pm$ 2 mm and 3.12 $\pm$ 0.14 g compared to 130 $\pm$ 1 mm and 2.87 $\pm$ 0.07 g for unmetamorphosed larvae ($N = 79$). The minimum size of a metamorphosing animal was 118 mm and 2.46 g. There were no trends in the length and weight of animals at different stages of metamorphosis in early August (Fig. 3a, b). However, the CF of metamorphosing animals at stages 2 and 3 was significantly greater than the CF of nonmetamorphosing larvae (Fig. 3c). The minimum CF of metamorphosing animals was 1.30 and the mean ($\pm$ SE) was 1.50 (0.027) compared to a minimum of 1.05 and mean of 1.28 (0.011) for nonmetamorphosing larvae.

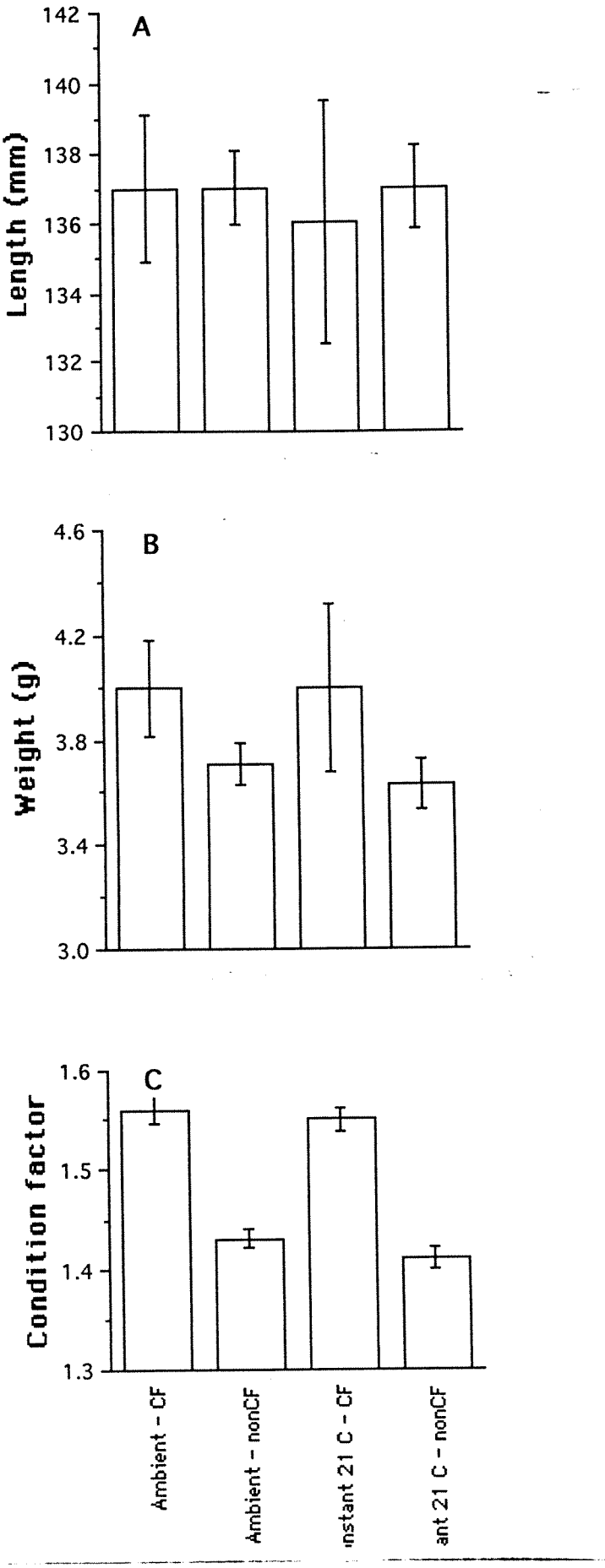

Figure 1. Initial lengths (A), weights (B), and condition factors (C) of presumptive nonmetamorphosing (nonCF) and presumptive metamorphic (CF) larval sea lampreys in ambient and constant 21 °C temperature regimes. Data are means ± 1 SE.

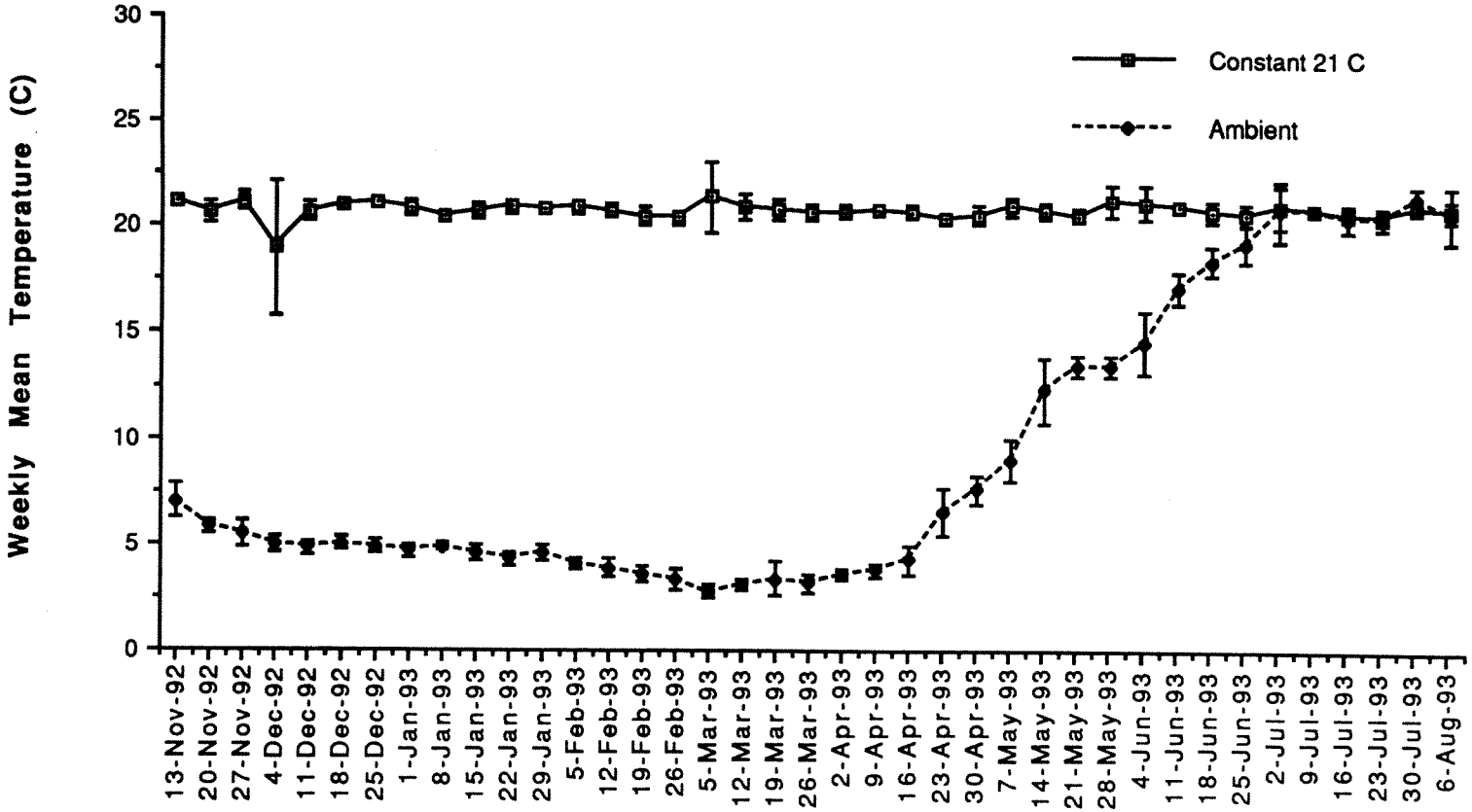

Figure 2. Mean weekly water temperatures experienced by larval sea lampreys between November 1992 and August 1993. Data are means $\pm$ SD.

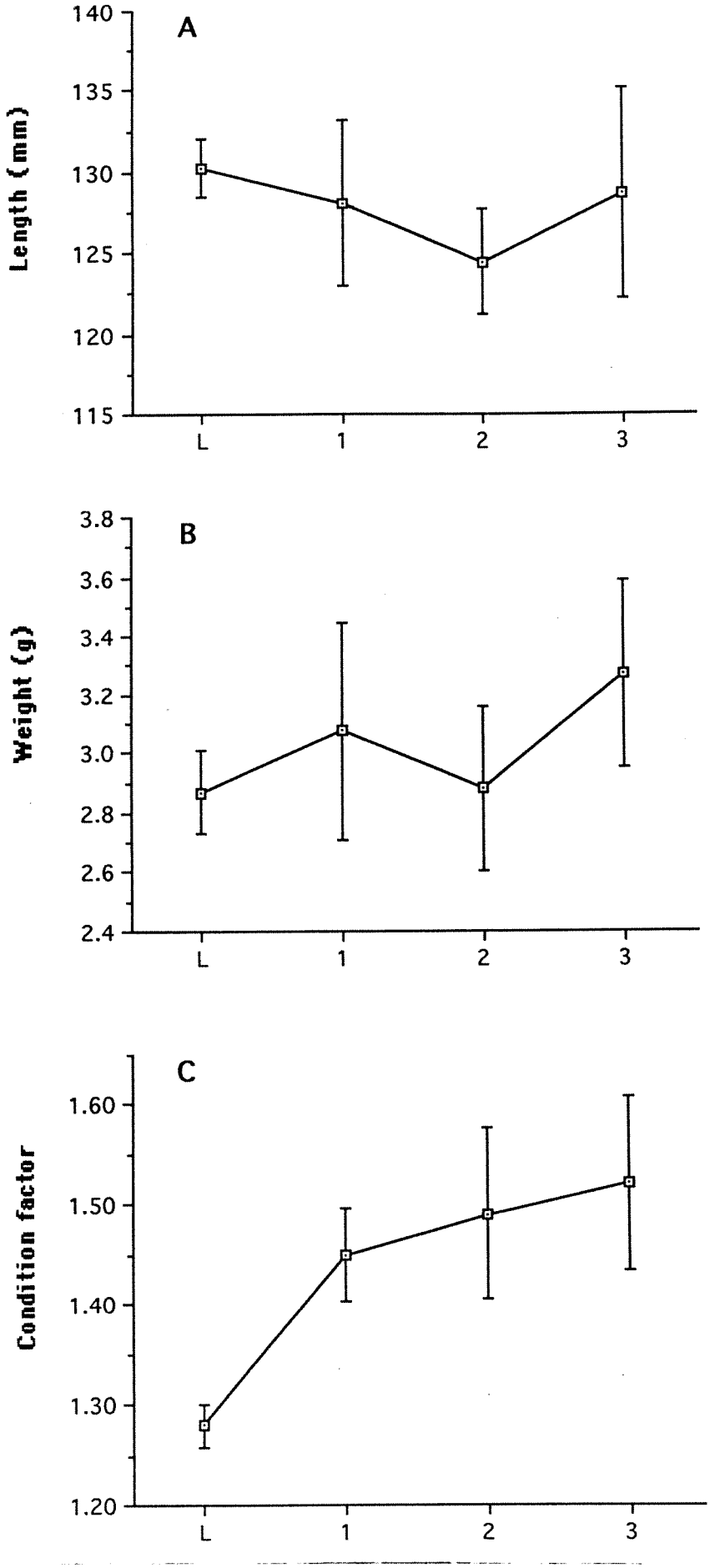

Figure 3. Changes in the length (A), weight (B), and condition factor (C) of larval (L) and metamorphosing (1, 2, 3) sea lampreys in August 1993. Data are means $\pm$ 95% CL.

Based on the size and CF of larval sea lampreys in the fall, we expected 10 and 11 larvae in the constant and ambient temperature regimes respectively, to enter metamorphosis the following July (Table 1) and we had marked these individuals with latex dye. About 38% of the presumptive metamorphic larvae marked in the fall (8 of 21) had entered metamorphosis, with more presumptive metamorphic larvae entering metamorphosis in the ambient temperature regime (7/11, 64%) than in constant temperature conditions (1/10, 10%). Although the prediction in the ambient temperature regime was not significantly different from the number of metamorphosing animals observed, significantly fewer larvae entered metamorphosis in the constant temperature than expected (Table 1). Presumptive nonmetamorphic larvae were not expected to enter metamorphosis and while the result in the constant temperature was consistent with this prediction, significantly more larvae entered metamorphosis in the ambient regime than expected (22/44, 50%; Table 1).

Table 1. Comparison of predicted and observed metamorphosis among presumptive metamorphic and nonmetamorphic larval sea lampreys held in an ambient and constant 21 °C temperature regimes for nine months.

Presumptive status		Temperature regime: Ambient	Temperature regime: Constant
Metamorphic (CF $\geq$ 1.50)	Observed	7	1*
	Predicted	(11)	(10)
	G value	8.4	55.6
Nonmetamorphic (CF < 1.50)	Observed	22*	0
	Predicted	(0)	(0)
	G value	73.0	3.3

* Observed value is signficantly different from expected ($P = 0.05$). G values were compared to $\chi^2_{0.05,[4]} = 9.488$ for the test of significance.

Larval sea lampreys exhibited negative changes in length of 3.5 to 7.6% and from 20 to 23% of their weight between November 1992 and August 1993 (Figs. 4a and b respectively). Results of the repeated measures ANOVA indicated that temperature but not metamorphic status influenced the length and weight of a larva over the winter (November to March) and between May and August ($P < 0.002$) but during the short interval between March and May neither factor was a significant influence on larval length or weight ($P \geq 0.09$). There was little change in the length or weight of presumptive metamorphic and nonmetamorphic larvae in the ambient temperature regime prior to May, but between May and August these groups displayed the largest rate of change observed. In contrast, larvae of these two groups in the constant temperature underwent continuous negative changes in length and weight throughout the study. There were no significant

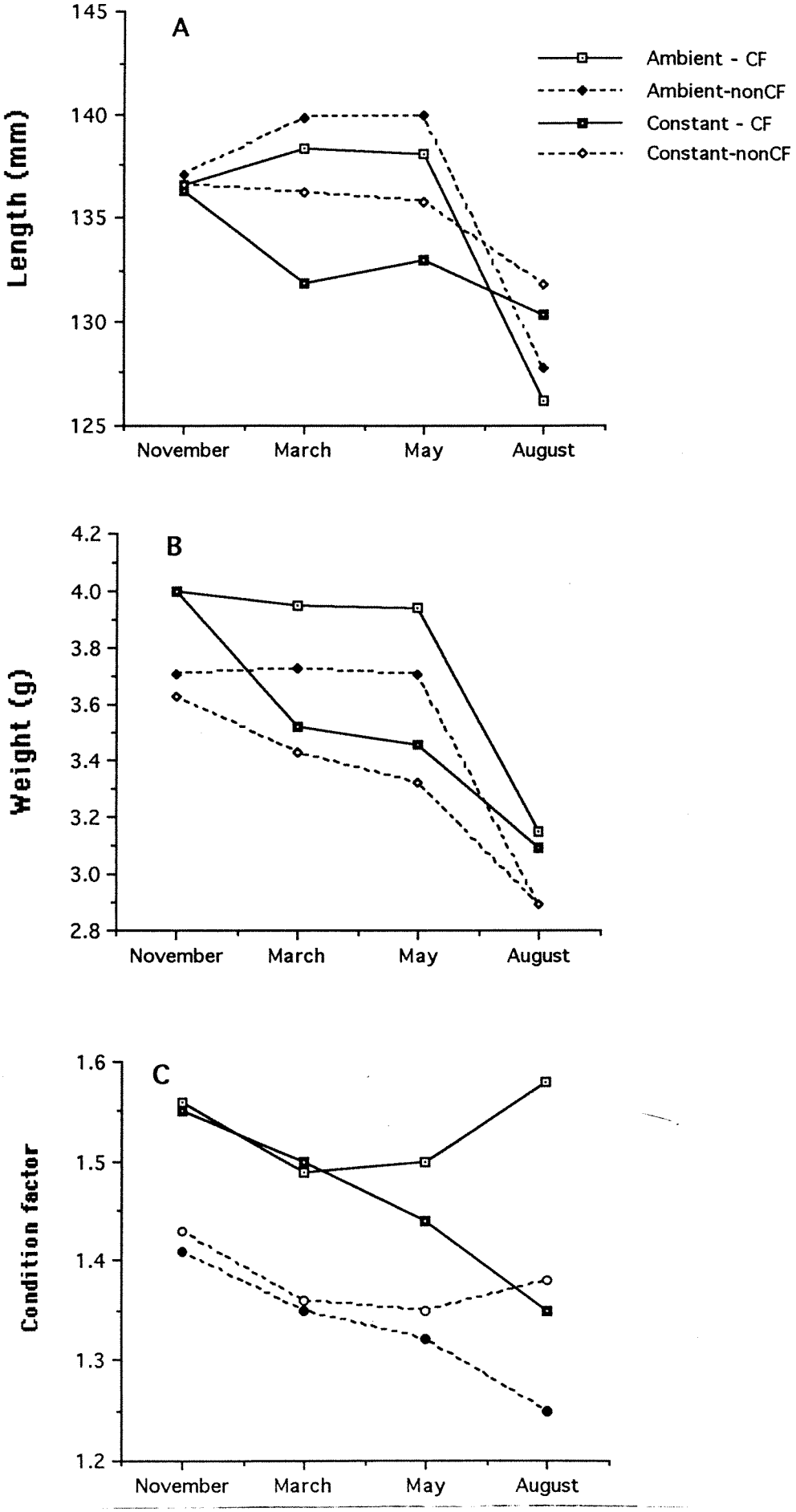

Figure 4. Changes in the length (A), weight (B), and condition factor (C) of presumptive non-(nonCF) and metamorphic (CF) larval sea lampreys between November 1992 and August 1993. For clarity, only means for each sample are shown.

differences in the length of larvae in any of the groups in November, March, May, or August ($P > 0.05$) but the final lengths in August were significantly lower than the initial lengths in November (Fig. 4a). The weight of presumptive metamorphic larvae, which was significantly greater than that of presumptive nonmetamorphic larvae at the beginning of the study, was not significantly different among groups by the end of the study ($P > 0.05$). However, the weights of larvae in all groups were significantly lower in August compared to the initial weights in November. By May, only six larvae in the ambient regime met the three criteria for entering metamorphosis, i.e., ≥ 120 mm and ≥ 3.0 g in size and with a CF ≥ 1.50, five were marked as presumptive metamorphic larvae in the fall and three of these larvae entered metamorphosis by August. In the constant temperature, four larvae had CFs ≥ 1.50 in May, all were considered presumptive metamorphic in the fall, but none of them entered metamorphosis by August.

Changes in CF tended to reflect both temperature and the metamorphic status of the larvae (Fig. 4c). There was little difference in the CF of presumptive metamorphic or nonmetamorphic larvae in the two temperature regimes between November and March. Presumptive metamorphic larvae had significantly higher CFs than presumptive nonmetamorphic larvae during this period. Between March and August the CFs of preumptive metamorphic and nonmetamorphic larvae in the constant temperature regime tended to decrease while the CFs of larvae in the ambient regime tended to increase relative to values at the end of the winter (March). These changes in CF may be related to the loss of weight between November and May as body reserves of lipid are used as an energy source. During the spring the relatively larger changes in length relative to weight in premetamorphic larvae may account for the slight increase in CF of these animals by August.

Significance: 1. Temperature but not metamorphic status influenced changes in length and weight; larvae held in the ambient temperature changed little between November and May but experienced rapid negative changes in length and weight between May and August whereas larvae in the constant temperature underwent negative changes in length and weight continuously between November and August.

2. The incidence of metamorphosis was influenced significantly by temperature ($P < 0.05$), confirming previous findings. Approximately 53% of the larvae in the ambient regime (29/55) entered metamorphosis but only 2% (1/55) in the constant temperature.

3. Our predictions of metamorphosis based on CF were consistent with observed metamorphosis in two cases ($P > 0.05$); seven premetamorphic larvae metamorphosed in the ambient temperature regime and there was no metamorphosis among nonmetamorphic larvae in constant temperature. In the other two cases our predictions were significantly different than observed ($P < 0.05$): we attribute this response to the effects of temperature on metabolic processes.

4. Larvae with a CF of 1.50 or greater in the fall will enter metamorphosis the following July, but there a CF of 1.45 or greater in the fall might be more appropriate for this population.

2. Short-term Influence of Temperature and Larval Density on Metamorphosis

Objective: Most of our studies to date have employed either 13 and/or 21 °C, two points on a continuous scale of temperature. These data are not sufficient for determining the relationship between temperature and the incidence of metamorphosis. Here we employ an intermediate temperature and a temperature below 13 °C in an attempt to establish the type of response to temperature and the approximate lower temperature limit for metamorphosis to occur. Studies to date, with the exception of 1 above, have used larval densities of 164 larvae•m^{-2} (30 larvae per tank). This density is much higher than is normally found in wild populations, except in particularly good "beds" of larval habitat. Thus, we examined the short-term effects of lower larval densities on metamorphosis in this study. The objective of this study was to determine the short-term effects of temperatures other than 13 and 21 °C and larval density on the incidence of metamorphosis.

Design: Since a long-term experiment was in progress at Scarborough College from November 1992 to August 1993 (study 1 above) we used the system developed for previous studies at the Hammond Bay Biological Station (Youson et al. 1993; Holmes et al. 1994). This system consisted of 32 aquaria housed in enclosed cabinets and supplied with aerated, unfiltered Lake Huron water at rates of 4-9 L•min^{-1}.

Larval sea lampreys were collected from the Pigeon River, MI, in early June 1993. This stream was an alternate choice as our first collection stream, the Rifle River, MI, was flooding until late June. We eventually got into the Rifle River in early July, but this was much too late for our laboratory studies of metamorphosis.

Although we collected approximately 900 larvae, only 64 met our original selection criteria of $\geq$ 120 mm and $\geq$ 3.0 g. Consequently we used lower selection criteria of $\geq$ 110 mm total length and $\geq$ 2.0 g weight for anadromous sea lampreys (Potter et al. 1978). Larvae were randomly sorted into 28 aquaria on June 14, 1993 and remained undisturbed until July 28 when total lengths ($\pm$ 1 mm) and weights ($\pm$ 0.01 g) were measured and stage of metamorphosis was determined. To confirm our staging of metamorphosis, the experiment was continued until August 10 when lengths and weights were measured and the stages of metamorphosis were redetermined.

Larval sea lampreys were exposed to fixed temperatures of $\approx$ 9, 13, 17, and 21 °C and two densities, 5 larvae per tank (27 larvae•m^{-2}) and 12 larvae per tank (66 larvae•m^{-2}) for two months. At each temperature 7 tanks were used, 3 for each density, and a seventh tank containing replacement larvae. Photoperiod was held constant at 15 h light:9 h dark and larvae were fed baker's yeast once per week. Each tank received 50.4 g of yeast per feeding (the same quantity as in previous studies) which translates to 1.05 g yeast•larva^{-1}•wk^{-1} in the high density tanks and 2.52 g yeast•larva^{-1}•wk^{-1} in the low density tanks.

Results: The data on the incidence of metamorphosis are too meagre for an analysis of variance using temperature and density as the independent factors. Within each factor we used a G-test to compare our predictions of metamorphosis, based on presumptive metamorphic larvae being at least 120 mm and 3.0 g in size and with a CF $\geq$ 1.50, with observed metamorphosis.

The incidence of metamorphosis was extremely low, consisting of 6 animals at 21 °C (12%, 6/51), 5 animals each at 17 and 13 °C (10%, 5/51), and 4 animals at 9 °C (8%, 4/51). These values are much lower than expected based on our previous studies of temperature and metamorphosis for the GLFC (Youson et al. 1993; Holmes et al. 1994); the differences may be

attributable to our selection criteria for larvae and/or spring weather conditions. We used larvae that were at least 110 m and 2.0 g in size, which is lower than the criteria of 120 mm and 3.0 g established for landlocked sea lampreys. Consequently we knew the majority of animals were unlikely to enter metamorphosis. Even so, we expected that 31% of the larvae (64/204) would metamorphose, but observed only 10% were metamorphosing when the study was terminated in August. Second, the spring was cool and damp, significant warming did not occur until about a week prior to our collections on June 6-9. This perhaps may have inhibited metamorphosis in some way in the laboratory. Although there is a tendency for the incidence of metamorphosis to increase with temperature, a more extensive dataset is required before sound conclusions can be drawn with respect to the nature of this relationship.

There was a tendency for more animals to more animals to metamorphose in the low density tanks compared with the high density tanks at a given temperature (Table 2). Furthermore, our predictions of metamorphosis did not differ significantly from observed metamorphosis in the low density tanks (G-test, $P > 0.05$). In the high density tanks, our predictions were significantly higher than observed metamorphosis ($P < 0.05$) in all but one temperature (13 °C). Placing larvae of the appropriate size in a high density environment about one month prior to the normal commencement of metamorphosis appears to inhibit metamorphosis compared to larvae in a low density and relative to our predictions. However, this finding is qualified because we do not know the effective density of larvae in the habitat from which they were collected.

Table 1. Comparison of observed and predicted metamorphosis. Metamorphosis predictions are based on larvae being ≥ 120 mm and 3.0 g in size and with a CF of 1.50 or greater in June. Values on the same line with an asterisk (*) are significantly different ($P \leq 0.05$).

	Low density		High density	
Temperature	Observed	Predicted	Observed	Predicted
21	4	4	2*	10*
17	3	6	3*	11*
13	3	7	2	6
≈9	3	5	1*	14*

Significance: 1. The incidence of metamorphosis may be inhibited among larvae of the appropriate size in high density habitats (66 larvae•m^{-2}) compared to larvae in low density habitats (27 larvae•m^{-2}).

2. Low density and high temperature (21 °C) appear to be the most favorable conditions for metamorphosis in the laboratory.

3. The incidence of metamorphosis tends to increase with temperature but our data are too meagre to be certain of this interpretation.

4. This study should be repeated using larvae that are at least 120 mm and 3.0 g in size and a broader range of temperatures, e.g., from 5 to 25 °C.

3. Induction of Metamorphosis

(This report summarizes a paper published in the *Journal of Experimental Zoology*. A reprint has been appended to this report)

Objectives: Several attempts to induce metamorphosis in lampreys using drugs have met with varying degrees of success. The age of the lampreys and species-specific responses to different drugs are confounding factors. The purpose of this study was to (1) determine if morphological changes consistent with metamorphosis can be induced in several year-classes of sea lampreys by potassium perchlorate ($KClO_4$); and to (2) determine if the minimum size of 120 mm and 3.0 g combined with a CF of 1.50 are necessary prerequisites for inducing metamorphosis.

Design: Larval sea lampreys were divided into three size-groups on the basis of length: 65-95 mm, 110-119 mm, and $\geq$ 130 mm. The two former groups comprised larvae collected from Brown Creek, a Lake Huron tributary, in late September 1992, while the latter group was collected in the Great Chazy River, NY, in May 1992. Larvae from each size-group were exposed to controls (dechlorinated tap water) and 0.01% and 0.05% $KClO_4$ from November 20, 1992 to March 17, 1993 at Scarborough College. There were three replicate tanks for each size-group treatment combination with 10 larvae•tank^{-1} (125 larvae•m^{-2}) for a total of 27 tanks and 270 larvae (90 per size-group). The tanks were provided with 5 cm of sand and 12 L of water, which was changed every two weeks because the tanks were static. Water temperatures varied with room temperature between 10 and 19 °C, with means of 15-16 °C in individual tanks. Lengths ($\pm$ 1 mm) and weights ($\pm$ 0.01 g) were measured and CF calculated as weight (g)/(length (mm))^3 x 10^6 at the beginning and end of the study. Metamorphosing stages were determined using the descriptions in Youson and Potter (1979).

Results: Only 19% of the larvae in the largest size-group ($\geq$ 130 mm) met or exceeded the criteria for selecting presumptive spontaneous metamorphosing animals (120 mm, 3.0 g, and CF $\geq$ 1.50) in landlocked populations of sea lampreys (Youson et al. 1993).

Metamorphosis was observed in all size-groups and both concentrations of $KClO_4$ but not in the control groups. Size and exposure to $KClO_4$ strongly influenced the incidence of metamorphosis (ANOVA, $P = 0.0001$). The concentration of $KClO_4$ used did not have any detectable effect on the induction of metamorphosis ($P > 0.05$). The incidence of metamorphosis increased from 22% (13/60) in the 65-95 mm group, to 52% (31/60) in the intermediate size-group, to 98% (59/60) among the largest animals. Metamorphosing animals were significantly larger than unmetamorphosed animals in each size-group (t-test, $P < 0.05$). The smallest metamorphosing animal at stage 2 or beyond was 84 mm long, weighed 0.71 g, and had a CF of 1.20. In the 110-119 and $\geq$ 130 mm groups the smallest metamorphosing animals were 107 mm and 1.46 g in size with a CF of 1.19, and 122 mm and 2.1 g with a CF of 1.16 respectively.

Metamorphosing animals in the ≥ 130 mm group were at stages 1 to 6 in their development, with 59% at stage 3 or beyond. In contrast, 77% of the metamorphosing animals int he 110-119 mm group were at stages 1 or 2 and the highest stage observed was stage 3. Of the few animals metamorphosing in the smallest size-group (N = 13), all were at stages 1 or 2.

Significance: 1. $KClO_4$ will induce metamorphosis in sea lampreys at a time when spontaneous metamorphosis does not occur.

2. The induction of metamorphosis depends on the size of the larvae but not the concentration of $KClO_4$ tested. The size-effect presumably relates to the fact that larger (older) larvae have higher concentrations of thyroid hormone levels in their sera and so are better prepared to respond to $KClO_4$.

3. A minimum size of 120 mm and 3.0 g and a CF ≥ 1.50 are not necessary for inducing metamorphosis. Much smaller animals in all size-groups were induced to metamorphose by $KClO_4$.

4. The ability to induce metamorphosis will be useful for studies of developmental phenomena occurring during metamorphosis. The fact that size and CF are not prerequisites for induction should be explored as an alternative population control strategy, particularly if it can be shown that induced animals do not survive metamorphosis and begin feeding parasitically.

4. Serum Concentrations of Thyroid Hormones in $KClO_4$-treated Larval Sea Lampreys

(The following data have been submitted to the *Journal of Experimental Zoology*)

Objectives: Since serum thyroid hormone levels fall during spontaneous metamorphosis in Petromyzon marinus and administration of the antihyperthyroid agent, potassium perchlorate ($KClO_4$), to ammocoetes induces the onset of metamorphosis, we investigated whether this drug results in a similar depression of systemic levels of thyroid hormones. As the degree of response to the inducing agent is influenced by age (see 3 above) and there are increasing serum levels of both thyroxine (T_4) and triiodothyronine (T_3) with increasing age (Youson et al., manuscript submitted), we measured serum T_4 and T_3 concentrations in animals from the inducement experiment (Holmes and Youson 1993 and 3 above). We hoped to provide some explanation for the variable age-related response.

Design: Details of the experimental protocol for inducement of metamorphosis in landlocked sea lampreys are provided in Holmes and Youson (1993) and 3 above. Blood samples were collected from 148 sea lampreys and allowed to clot overnight at 4 °C, centrifuged, and the serum stored at -70 °C for 2 months. The remaining animals (N = 120) were maintained until the end of July ($KClO_4$-treated) or mid-August (controls) 1993 to assess whether metamorphosis would be completed after inducement and if controls entered spontaneous metamorphosis.

Radioimmunoassays (RIAs) for T_4 and T_3 were performed using the Amersham Amerlex RIA kits for these two hormones. All samples were measured in duplicate but in some cases, because of the small serum volume in the smallest animals, samples were pooled or only one

hormone was measured. Blood from 16 larvae in the 65-95 mm group treated with 0.05% $KClO_4$ was pooled into 2 samples to obtain sufficient serum for T_4 and T_3 analysis. As a result, sample sizes were 129 for T_3 and 123 for T_4. The intra- and inter-assay coefficients of variation for both hormones were < 7% and assay sensitivities were 5 and 0.2 nmoles•L^{-1} of serum for T_4 and T_3, respectively.

Results: Serum T_4 and T_3 concentrations in control larvae increased with the size of the larvae (Figs. 5a, b). Although levels of each hormone were not significantly different in the two largest groups ($P > 0.05$), T_4 (77.93 nmoles•L^{-1}) in the ≥ 130 mm group and T_3 in the two largest groups, 22.13 and 23.55 nmoles•L^{-1} respectively, were signficantly higher ($P < 0.05$) than in the smallest size-group (T_4 = 56.03, and T_3 - 15.44 nmoles•L^{-1}). In all cases but one (T_4, 65-95 mm group, 0.01% $KClO_4$), there was no significant difference in either serum T_4 or T_3 concentration in unmetamorphosed larvae and metamorphosing animals of all three size-groups (t-test, $P > 0.05$). There also were no significant differences between serum hormone concentrations in animals at the various stages (1 to 5) of metamorphosis within each size-group ($P > 0.05$). Therefore, with this one exception where larval data were used, means were calculated from the combined data of unmetamorphosed and metamorphosing individuals of each size-group and treatment combination. No difference was seen in serum T_4 and T_3 levels when size-groups treated with either 0.01% or 0.05% $KClO_4$ were compared (t-test, $P > 0.05$).

$KClO_4$ significantly lowered ($P < 0.05$) the serum T_4 concentration of 65-95 mm animals in 0.01% and in 110-119 mm animals in both concentrations but had no effect on T_4 levels in the ≥ 130 mm group, compared to the appropriate controls for each size-group. The declines in T_4 from control levels ranged from 30-32%, 27-31%, and 18-22% in the smallest to largest size-groups respectively. Serum T_3 levels dropped significantly ($P < 0.05$) in all treated groups, with the decrease from control levels being from 91 to 95%. Since the means for the two hormones within each size-group did not differ signficantly ($P > 0.05$) following the two treatment concentrations, we calculated and compared combined treatment means (± 1 SE) for T_4 and T_3 within the three size-groups. For T_4, 62.5 ± 2.98 nmoles•L^{-1} ($N = 38$) in the ≥ 130 mm group was significantly higher ($P < 0.05$) than the 48.85 ± 2.03 nmoles•L^{-1} ($N = 37$) and the 44.14 ± 4.09 nmoles•L^{-1} ($N = 18$) for the 110-119 mm group and 65-95 mm groups respectively, which did not differ from one another ($P > 0.05$). For T_3, the 1.67 ± 0.81 nmoles•L^{-1} ($N = 37$) in the largest size-group was significantly higher ($P < 0.05$) than the 1.28 ± 0.12 nmoles•L^{-1} ($N = 22$) in the smallest size-group, but neither of these values were significantly different ($P > 0.05$) from the 1.55 ± 0.09 nmoles•L^{-1} ($N = 39$) in the 110-119 mm group.

Significance: 1. There is an increase in the concentration of T_4 and T_3 with increasing size (age) as reported earlier in a single population (see our 1992 contract report).

2. $KClO_4$ depresses serum T_3 levels by 91–95% in metamorphosing and unmetamorphosed sea lampreys of all size-groups, but serum T_4 was only decreased by 18-22% in the ≥ 130 mm group when a large proportion of the animals (59%) were at stage 3 or later in metamorphosis. However, by stage 3 in spontaneous metamorphosis of this species, serum T_4 has decreased 74% and T_3 by 91% from larval values. This result implies that suppression of the activity of the endostyle, either directly or indirectly by an antithyroid agent, and an "adequate quantity" of available T_3 are necessary to initiate metamorphosis. Neither the endogenous suppressor of endostylar activity nor the quantity of available T_3 that is adequate are known at this time.

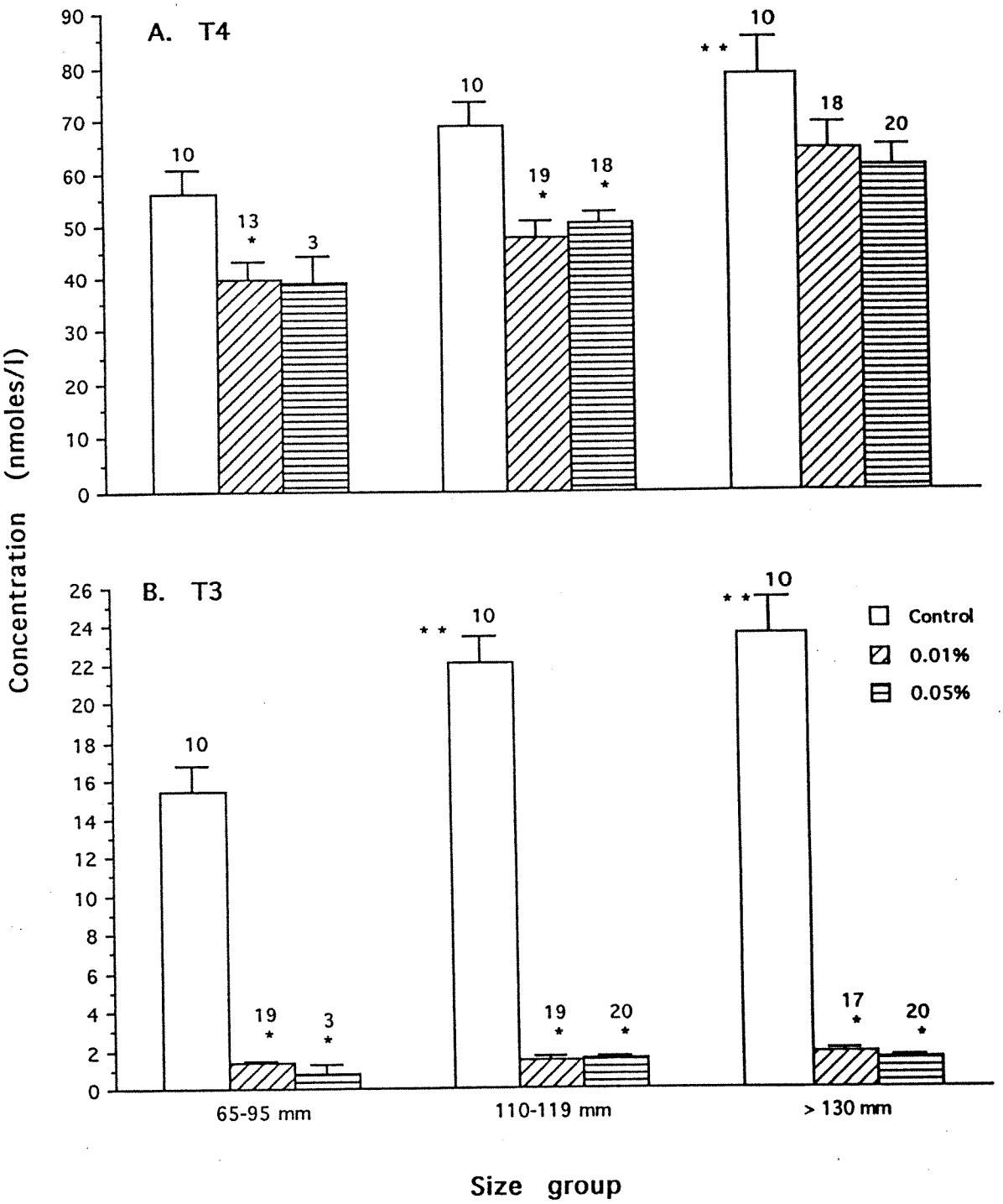

Figure 5. Comparison of the means (± 1 SEM) sera concentrations (nmoles•L^{-1}) of thyroxine (T_4) and triiodothyronine (T_3) in control and $KClO_4$-treated (0.01 and 0.05%) larval sea lampreys of three size-groups (65-95, 110-119, and ≥ 130 mm). The T_4 data in the 0.01%, 65-95 mm group represents unmetamorphosed larvae only, whereas all other means were calculated from data for umentamorphosed and metamorphosing individuals combined. Significant differences ($P < 0.05$) between treatments and controls within each size-group (*) are indicated. Among the control groups, concentrations which are significantly higher ($P < 0.05$) than those in the 65-95 mm group are shown (**). Numbers above the bars indicate sample sizes.

3. None of the animals that we retained for 10 months under $KClO_4$ treatment showed a complete (normal) development. We believe this to be due to the fact that induced metamorphosis does not stimulate the conversion of T_4 to T_3 through the monodeiodinase system. Therefore, we might assume that peripheral monodeiodination of T_4 to T_3 is an essential event in lamprey metamorphosis, for it assures that sufficient T_3 is available to aid in development.

5. Time-series Analysis of Serum Thyroid Hormone Concentrations

Objectives: Previous work has shown that exposure to $KClO_4$ induces metamorphosis in sea lampreys, presumably by suppressing thyroid hormone (TH) levels in the serum. The length of the exposure period necessary to induce metamorphosis is not known. Thus, the objective of this study is to assess changes in concentrations of thyroxine (T_4) and triiodothyronine (T_3) over time in larval sea lampreys exposed to potassium perchlorate ($KClO_4$), an antihyperthyroid drug, and to correlate external morphological changes with TH concentrations.

Design: The study began at the Hammond Bay Biological Station (HBBS) using larvae collected from the Pigeon River, MI. Larvae between 100 and 110 mm long were randomly sorted into 12 tanks ($\underline{N}$ = 120) at a density of 10 larvae•tank^{-1} (125 larvae•m^{-2}). These larvae were chosen because they were too small to enter metamorphosis spontaneously, i.e., in the absence of an inducing agent. The tanks were provided with 7.5 cm of sand and 10 L of unfiltered Lake Huron water. Because the tanks were static, the water was changed and the sand cleaned every two weeks. Larvae were fed baker's yeast once per week at a rate of approximately 0.5 g yeast•larva^{-1}•wk^{-1}. We used the natural photoperiod at the HBBS, which at the start of the study was 15 h light: 9 h dark.

Larval sea lampreys were assigned to control (6 tanks) or 0.05% $KClO_4$ (6 tanks) groups. At approximately logarithmic intervals of 1 week, 4 weeks, 8 weeks, and 16 weeks after the start of the experiment one control and one $KClO_4$ tank were sampled. During the sampling the total length ($\pm$ 1 mm) and weight ($\pm$ 0.01 g) of a larva were measured, sex and the stage of metamorphosis were determined, and blood was collected by caudal severance after anesthetizing the animals with MS-222. The blood from each animal was separated during collection and subsequent analysis. Blood was collected in heparinized capilliary tubes and stored at $\approx$4 °C overnight. After 12-16 h, the tubes were centrifuged for 3 minutes and the serum was collected and stored at -70 °C until the TH analysis was performed. Thyroid hormone concentrations were determined using Amerlex-M T_4 and T_3 radioimmunoassay (RIA) kits supplied by Kodak. The carcasses of all animals were stored at -70 °C for later examination.

The study began on June 16 and was terminated on October 19, 1993. Blood was collected from ten larvae on June 17 to provide baseline values of serum TH concentrations against which subsequent samples would be compared. On August 16 the larvae were transported to our laboratory at Scarborough College. During this transfer the larvae were provided with the appropriate treatment water (control or 0.05% $KClO_4$). At Scarborough conditions were essentially the same as at the HBBS except the photoperiod was derived from fluorescent lights and was held constant at 15 h light:9 h dark and the animals were held in dechlorinated tap water.

Results: The RIAs have not been performed so the serum TH concentration data are not available yet. Thus, this report will present data concerning only the external changes of

metamorphosis that were observed.

Definitive external changes consistent with metamorphosis (at least stage 2 characterisitcs) were first observed in animals after 8 weeks exposure to $KClO_4$. At this time 30% of the animals in $KClO_4$ were at stage 2 and a further 20% were at stage 1 of metamorphosis. After 16 weeks exposure to 0.05% $KClO_4$, 83% of the animals were in the early stages of metamorphosis (stages 1-3), with 21% of those animals (5/24) at stage 1 and 79% at stages 2 or 3. No external signs of metamorphosis were observed among control larvae at any point during the study.

Although there are no significant trends in the length or weight of control animals (Fig. 6), CF tended to decrease after 16 weeks. Larvae exposed to $KClO_4$ tended to undergo negative changes in length, weight, and CF compared to control animals. These changes were most pronounced with respect to weight and, as a consequence, CF. Furthermore, after 16 weeks unmetamorphosed larvae in $KClO_4$ were significantly smaller in size (length and weight) than metamorphosing animals (t-test, $P < 0.05$).

Significance: 1. Definitive external signs of metamorphosis in sea lamrpeys became visible 4 to 8 weeks after the start of continuous exposure to $KClO_4$. That $KClO_4$ is responsible for these changes is supported by the absence of such signs at any time among untreated control larvae of the same size, 100-110 mm.

2. Serum has been collected and will be analyzed for TH levels in the near future. These data should provide a useful time series of changes in hormone levels due to $KClO_4$ that can be correlated with the concomittant external morphological changes.

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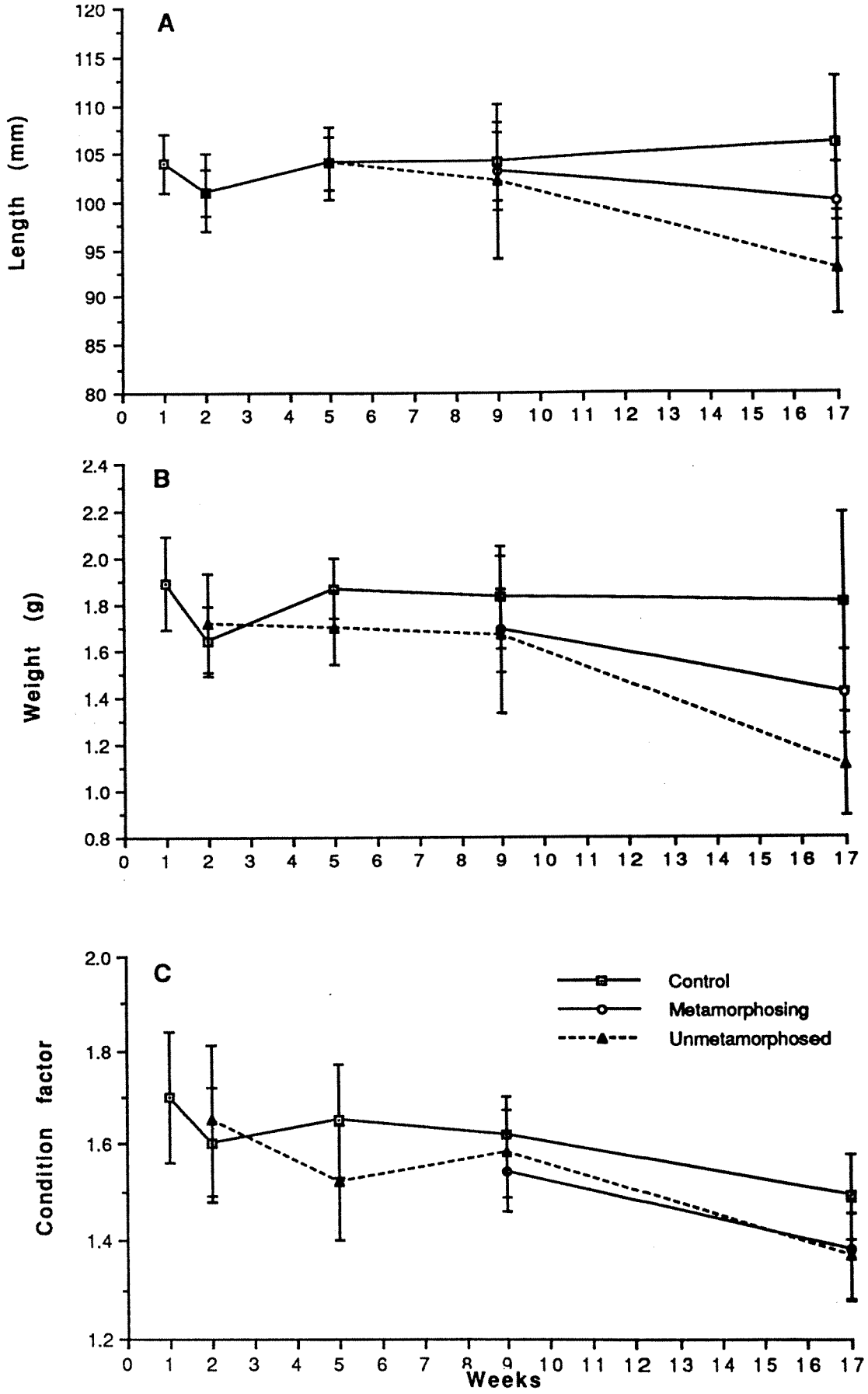

Figure 6. Changes in the length (A), weight (B), and condition factor (C) of control, metamorphosing, and nonmetamorphosing sea lampreys in 0.05% $KClO_4$ from June to August

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