*Comp. Biochem. Physiol.* Vol. 67A, pp. 581 to 586

0300-9629/80/1201-0581$02.00/0

# DAILY RHYTHMS OF AMINO ACID LEVELS IN THE PLASMA OF GOLDFISH (*CARASSIUS AURATUS*)

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(*Received* 17 *March* 1980)

**Abstract**—1. In order to determine the effect of photoperiodicity on the diurnal changes of plasma amino acid levels in the goldfish, *Carassius auratus*, groups of fish were maintained under short day, long day and natural day schedules for 2.5 months.

2. After a 48-hr fast, five fish were killed at 3-hr intervals throughout a 24-hr period and amino acid levels were measured in plasma by a radiochemical method.

3. Except for val, lys and tyr, amino acids showed circadian rhythms in at least two photoperiods. In some of these amino acids (ser, arg, asp, gln, met, leu + ile and his), changes during the day were dependent on the photoperiods, while the rhythms of others seems independent of the type of photoperiod.

4. The highest value of most amino acids showing unimodal rhythm appeared at the onset of the dark phase. The bimodal change found in several amino acids may be related to reported diurnal variations of certain hormones such as corticoids and prolactin that may control these changes, as suggested for other metabolic parameters (Delahunty *et al.*, 1978).

## INTRODUCTION

Diurnal rhythms in goldfish have been studied for their effects on different physiological parameters, including metabolites (Delahunty *et al.*, 1978) and hormones (Leatherland & Mckeown, 1973; Sauerbeir & Meyer, 1977) and the effect of hormones on biochemical aspects has also been reported (Leatherland & Holub, 1978). It has been established that some of these parameters are affected by photoperiodicity (Shapiro & Hoffman, 1975; Mckeown & Peter, 1976). To our knowledge, no studies have been performed on the effects of photoperiods on the circadian rhythms of individual amino acids in teleosts. Using a highly sensitive radiochemical technique for measurement of amino acids with labelled dansyl-chloride (Arola *et al.*, 1976), this study has been made in goldfish maintained under different photoperiods for two and a half months.

## MATERIALS AND METHODS

One hundred and twenty goldfish of both sexes weighing 11.77 ± 0.40 g were used. The fish were obtained from the Barcelona Zoo and maintained in the laboratory in constantly running, aerated tap-water at 12 ± 2°C. The fish were divided into three groups and exposed to different photoperiods: (a) short day photoperiod (SD) with the lights on from 8:00 to 17:00 h; (b) long day photoperiod (LD) with the lights on from 5:00 to 20:00 h; and (c) natural day photoperiod (ND) with sunrise at 7:34 and sunset at 15:35, at the beginning of the experiment (December 2) and sunrise at 6:55 and sunset at 17:34 at the end (February 14). All groups were run in parallel and the fish were fed twice daily at 10:00 and 16:00 with commercial pellet food (Tetra-min). Subjects were starved for 48 hr at the end of the experiments after which five fish were killed every 3 hr throughout a 24-hr period. In each fish, a blood sample from a caudal vein was collected into a heparinized syringe and immediately centrifuged at 3000 rev/min for 20 min at 4°C. Plasma aliquots were stored at −75°C until assayed. The samples were deproteinized with acetone in small-bore capillary tubes (Arola *et al.*, 1977). Individual amino acids were measured in the protein free supernatants by means of a dansyl-chloride radiochemical method (Arola *et al.*, 1976), using norvaline as internal standard. When the spots of two amino acids overlapped in the chromatograms, their composite value was calculated. One-way analysis of variance (Sokal & Rohlf, 1969) was used to determine whether any amino acid amounts changed as a function of the sampling time. Two-way analysis of the variance (Sokal & Rohlf, 1969) was used to compare the absolute levels of each measured amino acid between two experimental groups.

## RESULTS

*Amino acids showing circadian rhythms in at least two photoperiods.*

In Fig. 1 the values are summarized of those amino acids in goldfish plasma which changed during the day, depending on the photoperiods (showing significant variation between at least two photoperiods) and which had a significant ($P < 0.01$) diurnal variation when the fish were exposed to at least two photoperiods. These amino acids are serine, arginine, aspartate, glutamine, methionine, leucine + isoleucine and histidine. In most cases there seemed to be an inductor scotophase as their levels tended to increase by the end of the light period, while there was a consistent decrease by the end of the dark phase. Serine levels showed a non significant, unimodal rhythm in goldfish exposed to the long day (LD) photoperiod while it was significant and bimodal when exposed to

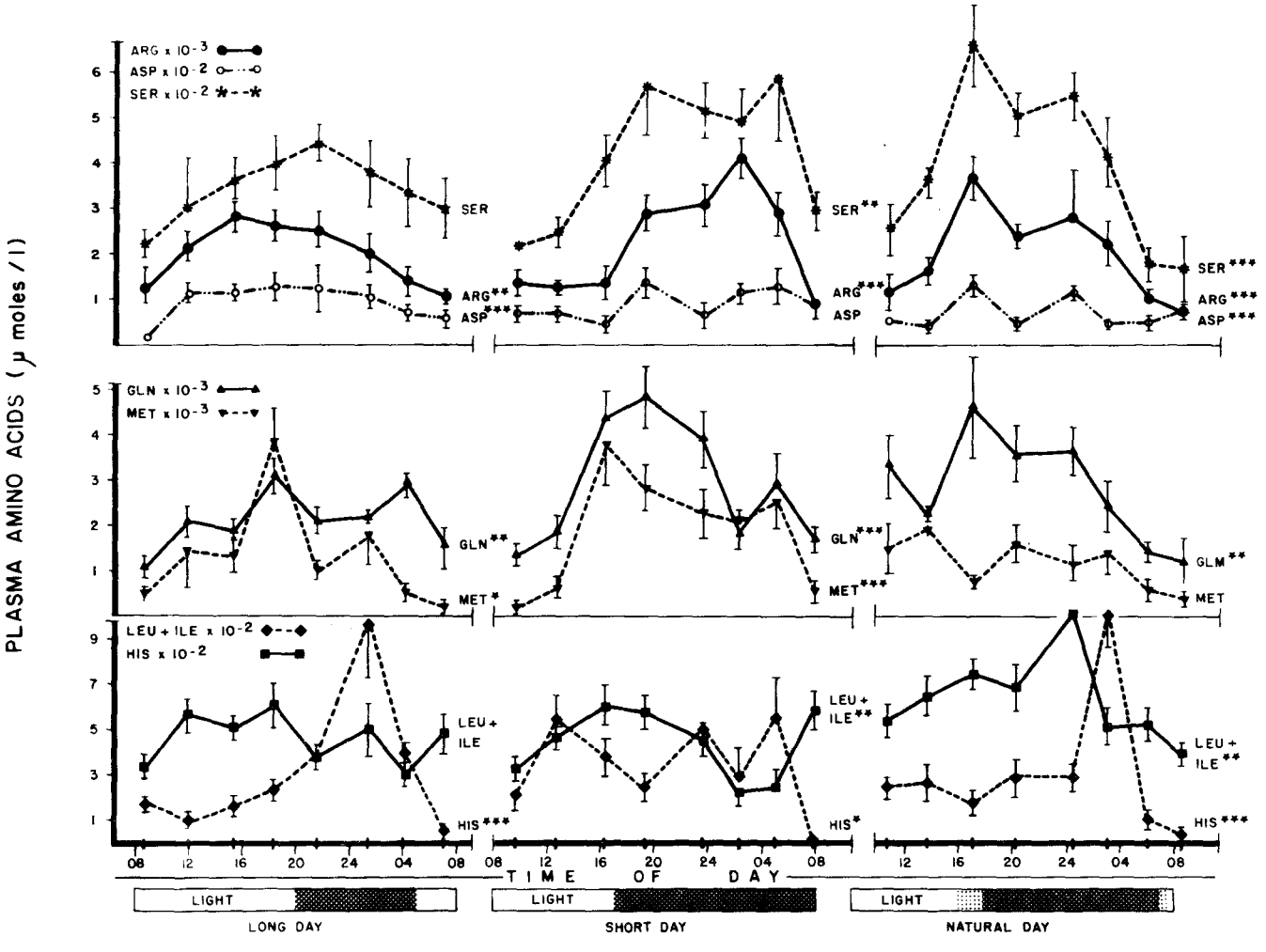


Fig. 1. Plasma level of those amino acids showing circadian rhythms in at least two photoperiods which change is dependent on the photoperiod in goldfish exposed to long-day, short-day or natural-day light schedule. Values correspond to means ± SEM of five animals. The significance of each amino acid change as function of the sampling time is denoted by asterisks: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. No asterisks correspond to not significant.

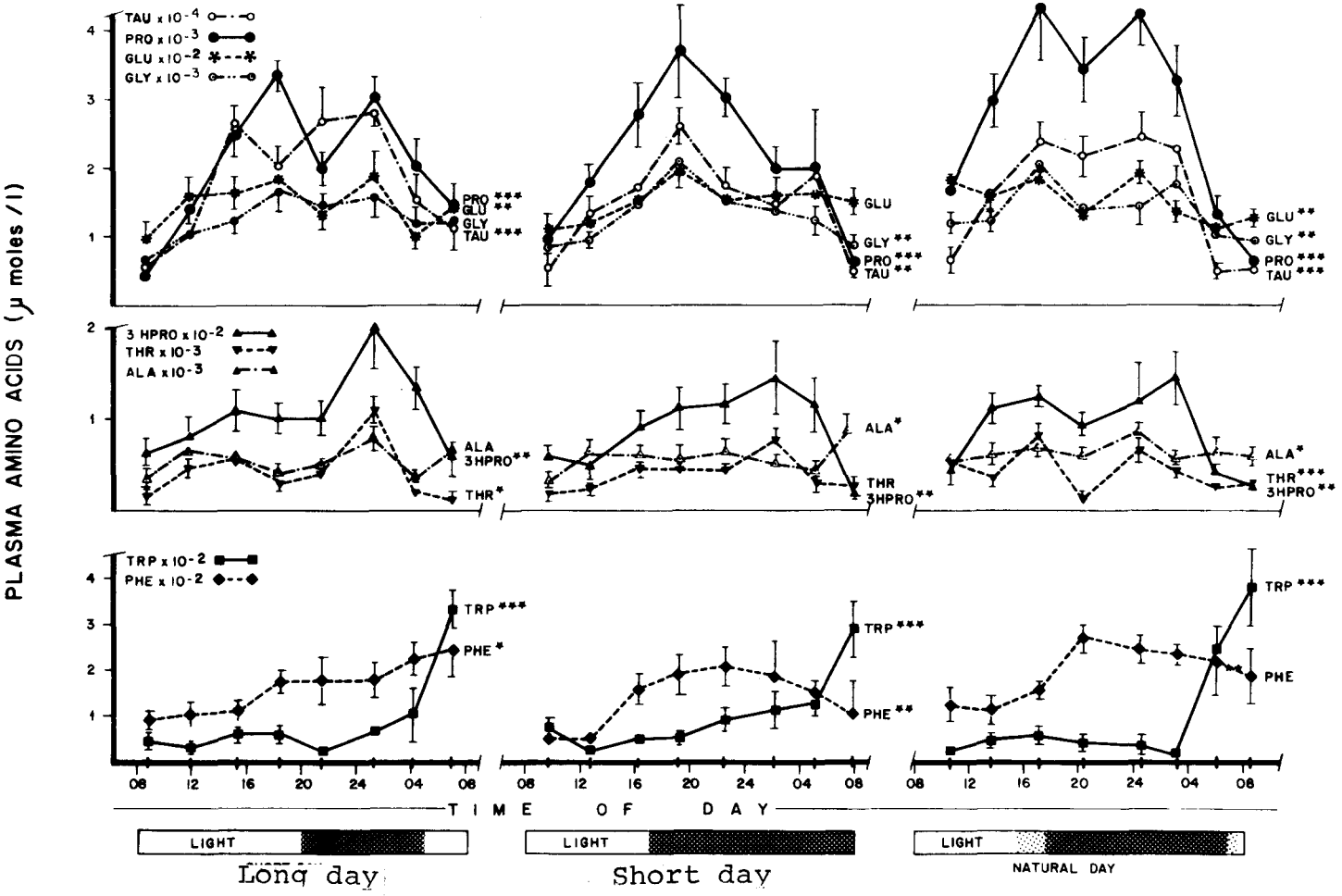


Fig. 2. Plasma level of those amino acids showing circadian rhythms in at least two photoperiods which change seem independent of the type of photoperiod in goldfish exposed to long-day, short-day or natural-day light schedule. Values correspond to means ± SEM of five animals. The significance of each amino acid change as function of the sampling time is denoted by asterisks: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. No asterisk correspond to not significant.

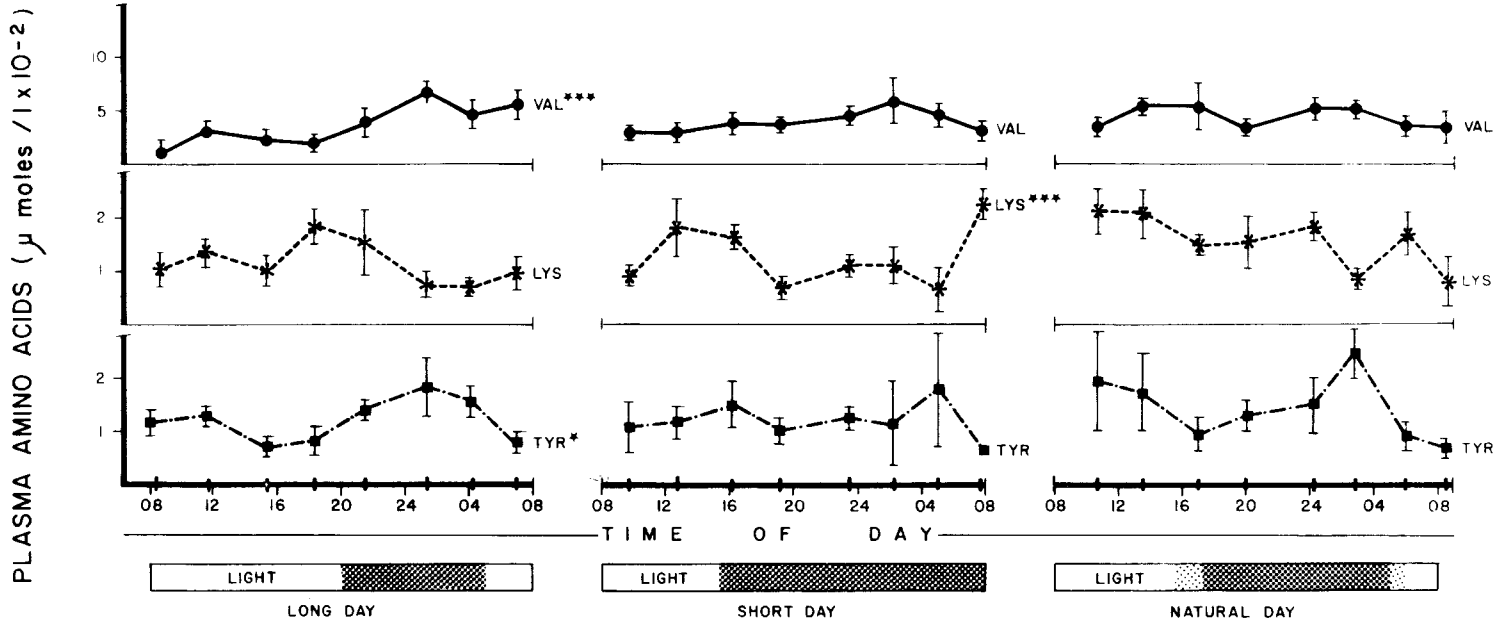


Fig. 3. Plasma level of those amino acids without circadian rhythms in at least two photoperiods in goldfish exposed to long-day, short-day or natural-day schedule. Values correspond to means ± SEM of five animals. The significance of each amino acid change as function of the sampling time is denoted by asterisks: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. No asterisk correspond to not significant.

both short day (SD) photoperiod ($P < 0.01$) and natural day (ND) ($P < 0.001$). The serine levels also differed between photoperiods, being higher in the SD than in the LD ($P < 0.05$). Arginine levels showed significant diurnal variations in goldfish exposed to the three photoperiods, having unimodal change with the LD and SD and bimodal with ND. Arginine levels did not differ among the photoperiods although the sampling time affected this parameter in a different manner in the LD and SD ($P < 0.001$). Aspartate diurnal variations were highly significant ($P < 0.001$) when goldfish were exposed to both LD and ND photoperiods, while they were not significant when exposed to the SD photoperiod. As also seen in Fig. 1, the daily change of plasma aspartate levels was unimodal with the LD while it was bimodal with both SD and ND photoperiods. The daily variation of plasma methionine levels was significant with the LD ($P < 0.05$) and SD ($P < 0.001$) photoperiods but not with the ND. As with other amino acids of this group, the levels of methionine differed with the photoperiod, being significantly higher in the SD than in the LD ($P < 0.01$). Glutamine concentration in goldfish plasma rose more than other amino acids in this group (Fig. 1) at certain times during the dark phase when the animals were exposed to SD or ND photoperiods. Here again, the levels observed with the SD photoperiod were higher than those with the LD ($p < 0.001$). In the three photoperiods studied, the daily variations of glutamine levels were significant and with bimodal change. The methodological system used in the present study did not allow separation of leucine and isoleucine, thus their combined value is presented. The plasma level of these amino acids showed significant diurnal variation when goldfish were exposed to the SD and ND photoperiods, but not to LD (Fig. 1). The levels of leucine plus isoleucine did not differ between the LD and SD photoperiods and unlike other amino acids from this group, leucine + isoleucine levels showed induction during the light, not the dark period. Histidine showed significant diurnal variations in the plasma of goldfish exposed to the three photoperiods (Fig. 1). These levels were affected by sampling times and different photoperiods, demonstrating that their rhythm was dependent on the specific photoperiod.

Other amino acids showed significant diurnal variations when goldfish were exposed to at least two photoperiods, but these changes seem independent of the type of photoperiod; their plasma levels are summarized in Fig. 2. Some of these amino acids showed unimodal circadian rhythms, such as glutamate under SD, glycine under both LD and SD, and tryptophan and phenyl-alanine under the three photoperiod exposals studied, while others showed bimodal rhythms (Fig. 2).

*Amino acids without circadian rhythms in at least two photoperiods*

Plasma levels of valine, thyrosine and lysine varied significantly ($P < 0.05$) throughout a 24 hr period in goldfish exposed to only one of the photoperiods studied, but not in the other two (Fig. 3). Thus valine and thyrosine levels changed significantly in the LD photoperiod while lysine varied only in the SD (Fig. 3).

Table 1. Qualitative changes of plasma level of amino acids in the goldfish exposed to three photoperiods

| Atypic | With rhythms (at least in two photoperiods) | | | | Without rhythms (at least in two photoperiods) |
|---|---|---|---|---|---|
| | Dependent on the photoperiod | | Independent on the photoperiod | | |
| | Unimodal rhythm | Bimodal rhythm | Unimodal rhythm | Bimodal rhythm | |
| His (LD***, SD*, ND***) | Ser (LD)<br>Arg (LD**, SD***)<br>Asp (LD***) | Ser (SD**, ND***)<br>Arg (ND***)<br>Asp (SD, ND***)<br>Met (LD*, SD**, ND)<br>Gln (LD**, SD***, ND**)<br>Leu + Ile (LD, SD**, ND**) | Glu (SD)<br>Gly (LD, SD**)<br>Pro (SD***)<br>3-H-Pro (LD**, SD**)<br>Trp (LD***, SD***, ND***) | Glu (LD**, ND**)<br>Gly (ND**)<br>Tau (LD***, SD**, ND***)<br>Pro (LD**, ND***)<br>Thr (LD*, SD, ND***)<br>3-H-Pro (ND**)<br>Phe (LD*, SD**, ND)<br>Ala (LD*, SD, ND*) | Tyr (LD*, SD, ND)<br>Val (LD***, SD, ND)<br>Lys (LD, SD***, ND) |

Significant diurnal variation for each particular amino acid at each photoperiod is shown by asterisk: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$; no * = not significant, LD = long day, SD = short day, ND = natural day.

## DISCUSSION

Table 1 summarizes changes in levels of different amino acids in the plasma of goldfish exposed to the three photoperiods. This summary shows that most of the circulating amino acids in goldfish exhibited a diurnal variation. Few of them were dependent on the photoperiod, while most were independent of the type of light–dark schedule at which the fish were maintained. The highest value of most amino acids showing unimodal rhythm appeared between 17:00 to 20:00 h which corresponded to the onset of the dark phase in the three photoperiods studied. The values were usually elevated during the first part of the dark phase and began to decrease near the end of this period. These changes may be related to the behavior of these fish which are known to be more active during the day (Spencer, 1939). If this is true, the values may also be related with known daytime changes of liver glycogen levels in goldfish (Delahunty *et al.*, 1978) and indicate an augmented energy and substrate consumption during active day time periods, producing low levels of these metabolites. The rise of most amino acids studied began at 11:00–12:00 hr which was just after the first daily feeding. Thus, although the goldfish were sacrificed after a fasting period of 48 hr, the feeding schedule apparently produced an internal cycle which, without food, caused either the release of amino acids from peripheral tissues (mainly skeletal muscles) to blood, or a decrease in their endogenous consumption by the liver. Feeding times in the goldfish have also been reported to influence liver glycogen, plasma lipids, plasma corticoids, hypothalamic 5-HT and plasma glucose (Delahunty *et al.*, 1978), although the last effect has not always been observed (Chavin & Young, 1970).

Some of the amino acids studied showed unimodal diurnal variations but most had a bimodal rhythm. It has recently been reported that other metabolic parameters such as plasma cholesterol and liver triglyceride levels also exhibited a bimodal variation in goldfish (Delahunty *et al.*, 1978) and in another cyprinid, *Notemigonus crysoleucas* (Pardo & De Vlaming, 1976). The factors controlling these changes may be diverse but the bimodal variations of plasma corticoid levels in goldfish (Delahunty *et al.*, 1978) could be of pivotal importance. Some influence may also be exerted by changes in prolactin levels and although the circadian effects on metabolism of exogenous prolactin administration in the goldfish are limited (Leatherland & Holub, 1978), the augmented level of both plasma (Leatherland & Mckeown, 1973; Mckeown & Peter, 1976) and pituitary prolactin (Delahunty *et al.*, 1978) may determine the high peak of plasma concentration of certain amino acids.

Comparison has been made between the amino acid values obtained in goldfish in the present study and values previously reported in the rat (Arola *et al.*, 1976; Palou *et al.*, 1977) with the same methodological procedure. Except for the high values of arginine in goldfish versus rat plasma, amino acid levels in goldfish sacrificed at the beginning of the light period are similar to those in the rat obtained at the same time (Palou *et al.*, 1977). It is not known whether amino acids in rat plasma show diurnal variations, but the plasma levels of most amino acids (arginine, glutamine, glycine, histidine, proline, taurine, and threonine) in goldfish during dark periods are much higher than any reported for the rat. Whether this difference is due to an enhanced protein breakdown and specific augmented catabolism of other amino acids or to reduced utilization of these amino acids in goldfish during dark periods remains to be established.

*Acknowledgement*—The authors wish to thank Caroline S. Delgado for her editorial help.

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