and auditory stimuli. However, slight differences in P300 distribution with different modalities have also been found in humans (*12*). In contrast, there are profound differences in the scalp distribution of earlier components of the AEP with changes in modality. Thus, it is likely that spatially adjacent structures contribute to the P300 component evoked by stimuli of different modalities in both the human and the cat, whereas separate neural structures are responsible for the generation of the earlier components of the AEP.

Previous research on P300 has been done exclusively on humans. However, localization of the neural generators contributing to P300 will require depth recording and lesion experiments which can be done more easily in nonhuman subjects. Likewise, investigation of neural information-processing mechanisms reflected in the P300 waveform will require detailed study of unit responses and anatomical pathways, which must be carried out in animals. This demonstration in cats of a late positive component in the AEP that behaves comparably to the human P300 provides the opportunity to obtain this important anatomical and physiological information. It is not necessarily the case that precisely the same neural processes give rise to P300 in cats and humans. However, the demonstrated similarity in the behavior of the component between species suggests that the processes might be comparable.

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References and Notes

1. S. Sutton, M. Braren, J. Zubin, E. R. John, *Science* **150**, 1187 (1965); S. Sutton, P. Tueting, J. Zubin, E. R. John, *ibid.* **155**, 1436 (1967).
2. E. Callaway, P. Tueting, S. Koslow, Eds., *Brain Event-Related Potentials in Man* (Academic Press, San Francisco, 1978); E. Donchin, in *Evoked Potentials in Psychiatry*, H. Begleiter, Ed. (Plenum, New York, 1979), p. 13; R. L. Price and B. D. Smith, *Physiol. Psychol.* **2**, 179 (1974).
3. E. R. John, *Science* **177**, 850 (1972); E. S. Boyd, E. H. Boyd, L. E. Brown, *Electroencephalogr. Clin. Neurophysiol.* **42**, 341 (1977).
4. E. Courchesne, S. A. Hillyard, R. Galambos, *Electroencephalogr. Clin. Neurophysiol.* **39**, 131 (1975); K. C. Squires, E. Donchin, R. I. Herning, G. McCarthy, *ibid.* **42**, 1 (1977); T. W. Picton and S. A. Hillyard, *ibid.* **36**, 191 (1974); W. T. Roth, J. M. Ford, B. S. Kopell, *Psychophysiology* **13**, 311 (1976); C. C. Duncan-Johnson and E. Donchin, *ibid.* **14**, 456 (1977); G. McCarthy and E. Donchin, *ibid.* **13**, 581 (1976); L. R. Hartley, *Q. J. Exp. Psychol.* **22**, 531 (1970).
5. R. Simpson, H. G. Vaughan, W. Ritter, *Electroencephalogr. Clin. Neurophysiol.* **40**, 33 (1976); *ibid.* **42**, 528 (1977); *ibid.* **43**, 864 (1977).
6. T. D. Oleson, I. S. Westenberg, N. M. Weinberger, *Behav. Biol.* **7**, 829 (1972); T. D. Oleson, D. S. Vododnick, N. M. Weinberger, *ibid.* **8**, 337 (1973).
7. Tonal stimuli were 50-msec, 85-dB (sound pressure level) tone bursts of either 7 kHz (background stimulus) or 3 kHz (signal) delivered monaurally to the cats' left ears. Light flashes (10 msec, 32 mcd) were from a light-emitting diode (LED) placed 2.0 cm in front of the subjects' right eyes. The tail shock that followed signals during conditioning was a train of 25 current pulses (3 mA, 2 msec), delivered at intervals of 10 msec. Total train duration was 250 msec.
8. The pupillometer was similar to that used by T. D. Oleson, J. H. Ashe, and N. M. Weinberger [*J. Neurophysiol* **38**, 1114 (1975)]. It detected changes in the amount of light reflected by the iris as the cat's pupil dilated and contracted. Light was generated by three LED's and reflected by the iris to a solar cell, which generated a d-c voltage proportional to the amount of light received. This voltage was amplified with operational amplifiers and recorded on a polygraph.
9. Immobilization controlled for stimulus variables (changes in sound field and middle ear muscle contractions) and artifacts of electrophysiological origin (muscle potentials and eye movements). Gallamine acts at the motor end plate of skeletal muscle, leaving the subject conscious but unable to move. For this reason, special care was taken to minimize the discomfort of the subjects. Experimental sessions were limited to 3 hours, and cats were used no more than twice per week. Expired CO_2, cardiac rate, electroencephalogram, and body temperature were monitored to ensure that the cats were maintained in a proper state. The cats we have studied with these methods thrive. Their general condition has been satisfactory and they show no signs of fear (piloerection or hissing) when brought into the experimental chamber.
10. Other work in our laboratory has shown the P300 component to be correlated strongly with the pupil response. Both responses decrease to pretraining levels if backward conditioning (in which the shock precedes the signal) is substituted for conditioning [G. R. Farley, thesis, University of California, Irvine (1980)].
11. The AEP's recorded from the cat with the more anterior electrode placement were less than half as large as those recorded from the other three cats. For this reason we normalized the data from each cat by setting the amplitude of the late positive component elicited by the tone signal at the .10 probability level to 100 percent. The amplitude of each of the subject's other responses was then recalculated as a percentage of that response. These normalized data were used in the analyses of variance. The outcome of an analyses of variance on the raw amplitude data was similar to that obtained with the transformed data. The effect of probability was significant [$F(2, 6) = 10.04$; $P < .025$), while the effect of modality and the modality by probability interaction were not significant.
12. N. K. Squires *et al.*, *J. Exp. Psychol.* **3**, 299 (1977); E. Snyder, S. A. Hillyard, R. Galambos, *Psycho-physiology* **17**, 112 (1980).
13. We thank J. K. Manago for technical assistance. Supported by PHS grant NS 11876-06.

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Lunar Phasing of the Thyroxine Surge Preparatory to Seaward Migration of Salmonid Fish

Abstract. *Anadromous salmonid fish show a distinct surge in plasma thyroxine during the smoltification period prior to their migration to the sea. Analysis of 27 groups of hatchery-reared salmon and anadromous trout indicates that thyroxine levels peak coincident with the new moon. The ability to predict migratory readiness by lunar calendar would have substantial implications for the efficient culture of this economically important protein resource.*

Each year salmon and closely related anadromous trout species hatch in fresh water and, at the end of an early but variable phase of growth and development, migrate to the ocean. In most species this migration is preceded by a dramatic and essential transformation in appearance, behavior, and metabolism known as smoltification. The sum of these developmental changes results in fish which are fully suited to life in the ocean (*1*). The ability to predict when this migration will occur has long been a major concern of agencies responsible for the maintenance and enhancement of salmonid fisheries.

Knowledge of the optimal time for release of hatchery-reared fish is necessary for the efficient operation of salmon enhancement programs. Thus, many phenomena, such as scale loss, changes in coloration, body size, and gill Na^+, K^+-adenosinetriphosphatase activities, have been used as indicators of migratory readiness, but none of these has been completely satisfactory. And, therefore, the problem of when to release hatchery stock has remained, despite the intensive investigative efforts by salmonid culture agencies and research laboratories.

Smoltification may be viewed as a partial analog of amphibian metamorphosis; indeed, the hormones regulating both these phenomena together with their pattern of release may be similar. Unlike amphibian metamorphosis, however, the changes associated with smoltification are of finite duration, and, if the smolted fish are prevented from migrating to seawater, they will revert (desmoltify) to an immature parr-like condition (*1*). After reversion has taken place, young salmon that are transferred to seawater are unlikely to survive or grow. Indeed, if salmon are introduced into seawater, either prior to or following the period of smoltification, mortality is high. Of those that survive, many do not grow but appear generally weak, a condition known as "stunting" (*2*). The accurate timing of the release of hatchery-raised fish is, therefore, clearly critical to the success of these fish in seawater and ultimately to their contribution to the salmonid fishery as a whole.

Although the stunting phenomenon

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may involve malfunction in several endocrine systems, thyroid hypoactivity may be the central cause (*2*). Endocrinologists have known for some time that the thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3), can stimulate, at least in part, virtually every phase in the smoltification process (*1*). It has been reported that during the smoltification of both coho and masu salmon, plasma T_4 rises to a distinct peak before returning to base levels (*3*). Survival of coho in seawater appears to be directly related to the proportion of this T_4 surge completed prior to seawater entry (*4*). This surge thus appears to be an excellent indicator of smoltification. Within a given locale, the T_4 peaks of various stocks will frequently, although not always, coincide. Moreover, the timing of the peaks varies with latitude and from year to year.

For the past several years in collaboration with the California State Department of Fish and Game, Washington Department of Fisheries, Oregon Department of Fish and Wildlife, and U.S. Fish and Wildlife Service, we have continued to monitor the patterns of plasma T_4 through the smoltification period in a large number of selected stocks of Pacific salmon and anadromous trout (*5*).

One of our objectives has been to find a practical and reliable method for timing hatchery releases. The results indicate that, in spite of the considerable variation noted above, there is a high degree of local interstock synchrony each year. Both of the California coho salmon stocks examined in 1980 showed T_4 peaks on 18 March. In 1979 five of six Washington and Oregon coho stocks sampled biweekly had peaks within the same week, and in 1978 eight of nine Washington and Oregon stocks peaked within 6 days of 1 May.

What accounts for this strong synchrony and the coincidence of T_4 peaks among many stocks as well? Earlier investigators (*1*) concluded that there was a strong endogenous component that times the onset of smoltification which is controlled, although not completely, by photoperiod. We believe that an additional external zeitgeber may be involved.

The lunar cycle meets the requirements for such a zeitgeber. It is a cue to which all stocks are exposed, its phase relation with photoperiod varies from year to year, and its period length is short enough to be used as a timing signal by more northern stocks that smoltify later than those in California.

When the possibility of a lunar zeitgeber first arose in discussion, we realized that our sampling frequency (every 2 weeks) was not ideally suited to establishing whether an association existed between a phase of the lunar cycle and the timing of the plasma T_4 peak. In this regime the true plasma T_4 peak could occur as much as a week away from the sample dates. Fortuitously, samples from the California stocks (Fig. 1) that we examined first were obtained close to the new and full moon. In the Mad River stock studied in 1979, the measured plasma T_4 peak occurred within 1 day of the new moon. In 1980 the peak occurred in both of the California stocks (Trinity River and Iron Gate) in the sample taken nearest the new moon (2 days) (Fig. 1). The peaks for Washington and Oregon stocks were also correlated with the occurrence of the new moon.

We reasoned that if a significant association existed between one phase of the lunar cycle and the plasma T_4 peak, the measured peaks should occur in one-half of the cycle more frequently than could be explained by chance. Of the 27 developing salmonid populations analyzed in 1978, 1979, and 1980, 21 showed peaks that fell within the 2 weeks around the new moon. Chi-square analysis showed these data to be highly significant ($\chi^2 = 8.34$; $P < .01$). This is particularly impressive since the sampling frequency itself introduced an error of plus or minus 1 week.

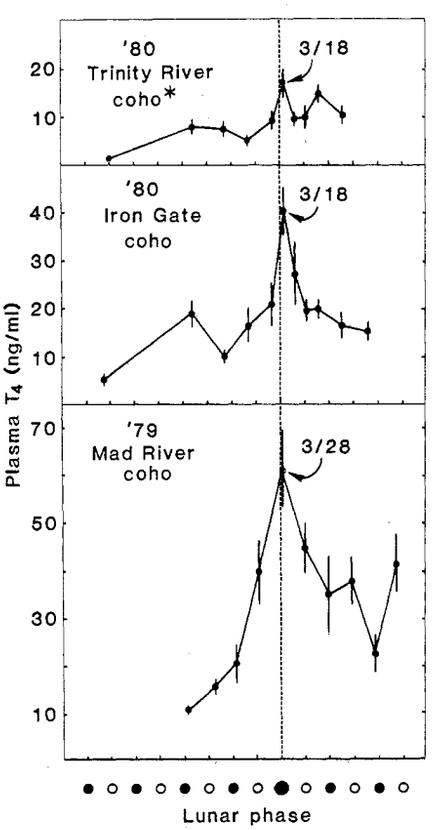

Fig. 1. Patterns of plasma T_4 in three California coho salmon stocks plotted on a lunar calendar. ●, new moon; ○, full moon; *, Trinity stock raised at Mad River Hatchery.

We next asked, given the sampling frequency, whether there was a significant tendency for the measured plasma T_4 peaks to occur on or near a more specific portion of the lunar cycle. In this regard, we did have one important advantage. Although the sampling was not as frequent as we would have preferred, we did sample 27 stocks, and these were measured at random with respect to lunar phase.

In the analysis, the T_4 peaks were handled as a discrete circular distribution about the lunar cycle (*6*), and the mean time for the occurrence of the T_4 peak was calculated as the vector resulting from the sum of individual vectors describing the time-directed T_4 peaks measured for each stock. Accordingly, the resultant time is independent of the reference frame chosen to describe the circular distribution. Plasma T_4 in the salmonids tested showed a significant tendency ($P < .01$) to peak exactly at the new moon with a confidence interval (99 percent) of plus or minus 3.8 days. Thus, the results indicate that the reversal of the trend of increasing plasma T_4 concentration is coincident with the time of the new moon.

At this time we can only speculate as to the potential advantages lunar phasing of the spring thyroxine peak might have for salmonids. Lunar phasing seems generally to coordinate the activities of individuals or to relate activity (or both) to some environmental phenomenon. A large number of behavioral events and physiological variations including spawning and migration display well-described lunar and semilunar periodicities (*7*). The semilunar spawning and hatching of the grunion *Leuresthes tenuis* on the Pacific Coast are well known, but only one of the many such examples among teleost fish (*8*).

Among salmonids, thyroid hormones play a demonstrated role in virtually every phase of development: in growth, in silvering, in the onset of migratory restlessness, in the acquisition of seawater preference, and others (*1*). There is evidence that group behavior, like schooling, may be adaptively important during the seaward migration of most, if not all, anadromous salmonids. Along this line, the migration of steelhead trout through a smolt-trapping system at Mad River essentially began and peaked within a week of the new moon in 1977 (*9*). In another study, behavior associated with seaward migration, although interpreted by the investigators to be dependent on a rise in water temperature, commenced in

Atlantic salmon within 3 days of the new moon in the spring of two different years (*10*). While initial movements may coincide with the new moon and T_4 peak, organized movement of migratory smolts into the estuary and beyond may be delayed. Mason has reported that the outward migration of coho salmon smolts peaked at the full moon (*11*). Inasmuch as this would approximately coincide with the completion of the major part of the T_4 surge (that is, 2 weeks after the peak), one would predict that this would be the period of maximal seawater readiness. Linking the T_4 surge to the lunar cycle offers an appealing model to explain the synchrony of thyroid-dependent developmental changes among and within various salmonid stocks. Moreover, seaward migration appears to be restricted to nighttime hours, and timing migration to coincide with the new moon phase, the darkest nights of the month, could also be advantageous by making it more difficult for predators to locate migratory smolts.

To date, T_4 peaks appear to occur in California stocks during the new moon phase closest to the vernal equinox and at the subsequent new moon in most Washington and northern Oregon stocks. Continued investigation is essential to define the timing for each stock with more precision.

The ability to predict migratory readiness by lunar calendar would minimize resorting to sampling or to complicated technology (such as changes in gill enzyme activities, blood hormone concentrations, or blood ion levels after seawater challenge) and thus would have substantial implications for efficient culture of this economically important protein resource.

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References and Notes

1. See reviews by M. Fontaine, *Adv. Mar. Biol.* **13**, 241 (1975); A. D. Woodhead, *Oceanogr. Mar. Biol. Annu. Rev.* **13**, 287 (1975); W. S. Hoar, *J. Fish. Res. Board Can.* **33**, 1234 (1976); G. A. Wedemeyer, R. L. Saunders, W. C. Clarke, *Mar. Fish. Rev.*, in press; L. C. Folmar and W. W. Dickhoff, *Aquaculture*, **21**, 1 (1980); see also B. Baggerman, *J. Fish. Res. Board Can.* **17**, 295 (1960); *Can. J. Zool.* **41**, 307 (1963); D. Chua and J. G. Eales, *ibid.* **49**, 1557 (1971); J. G. Godin, P. A. Dill, D. E. Drury, *J. Fish. Res. Board Can.* **31**, 1787 (1974).
2. W. C. Clarke and Y. Nagahama, *Can. J. Zool.* **55**, 1620 (1977); H. A. Bern, in *Comparative Endocrinology*, P. J. Gaillard and H. H. Boer, Eds. (Elsevier, Amsterdam, 1978), pp. 97–100.
3. W. W. Dickhoff, L. C. Folmar, A. Gorbman, *Gen. Comp. Endocrinol.* **36**, 229 (1978); K. Nishikawa, T. Hirashima, S. Suzuki, M. Suzuki, *Endocrinol. Jpn.* **26**, 731 (1979).
4. L. C. Folmar and W. W. Dickoff, *Aquaculture*, in press.
5. Plasma samples were collected from the caudal vein of stunned or anesthetized fish whose tails had been severed. Plasma samples were frozen and transported to our laboratories in Berkeley or Seattle and stored until T_4 was measured by a specific radioimmunoassay method.
6. E. Batschelet, *Statistical Methods for the Analysis of Problems in Animal Orientation and Certain Biological Rhythms* (American Institute of Biological Sciences, Washington, D.C., 1965).
7. F. A. Brown, in *Comparative Animal Physiology*, C. L. Prosser, Ed. (Saunders, Philadelphia, 1973), pp. 429–456; W. B. Vernberg and F. J. Vernberg, *Environmental Physiology of Marine Animals* (Springer-Verlag, New York, 1972).
8. B. W. Walker, "Periodicity of spawning by the grunion, an atherine fish," thesis, University of California, Los Angeles (1949); M. H. Taylor, G. J. Leach, L. DiMichele, W. M. Levitan, W. F. Jacob, *Copeia* **1979**, 291 (1979).
9. T. H. Kerstetter, in *University of California Sea Grant College Program Annual Report, 1976–1977*, pp. 157–158.
10. S. M. Fried, J. D. McCleave, G. W. LaBar, *J. Fish. Res. Board Can.* **35**, 76 (1978).
11. J. C. Mason, *ibid.* **32**, 2542 (1975).
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