nies (Fig. 1A). Tumor colonies from the bone marrow of a patient with neuroblastoma grew as a sphere of large (> 25 μm), round, tightly packed cells (Fig. 1D). These colonies grew rapidly and continued to grow for 5 weeks. Ovarian adenocarcinoma cells retained their epithelial morphology (Fig. 1C), and the plating efficiency of these was high enough to indicate linear increase in colonies with increasing numbers of cells plated above 10^3 cells. The characteristic morphology and individual growth kinetics of each colony type have enabled us to distinguish between stem cell colonies of different tumor types and between tumor stem cell colonies and the occasional colonies of normal granulocyte-macrophage precursors. To date, we have studied the behavior of tumor stem cells from metastatic sites only. Clearly, studies of stem cells from the site of origin of the tumor and comparison to metastatic clones will be important and perhaps will elucidate the metastatic process.

We believe that application of such simple in vitro culture techniques for studies of human tumor stem cells from primary explants will prove of clinical importance. First, the technique permits characterization of many of the biophysical properties of tumor stem cells, such as sedimentation velocity, fraction in the S phase as determined by cell death as a result of treatment with tritiated thymidine, and surface antigenic features. Second, formation of in vitro colonies may prove a more sensitive indicator of occult metastatic disease than standard pathological studies. Third, such an assay could potentially be applied to develop individualized predictive trials of anticancer drugs in a manner analogous to techniques used for selection of antibacterial agents. Finally, full realization of the clinical application of bioassay of human tumor stem cell colonies with regard to their sensitivity to drugs, hormones, immunological agents, heat, and radiation could lead to major advances in clinical oncology.

ANNE W. HAMBURGER*
SYDNEY E. SALMON
Section of Hematology and Oncology, Department of Internal Medicine and *Cancer Center, College of Medicine, University of Arizona, Tucson 85724*

References and Notes

1. M. Ogawa, D. Bergsagel, E. McCulloch, *Blood* **41**, 7 (1973); *Cancer Res.* **31**, 2116 (1971); *Blood* **42**, 851 (1973).
2. G. Steel and K. Adams, *Cancer Res.* **35**, 1530 (1975).
3. C. Park, D. Bergsagel, E. McCulloch, *J. Natl. Cancer Inst.* **46**, 411 (1971).
4. R. McAllister and G. Reed [*Pediatr. Res.* **2**, 356 (1968)] have reported that trypsin-dispersed cells from 9 of 17 solid tumors of children formed colonies in soft agar with histological characteristics of the tumor of origin. A. Altman, F. Crusi, W. Rierdan, and R. Baehner [*Cancer Res.* **35**, 1809 (1975)] have reported the in vitro growth of rhabdomyosarcoma colonies from pleural fluid.
5. I. E. Smith, V. D. Courtenay, M. Y. Gordon, *Br. J. Cancer* **34**, 476 (1976).
6. P. Roper and B. Drewinko, *Cancer Res.* **36**, 2182 (1976).
7. Y. Namba and M. Hanoka, *J. Immunol.* **109**, 1193 (1972).
8. The techniques applied to provide stimulation of human myeloma stem cell colony formation deserve some comment. Our technique for preparation of conditioned medium was an extrapolation of the conditions described for primary induction of myeloma in vivo in BALB/c mice with intraperitoneal injection of mineral oil [M. Potter, *Physiol. Rev.* **52**, 631 (1972)]. In addition, Namba and Hanoka (*7*) have demonstrated that long-term culture of mouse myeloma MOPC 104 E cells could be established only by culturing cells with phagocytic cells or media conditioned by these cells.
9. A thiol such as 2-mercaptoethanol was necessary for tumor colony growth in approximately 80 percent of the experiments. We therefore incorporated it routinely in the final culture medium.
10. Colony growth of normal human granulocyte-macrophage progenitors is dependent on the presence of a specific humoral stimulus [colony-stimulating factor (CSF)] [T. Bradley and D. Metcalf, *Aust. J. Exp. Biol. Med. Sci.* **4**, 287 (1966); D. Pluznik and L. Sachs, *Exp. Cell Res.* **43**, 553 (1966); B. Pike and W. Robinson, *J. Cell. Physiol.* **76**, 77 (1971)]. Although no exogenous source of CSF is supplied in our culture system, adherent bone marrow cells can elaborate endogenous CSF. Depletion of these CSF-producing cells, before plating, by allowing adherence to plastic or uptake of carbonyl iron, did not reduce the number or size of myeloma colonies. In addition, antibody to CSF did not appear to reduce the number of myeloma colonies. Therefore, we conclude that colony growth in our system is not dependent on CSF and that the contamination of myeloma colonies by granulocyte colonies is minimal.
11. Supported by PHS grants CA-14102 and CA-17094 and by contract NIH-NCI-C-73-3713 from the National Cancer Institute. We thank Drs. B. G. M. Durie, D. S. Alberts, S. E. Jones, R. E. Lloyd, L. J. McMahon, T. S. Herman, N. Hammond, A. Eisenberg, and J. Rainey for clinical assistance and M. B. Kim, Y. M. Frutiger, and B. J. Soehnlen for technical assistance. Antiserum to CSF was kindly provided by Dr. R. K. Shadduck, Pittsburgh, Pa.
* Address reprint requests to A.W.H.

26 November 1976; revised 8 February 1977

Harvesting Natural Populations in a Randomly Fluctuating Environment

Abstract. *As harvesting effort and yield are increased, animal populations that are being harvested for sustained yield will take longer to recover from environmentally imposed disturbances. One consequence is that the coefficient of variation (the relative variance) of the yield increases as the point of maximum sustained yield (MSY) is approached. When overexploitation has resulted in a population smaller than that for MSY, high effort produces a low average yield with high variance. These observations accord with observed trends in several fish and whaling industries. We expect these effects to be more pronounced for a harvesting strategy based on constant quotas than for one based on constant effort. Although developed in a MSY context, the conclusions also apply if the aim is to maximize the present value of (discounted) net economic revenue.*

The conventional theory of harvested populations (*1–5*) is based on equations in which the various environmental and biological parameters are treated as constants. But environmental randomness can have important effects on the dynamics of animal populations (*6, 7*).

Most animals that are harvested have a net population growth rate that is density dependent. In the unharvested state, the population is maintained around an equilibrium value, K, at which gains from recruitment balance losses from natural mortality. Harvesting constitutes an additional source of mortality; if the harvesting rate is steady and not too high, the population will settle to a new equilibrium value, $N^* < K$, at which the increased intrinsic growth rate balances the losses resulting from harvesting. To maximize this sustained yield, one seeks to determine the intrinsic population growth curve and to harvest at the rate that keeps the population at the maximum of the curve.

As a deliberately oversimplified example (*1, 3, 4*), consider a population $N(t)$ for which the intrinsic net growth rate is logistic, and where the rate of harvesting (the yield per unit time) is EN; here E represents the harvesting effort, and it is assumed that the catch per unit effort is linearly proportional to N. The net growth rate is then

$$dN/dt = rN(1 - N/K) - EN \quad (1)$$

This is illustrated in Fig. 1, in which the intrinsic growth rate rises and falls parabolically as N increases from 0 to the carrying capacity (K), while the harvesting losses increase linearly. For a strategy that keeps effort constant, the equilibrium population is

$$N^*(E) = K(1 - E/r) \quad (2)$$

and the sustained yield (Y) as a function of E is

$$Y(E) = EK(1 - E/r) \quad (3)$$

The maximum sustained yield (MSY) is attained for $E = r/2$, at which point $Y_{MSY} = rK/4$ and $N^* = K/2$. The logistic growth curve in Eq. 1 is only one of many broadly similar forms that have

been proposed in the fisheries literature (*8*). More detailed discussions incorporate the full age structure of the population (*2, 3, 9*).

We now turn to the dynamics and ask what happens if the population is disturbed from the equilibrium value $N^*(E)$ of Eq. 2. A qualitative answer (*1–4*) follows from the observation that $dN/dt > 0$ if $N < N^*$ and $dN/dt < 0$ if $N > N^*$, so that the population tends to return to N^* (Fig. 1). A more quantitative answer may be given in terms of the characteristic return time, T_R (*10*), which describes the typical time it takes the system to recover from a small disturbance. For the logistic equation without harvesting, $T_R = 1/r$. For the population of Eq. 1, harvested at constant E, $T_R(E)$ is given by

$$T_R(E)/T_R(0) = (1 - E/r)^{-1} \quad (4)$$

As E increases, the population becomes more sluggish, taking longer to recover from disturbances. At the MSY point, T_R is twice that for the natural population, and T_R continues to increase as E increases beyond the MSY point. When Eq. 3 is used to express the yield Y as a function of E, T_R may alternatively be expressed as a function of Y, for a harvesting strategy of constant E (*11*) (Fig. 2).

If the environment is randomly varying, the population is subject to a continuous spectrum of disturbances. The relative severity of the consequent population fluctuations depends on the relation between T_R and a diffusion time T_D (which is inversely proportional to the variance of the environmental fluctuations); the larger T_R, the more severe the population fluctuations (*7*). To see this explicitly, replace Eq. 1 by the stochastic differential equation

$$dN/dt = [r(t) - E]N - r_0 N^2/K \quad (5)$$

Here $r(t) = r_0 + \gamma(t)$, where r_0 is the mean value, and $\gamma(t)$ is white noise with mean zero and variance σ^2. This is only one of several ways of introducing random noise into Eq. 1 (*12*); it has the biological motivation of putting the environmental noise into the density-independent term in Eq. 1 (*13*). Equation 5 also applies to the case in which the intrinsic growth remains deterministic, but the harvesting effort is varying randomly (with mean E and variance σ^2). The equilibrium population is now described by a probability distribution, $f(n)$, which may be calculated [according to the Ito calculus (*14*)] as a function of E and the environmental noise level σ^2 (*15*). By calculating statistical moments of this probability distribution, quantities such as the average yield and the coefficient of variation of the yield may be found (*15*) (Fig. 3). In particular, note the way the coefficient of variation (CV) of the yield increases with E.

So far we have dealt with the commonly discussed case of a harvesting strategy of constant E. An alternative strategy is to harvest for constant yield, Y, in which case the population dynamics is described not by Eq. 1, but by (*16*)

$$dN/dt = rN(1 - N/K) - Y \quad (6)$$

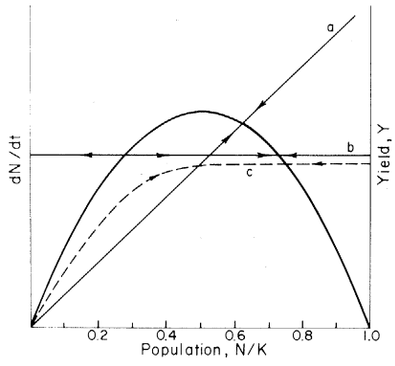
As can be seen from Eq. 6 or from Fig. 1, there are two equilibrium points for a given Y (provided $Y < Y_{MSY}$); one is stable, one unstable. Although the MSY point is still at $N^* = K/2$ and is still $Y_{MSY} = rK/4$, the dynamical behavior of the system under this constant Y strategy is more fragile than that of Eq. 1. Qualitatively (*16*), it may be observed that the population will return to N^* after small disturbances but will collapse to zero if perturbed below the lower (unstable) equilibrium point (Fig. 1). Quantitatively we note that the characteristic return time is

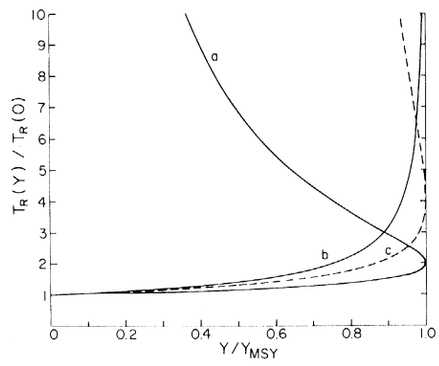
$$T_R(Y)/T_R(0) = (1 - Y/Y_{MSY})^{-1/2} \quad (7)$$

In contrast to Eq. 4, T_R becomes indefinitely large as Y tends to the MSY value. Under a strategy of constant yield, the analog of the stochastic differential Eq. 5 does not have an equilibrium probability distribution; the population fluctuations become larger as time goes on, and the system is doomed to eventual extinction.

A more realistic version of a "constant yield" harvesting strategy will acknowledge that fluctuations to low N imply impractically high values for E, and will include some upper limit to the harvesting effort. Such a strategy is illustrated by the dashed curve in Fig. 1, which aims at constant Y for high N, but accepts a lower yield at low N. The dynamic features of these "modified constant yield" strategies are intermediate between those for constant E and for constant Y (*17*). Figure 2 testifies to this argument by showing $T_R(Y)$ for a typical strategy of this sort.

The simple discussion in terms of T_R captures the essentials of the more detailed analysis in terms of stochastic differential equations. That is, Fig. 2 lays bare the basic features of Fig. 3 and (*15*). All the models in this report are excessively simple, and more realistic discussions will need to incorporate the ef-





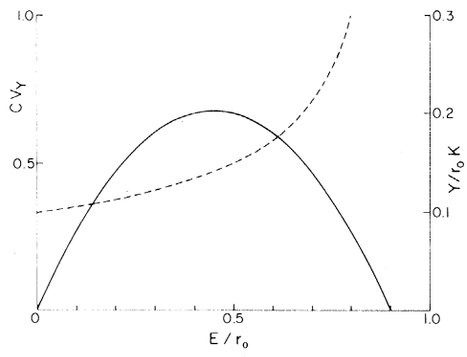


Fig. 1 (upper left). The parabolic curve illustrates the logistic curve for intrinsic population growth, dN/dt, as a function of N. Curves a, b, and c illustrate the population mortality rate (or, equivalently, the yield obtained) under harvesting strategies of constant effort (curve a), constant yield (curve b), and a more realistic modified constant yield strategy (curve c). The points where the harvesting curves intersect the intrinsic growth curve are where sustained yield is possible, and the arrows indicate the way the population will change (under the various strategies) if displaced from the sustained yield point. Fig. 2 (upper right). The characteristic return time, $T_R(Y)$, is shown as a function of yield, Y, for the intrinsic growth curve of Fig. 1 and harvesting strategies of constant E (curve a), constant Y (curve b), and modified constant yield, using the form given in (*17*) with $\epsilon = 0.5$ (curve c). For curves a and c, the lower part of the curve applies if Y is obtained by maintaining the harvested population N^* above the MSY value, and the upper part of the curve applies if N^* is below MSY. Y is plotted as a ratio to $Y_{MSY} = rK/4$. Fig. 3 (lower left). Average yield (solid curve), and the coefficient of variation (CV_Y, dashed curve) of the yield, as functions of the harvesting effort E, for a population which obeys the stochastic differential Eq. 5. The variance in $r(t)$ is here $\sigma^2/r_0 = 0.2$.

fects of age structure (*2, 3, 9*) and of time delays in the population's regulatory processes (*18, 19*). We think that the simple qualitative insights provided by $T_R(Y)$ will continue to be a reliable guide in these more complicated situations, in which the exact analysis will necessarily be numerical.

The main points that emerge from this analysis are:

1) For a population harvested for sustained yield in a randomly fluctuating environment, the relative variability in the population magnitude, and thence in the yield, increases systematically as the harvesting effort increases. That is, the predictability of the catch tends to decrease as the catching effort increases, particularly when overexploitation has resulted in the population's being kept below the MSY level. This appears to be a feature of many fisheries over the past 30 years (*20*) and of some whaling industries (*21*).

2) These effects are relatively more pronounced under a harvesting strategy that seeks to keep the yield constant (a strategy of constant quotas), than under a strategy of constant effort (for example, a fixed total number of fishing hours). Although this conclusion is based on oversimplified models, it is likely to remain true in more sophisticated and realistic studies. If so, it holds implications for the laws regulating fisheries and other harvested populations.

3) We have assumed that the policy aim is to maximize Y. As Clark (*4, 5*; see also *22*) has emphasized, in practice the aim will often rather be to maximize the present value (PV) of discounted net economic revenue. Unless there is a combination of high harvesting costs and a low discount rate, the sustained population value will be below the MSY point. It follows that, in a simple analysis, the characteristic return time will typically be longer, and the population fluctuations relatively more severe, if PV rather than Y is maximized. However, a more realistic accounting of the economic costs of harvesting is likely to introduce feedback mechanisms which help to stabilize the system (*4, 5, 19, 22*).

4) In general, given the environmental unpredictabilities of the real world, stability considerations suggest that it is usually undesirable to use nonfeedback control policies (such as MSY) to manage natural resources.

J. R. BEDDINGTON
Biology Department, University of York, York, Y01 5DD England
ROBERT M. MAY
Biology Department, Princeton University, Princeton, New Jersey 08540

References and Notes

1. M. B. Schaefer, *Inter-Am. Trop. Tuna Comm. Bull.* **1** (No. 2), 27 (1954).
2. R. J. H. Beverton and S. J. Holt, *Fish. Invest. Minist. Agric. Fish. Food (G.B.) Ser. II Salmon Freshwater Fish.* **19**, 1 (1957).
3. D. H. Cushing, *Marine Ecology and Fisheries* (Cambridge Univ. Press, Cambridge, 1975).
4. C. W. Clark, *Mathematical Bioeconomics* (Wiley, New York, 1976); *Science* **181**, 630 (1973).
5. The background papers for the Food and Agriculture Organization Scientific Consultation on Marine Mammals, Bergen, Norway, 31 August to 9 September 1976, contain extensive reviews of harvesting theory. One major recommendation is that the effects of environmental randomness be explored for fisheries models [C. W. Clark, *FAO Document ACMRR/MM/SC/65* (1976)].
6. R. M. May and R. H. MacArthur, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1109 (1972); J. Roughgarden, *Am. Nat.* **109**, 713 (1975).
7. R. M. May, *Stability and Complexity in Model Ecosystems* (Princeton Univ. Press, Princeton, N.J., ed. 2, 1975).
8. S. J. Holt, *FAO Document ACMRR/MM/IV/4* (1975); *FAO Document ACMRR/MM/EC/29* (1975).
9. J. R. Beddington, in preparation.
10. R. M. May, G. R. Conway, M. P. Hassell, T. R. E. Southwood, *J. Anim. Ecol.* **43**, 747 (1974). T_R is essentially the reciprocal of the real part of the dominant eigenvalue. T_R is further defined and discusse ➡ J. R. Beddington, C. A. Free, J. H. Lawton [*ibid.* **45**, 791 (1976)] ➡ D. Ludwig [*SIAM (Soc. Ind. Appl. Math.) Rev.* **17**, 605 (1975)].
11. This formula is

$$\frac{T_R(Y)}{T_R(0)} = \frac{2}{1 \pm \sqrt{1 - Y/Y_{MSY}}}$$

The + sign applies if the sustained yield is obtained by maintaining N^* above the MSY point; the − sign pertains if N^* is below the MSY point.
12. A. R. Kiester and R. Barakat, *Theor. Popul. Biol.* **6**, 199 (1974); H. C. Tuckwell, *ibid.* **5**, 345 (1974); M. Feldman and J. Roughgarden, *ibid.* **7**, 197 (1975); N. Keiding, *ibid.* **8**, 49 (1975).
13. H. S. Horn, *Ecology* **49**, 776 (1968).
14. When white noise in a differential equation is obtained by first letting the noise correlation time tend to zero, and then letting the time step tend to zero, the Ito calculus is appropriate; if the limits are taken in the opposite order, the Stratonovich calculus applies [(*7*), pp. 203–205 and 229–231; R. M. Capocelli and L. M. Ricciardi, *Theor. Popul. Biol.* **5**, 28 (1974); (*12*)]. We use the Ito calculus on the grounds that Eq. 5 is an approximation to an age-structured fish population, with population growth taking place in discrete time steps; however, our general conclusions are not dependent on the choice between the two calculi.
15. The result is

$$f(n) = \beta[\Gamma(\alpha + 1)]^{-1}(\beta n)^{\alpha} e^{-\beta n}$$

where

$$\alpha = 2(r_0 - E - \sigma^2)/\sigma^2$$

and

$$\beta = 2r_0/(K\sigma^2)$$

Hence the average yield is

$$\langle Y \rangle = (KE/r_0)(r_0 - E - \tfrac{1}{2}\sigma^2)$$

and the CV of Y is

$$CV_Y = \left(\frac{\tfrac{1}{2}\sigma^2}{r_0 - E - \tfrac{1}{2}\sigma^2}\right)^{1/2}$$

16. F. Brauer and D. A. Sanchez, *Theor. Popul. Biol.* **8**, 12 (1975).
17. A yield curve that is modified to allow for limitation of effort (curve c in Fig. 1) is

$$Y = ZN/(N + \epsilon K)$$

where ϵ and Z are constants. Using this in Eq. 1 gives the dynamic equation

$$dN/dt = rN(1 - N/K) - ZN/(N + \epsilon K)$$

It is a routine algebraic exercise to find the equilibrium point for this system, and to show that T_R as a function of Y under this harvesting strategy is

$$\frac{T_R(Y)}{T_R(0)} = \frac{(1 + 2\epsilon \pm \rho)}{(1 \pm \rho)(\epsilon \pm \rho)}$$

with

$$\rho \equiv \sqrt{1 - Y/Y_{MSY}}$$

As in (*11*) the + signs apply if N^* is above the MSY value, the − signs if it is below. In the limit $\epsilon \to 0$, we recover Eqs. 6 and 7; in the limit $\epsilon \to \infty$ (and $Z/\epsilon K \to E$), we recover Eqs. 1 and 4; curve c of Fig. 2 shows the intermediate case $\epsilon = 0.5$.
18. F. Brauer, *Math Biosci.*, in press.
19. C. W. Clark, *J. Math. Biol.*, in press.
20. M. B. Schaefer, *Inter-Am. Trop. Tuna Comm. Bull.* **2**, 245 (1957); R. C. A. Bannister, *Rapp. P. V. Reun. Cons. Int. Explor. Mer*, in press.
21. For example, R. Gambell [*FAO Document ACMRR/MM/EC/9* (1975)] and T. Kasuya and N. Miyazaki [*FAO Document ACMRR/MM/EC/25* (1975)].
22. R. M. May, *Nature (London)* **263**, 91 (1976).
23. We thank C. W. Clark, S. J. Holt, and an anonymous reviewer for helpful comments and the King's College Research Centre, King's College, Cambridge, for its hospitality. This work is supported by grant DEB 75-10464 from the National Science Foundation.

8 November 1976; revised 19 January 1977

Pesticide Uptake into Membranes Measured by Fluorescence Quenching

Abstract. *Pesticides that contain chlorine have been shown to quench the fluorescence of carbazole-labeled phospholipids. Incorporation of these carbazole-labeled phospholipids into model membranes provides a system that allows the rapid determination of the uptake rates of chlorinated hydrocarbons into model membranes. This technique can be used in the determination of diffusion rates and partition coefficients of chlorine-containing organic compounds in model membrane systems, and hence may provide a method by which the bioaccumulation potential of synthetic chlorine-containing compounds can be estimated.*

Chlorinated hydrocarbons have played a valuable role in the control of insect-born diseases, and in increasing agricultural production through the control of crop-damaging insects (*1*). As a result, these molecules have been widely dispersed in the biosphere. Unfortunately, chlorinated hydrocarbons such as DDT and the PCB's (*2*) are persistent in the environment and accumulate in the food chain. Bioaccumulation of chlorinated hydrocarbons necessarily involves transport into and across cell membranes. Since many chlorinated hydrocarbons have very low water solubilities they are associated with particulate mat-