Table 1. Photosynthetic rates [milligrams of carbon per gram (dry weight) per hour] of submergent aquatic macrophytes that overwinter in a physiologically active vegetative state.

Organism	Photosynthetic rate			
	Midwinter		Midsummer	
	2°C	23°C	2°C	23°C
Bidens beckii	0.194 ± 0.076	1.02 ± 0.12	0.145 ± 0.037	1.22 ± 0.10
Elodea canadensis	0.368 ± 0.024	1.79 ± 0.13	0.192 ± 0.052	1.82 ± 0.17
Isoetes echinospora	0.166 ± 0.028		0.169 ± 0.011	0.920 ± 0.057
Isoetes macrospora	0.145 ± 0.021		0.088 ± 0.011	0.690 ± 0.035
Myriophyllum alterniflorum	0.323 ± 0.021	1.52 ± 0.10	0.145 ± 0.001	1.63 ± 0.18
Myriophyllum tenellum	0.042 ± 0.004	0.353 ± 0.208	0.070 ± 0.004	0.312 ± 0.045
Potamogeton amplifolius	0.210 ± 0.099	1.03 ± 0.212	0.153 ± 0.008	1.54 ± 0.20
Potamogeton praelongus	0.250 ± 0.071	1.48 ± 0.18	0.175 ± 0.035	1.50 ± 0.14
Potamogeton robbinsii	0.118 ± 0.055	0.433 ± 0.011	0.121 ± 0.016	0.805 ± 0.064
Sagittaria graminea	0.140 ± 0.028			0.820 ± 0.099

*Data are expressed as mean values ± the standard deviation.

new rhizomes were formed, and the vegetative plants began to die. Population densities of *Potamogeton amplifolius* and *P. robbinsii* remained stable yearround and showed minimal seasonal fluctuation. *Potamogeton amplifolius* typically did not fruit when growing at a depth of 3 m or more, and densities from one to ten plants per square meter were found at this depth. In shallow water this species fruited, and in the autumn the dieback in the shallow lake periphery was readily observed. Annual densities of *P. robbinsii* varied from 100 to 1000 plants per square meter at depths from 5 to 7 m.

To confirm that the vegetative plants found during the winter continued to be metabolically active, we removed plants in midwinter and compared photosynthetic rates (*3*) to those of plants from midsummer (Table 1). Plants gathered during the winter exhibited 10 to 20 percent the photosynthetic activity of summer rates. On a clear winter day with minimal snow cover on top of the ice, approximately 4300 lux (light intensity) penetrated to 5 m. Light saturation for photosynthesis by two species, *P. robbinsii* and *P. amplifolius*, was approximately 2300 lux. For *P. robbinsii*, at densities found in Smith Bay at 5 m, maximum midday productivity during the winter ranged from 1.65 to 16.5 mg of carbon fixed per square meter per hour as compared to summer productivity rates, which ranged from 13.2 to 132 mg of carbon fixed per square meter per hour. *Potamogeton amplifolius* exhibited a maximum midday winter productivity at 3 m from 0.27 to 2.68 mg of carbon fixed per square meter per hour as compared to a summer maximum of 4.82 to 48.2 mg of carbon fixed per square meter per hour.

Aquatic macrophytes are an important ecological and limnological component of the aquatic ecosystem (*1*). Their role in summer productivity and contribution to the annual recycling of nutrients is well established (*4*). The omission of references to winter growth of submergent macrophytes in recent books on macrophyte productivity (*5*) suggests that there is relatively little known about growth under ice cover. The best data are those of Rich *et al.* (*6*), who report that *Scirpus subterminalis* maintains substantial viable biomass throughout the winter under ice cover. Rich *et al.* found that the submersed annual macrophytes of Lawrence Lake, represented mainly by *Potamogeton illinoensis* and *P. praelongus*, die back in the fall. In Lake George many species, including *P. praelongus*, when growing at depths of 3 m or more, maintain high viable biomass. Continued winter growth of aquatic annuals in temperate climates is probably considerably more common than had been thought.

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Karyotypic Analysis and Evidence of Tetraploidy in the North American Paddlefish, *Polyodon spathula*

Abstract. *A modal chromosome number of 120 was obtained for the ancient fish*, Polyodon spathula *(Pisces: Chondrostei). The karyotype consists of 48 macrochromosomes and 72 microchromosomes. The microchromosomes are like those found in certain other primitive fishes as well as in reptiles and birds. The possibility that* Polyodon *is a species of tetraploid origin is strongly suggested by the fact that the 120 chromosomes are easily arranged into 30 groups of four homologs each. Evolutionary comparisions are made with other primitive fish groups.*

The most ancient living ray-finned fishes, subclass Actinopterygii, belong to the superorder Chondrostei which includes the paddlefishes, family Polyodontidae, and the sturgeons, family Acipenseridae. Representative polyodontids are found in fossil deposits ranging from the Upper Cretaceous to Recent. Evidence indicates that they evolved from the extinct palaeoniscid fishes of the Pennsylvanian to Cretaceous (*1*). The fossil record also indicates that these chondrostean ancestors were eventually replaced by the better adapted holostean fishes, represented today only by the relict gars and bowfin. Finally, the holosteans themselves were replaced by the superior teleostean fishes which swarm the freshwaters and oceans of today and number approximately 30,000 species.

From an evolutionary viewpoint, it is of interest to determine the chromosome number and morphology of the extant chondrosteans. One species of sturgeon,

Scaphirhynchus platorhynchus, has been karyotyped (*2*). The family Polyodontidae includes only two living species, one found in North America and one in China. Therefore, we began a study to analyze the chromosomes of the North American paddlefish, *Polyodon spathula*, hoping that such information would yield further knowledge about the nature of the karyotype of the ancient ray-finned fishes.

Two male paddlefish, 937 mm and 1027 mm in total length, were obtained from the Tennessee River in Alabama (*3*). Both specimens were injected with colchicine in order to accumulate mitotic chromosomes in metaphase. Injections of 10 ml and 40 ml of 0.01 percent colchicine were made into the dorsal musculature and peritoneal cavity, respectively. The fish were then placed into a large concrete holding pond and were killed after 7 hours. Tissue samples were taken from gill, liver, spleen, kidney, gut, and testes. These tissues were diced into 1 mm cubes, swelled in distilled water for 1 hour, and fixed in a mixture of methanol and glacial acetic acid (3 : 1 by volume) (*4*). Slides were then prepared by the air-dry method (*5*), and stained with silver (*6*). Photographic enlargements were made of well-spread metaphase chromosome plates. The chromosomes were classified either as macrochromosomes if definite arms and centromeres were present, or as microchromosomes if they were dot-like with no discernible arms or centromeres. Macrochromosomes were further classified as metacentric, submetacentric, or acrocentric depending on the position of their centromeres. Gill and kidney tissues proved to yield the best analyzable metaphase chromosome plates.

A modal chromosome number of 120 was obtained for both specimens (Table 1). The karyotype consists of 32 metacentric, 8 submetacentric, and 8 acrocentric chromosomes, and 72 dot-like microchromosomes (Fig. 1). Counts above 120 may be due to chromosome fragmentation during slide preparation, or to the occasional inclusion of cellular fragments or artifacts resembling microchromosomes. Low counts can be attributed to loss of chromosomes through the technique, or overlapping of chromosomes and therefore two or more chromosomes being counted as one.

Our findings of 120 chromosomes in *Polyodon* closely correlate with the findings of Ohno *et al.* (*2*) on another living chondrostean, the sturgeon, *Scaphirhynchus platorhynchus*. The sturgeon is reported to have 112 chromosomes with 50 metacentrics, 14 acrocentrics, and 48 microchromosomes. The holostean gar, *Lepisosteus productus*, has a karyotype of 68 chromosomes with 28 metacentrics, 14 acrocentrics, and 26 microchromosomes (*2*). However, the holostean bowfin, *Amia calva*, has only 46 chromosomes, with 20 of them being metacentric and 26 acrocentric, but none of them being microchromosomes (*2*). The diverse teleosteans have a very heterogeneous chromosome number ranging from 16 to 130, with the basic number appearing to be 48 (*4*, *7*). No microchromosomes have been found in the entire teleostean group. Microchromosomes in fishes have been found in lampreys, some sharks and rays, chimeras, chondrosteans, and holostean gars, but not in the holostean bowfin, teleosteans, brachiopterygians, or dipnoans (*2*, *8*). Microchromosomes are also routinely found in reptiles and birds, and are suspected as being present in one mammal (*9*). Although it is commonly believed that microchromosomes are a primitive characteristic, their occurrence and numbers are highly varied between orders and even families. If the hypothesis is correct that large numbers of microchromosomes are an indication of a primitive state, then it would appear that *Polyodon* with 72 microchromosomes is more primitive than its closest living ally, the sturgeon, with 48 microchromosomes.

Table 1. Distribution of chromosome counts obtained from *P. spathula*.

Cells analyzed (No.)	Chromosome count												
	111	112	113	114	115	116	117	118	119	120	121	122	123
						Specimen 1							
26	1		1	4	2	3	1	3	1	6	3		1
						Specimen 2							
5				1	1					2		1	

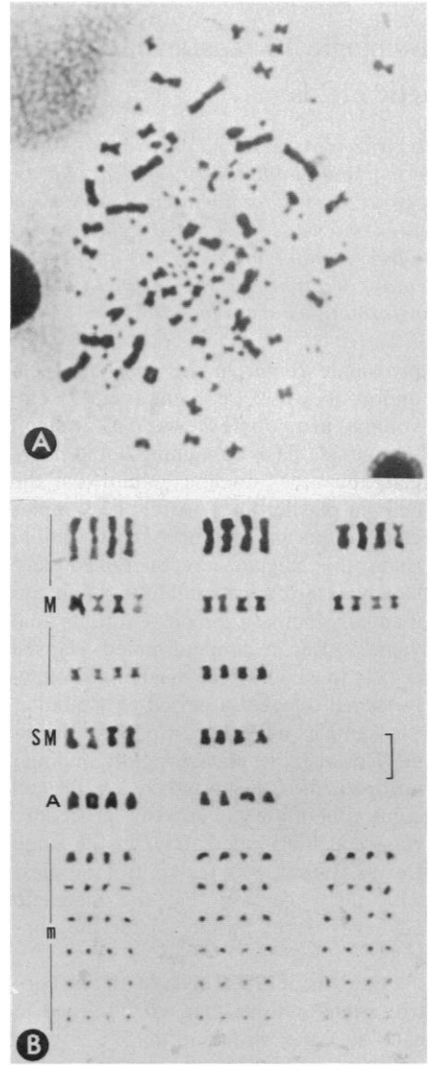

Fig. 1. (A) Mitotic metaphase chromosome spread from gill epithelium of *Polydon spathula*. (B) Chromosome pairs from (A) arranged in groups of fours to demonstrate the hypothesized tetraploidy. (*M*, metacentrics; *SM*, submetacentrics; *A*, acrocentrics, *m*, microchromosomes; scale bar equals 5 μm).

Ohno *et al.* suggested that the sturgeon might be a tetraploid on the basis of it being possible to arrange the first 64 chromosomes into 16 groups of four homologs (*2*). This character is highly evident in *Polyodon* (Fig. 1B), where all 120 chromosomes are clearly and easily arranged into groups of four homologs. If this hypothesis is upheld by further research, one might speculate that the entire actinopterygian fish lineage arose from their ancestral palaeoniscids through tetraploidization. We suggest that very important information might be obtained by a karyotypic analysis of the Chinese paddlefish, *Psephurus gladius*, which has been isolated from its North American relative since at least glacial times.

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Evidence of Morphological and Physiological Transformation of Mammalian Cells by Strong Magnetic Fields

Abstract. *Cultures of L-929 and WI-38 cells, frozen to 4.2°K and exposed for 4 to 8 hours to 5000-oersted magnetic fields, were markedly inhibited in their growth as compared to controls. In cultures grown on cover slips, approximately 7 days after exposure, morphologically distinct cells emerged and were propagated from generation to generation; 3 weeks later, in flask cultures, contact inhibition was abolished. It is concluded that under certain experimental conditions, strong magnetic fields induce morphological and physiological transformations of target cells.*

There have been numerous attempts to demonstrate significant effects of magnetic fields on several biological systems (*1*). Magnetic fields seemingly alter a number of physiological indicators in intact animals (*2*), as well as certain aspects of cellular metabolism (*3*), but no unequivocal effects of magnetic fields on intact mammalian cells have yet been demonstrated.

Under certain experimental conditions, we have found that strong magnetic fields induce an apparently irreversible transformation of cultured eukaryotic cells. Heteroploid L-929 and diploid WI-38 cells were grown in plastic Falcon flasks under standard conditions [Eagle's minimum essential medium (MEM) supplemented with 10 percent calf serum, 2 mmole of glutamine per milliliter, and antibiotics]. The cells were harvested by trypsinization and frozen at a rate of 1°C per minute to –90°C in the presence of 10 percent dimethylsulfoxide (DMSO) in MEM. The frozen samples were then placed in liquid nitrogen and transported to the magnet. They were exposed to magnetic fields of 5000 oersted for 4 to 8 hours, using a standard iron core electron paramagnetic resonance magnet with 12-inch pole faces and a 4-inch pole gap. The samples were placed in the approximate center of the pole gap (field uniformity was 0.1 percent over a 15-cm^3 volume) in a double Dewar flask system, the outside flask containing liquid nitrogen, the inner holding helium and the cells in plastic tubes. After the samples came to thermal and mechanical equilibrium, the magnet was energized very slowly; it took approximately 1 minute to manually increase the current to its final value. After a predetermined elapsed time (4 to 8 hours), the magnet was slowly turned off over a period of 1 minute. The samples were then lifted from the inner Dewar flask, placed rapidly in liquid nitrogen, and transported back to the cell culture laboratory. The same procedure was repeated immediately, in the same Dewar system, with the control samples, with the exception that the magnetic field was never turned on for the controls.

Four sets of L-929 cells (with one control group and one magnetized group in each set) and three sets of WI-38 cells were investigated at approximately equal intervals over a period of 14 months. After a set of target and control cells was obtained, the cells were thawed and allowed to grow, as described below, for periods of time ranging up to 2 months. Some variation of the exposure time to the magnetic field was attempted. Three sets of L-929 target cells and one set of WI-38 target cells were exposed to the magnetic field at a temperature of 4.2°K for 8 hours. The remainder of the experiments involved exposure to 5000-gauss magnetic fields at 4.2°K for 4 hours. (No differences were observed in the results described below for the different exposure times, with the techniques used in this study.)

Experimental and control frozen cells were thawed by immersion in a 37°C water bath. Thawed cell suspensions were then immediately diluted tenfold with MEM and allowed to stand undisturbed until most cells had settled to the bottom of a conical tube. The DMSO-containing medium was then aspirated and the cells were resuspended in fresh MEM supplemented with 10 percent calf serum, 2 mmole of glutamine per milliliter, and antibiotics. At this time, viability of the cells was determined by vital dye (trypan blue) exclusion, and the cells were counted in a hemocytometer. There were no statistically significant differences in viability of control [81 ± 6 percent (standard error of the mean)] and experimental (79 ± 5 percent) cultures. They were then seeded in plastic Falcon flasks and Leighton tubes at a concentration of 300,000 or 40,000 viable cells per flask or Leighton tube, respectively. Randomly selected cultures were demonstrated to be free of contamination by pleuropneumonia-like organisms (*4*).

Cells exposed to the magnetic field attached readily, but their rate of growth was markedly inhibited compared to that of the controls. Thus, while the controls had to be split at approximately 10-day intervals, there was no need to split experimental cultures for as long as 30 to 40 days. Moreover, the experimental cultures failed to maintain a consistent monolayer, leaving extensive free surfaces not covered by cells. In contrast to the controls, approximately 4 weeks after exposure to the magnetic field, the cell cultures formed tightly packed aggregates which, in the case of L cells, coalesced to form ridges several layers deep. Piling up of cells was likewise observed in the experiments with WI-38 cell cultures. The changes described here and depicted in Fig. 1 led us to conclude that in the cells exposed to the magnetic field, contact inhibition was abolished or markedly altered.

Before the loss of contact inhibition and about 7 days after exposure to a magnetic field, distinct cell types emerged in all experimental cultures. In the case of L cells, they were characterized by en-