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# CALIFORNIA FISH AND GAME

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## CONTENTS

	<i>Page</i>
Blood Lead Concentrations in Mallards from Delevan and Colusa National Wildlife Refuges.....David M. Mauser, Tonie E. Rocke, John G. Mensik, and Christopher J. Brand	132
Virulence of Four Isolates of Infectious Hematopoietic Necrosis Virus in Salmonid Fishes and Comparative Replication in Salmonid Fish Cell Lines .....Martin F. Chen, Candace M. Aikens, J. L. Fryer, and J. S. Rohovec	137
The Effects of Different Otolith Ageing Techniques on Estimates of Growth and Mortality for the Splitnose Rockfish, <i>Sebastes diploproa</i> , and Canary Rockfish, <i>S. pinniger</i> .....Christopher D. Wilson and George W. Boehlert	146
Growth and Longevity of Golden Trout, <i>Oncorhynchus aguabonita</i> , in their Native Streams .....Roland A. Knapp and Tom L. Dudley	161
Comparison of Efficiency and Selectivity of Three Gears Used to Sample White Sturgeon in a Columbia River Reservoir.....John C. Elliott and Raymond C. Beamesderfer	174
 NOTES	
Giant Bluefin Tuna off Southern California, with a New California Size Record .....Terry J. Foreman and Yoshio Ishizuka	181
Bobcat Electrocutions on Powerlines.....Richard D. Williams	187
BOOK REVIEWS .....	190
MISCELLANEA.....	192

## BLOOD LEAD CONCENTRATIONS IN MALLARDS FROM DELEVAN AND COLUSA NATIONAL WILDLIFE REFUGES<sup>1</sup>

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**Blood samples were taken from 181 (108 adult drakes and 73 individuals of mixed age and sex) mallards, *Anas platyrhynchos*, from Colusa and Delevan National Wildlife Refuges during late winter and summer of 1987. The percentage of birds with elevated lead concentrations was 28.7 for late winter and 16.4 for late summer. For summer trapped birds, a significantly greater proportion of males than females contained elevated lead levels. These findings indicate that lead poisoning may be a year-round event in certain areas of the Sacramento Valley.**

### INTRODUCTION

Lead poisoning in waterfowl, resulting from ingestion of lead shot, is an issue that has polarized both biologists and the hunting public for many years. It is one of the major diseases of wild waterfowl (Friend 1985) with annual losses estimated to be as high as 2-3 percent of the continent's population (Bellrose 1959). Although large outbreaks have been documented, it is believed that most losses occur as isolated cases (Sanderson and Bellrose 1986; NWHRC, unpubl. data). Lead poisoning is usually a chronic, debilitating disease, requiring 3 weeks or longer from ingestion of lead shot to death. Epizootics become apparent when local predators cannot consume sick and dead birds fast enough to prevent a visible accumulation of sick or dead birds.

Most studies on lead pellet ingestion and lead poisoning mortality are conducted during or just after the waterfowl hunting season (Bellrose 1959; NWHRC, unpubl. data). Few studies have been conducted during the summer or early fall. We provide information on blood lead concentrations in wild mallards, *Anas platyrhynchos*, in California's Sacramento Valley during late winter and summer.

<sup>1</sup> Accepted for publication March 1990.

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## STUDY AREA

Samples were collected at Delevan and Colusa National Wildlife Refuges (NWRs), in Glenn and Colusa counties of California. Both refuges are part of the Sacramento NWR complex and are administered by the U.S. Fish and Wildlife Service. Delevan and Colusa NWRs are comprised of 2280 and 1635 ha, respectively, of seasonally-flooded marsh (60%), watergrass fields, *Echinochloa cruzgalli*, (10%), permanent ponds (5–7%), rice (<2%) and uplands (20%). Each area is managed primarily for fall migrant and wintering waterfowl. Approximately 40% of each area is open to waterfowl hunting. Non-toxic (steel) shot has been required since the fall of 1986.

## METHODS

All birds used in the study were captured opportunistically and thus were not a random sample of mallards from the study area. In addition, most birds sampled were captured on previously hunted areas of the refuge (hunted since 1962).

During February 1987, 108 drake mallards were captured with baited funnel traps. These birds were captured for use as sentinels in a study of botulism on Sacramento NWR. Birds were bled within 24 hours of capture and placed in holding pens where they were fed a combination of rice and scratch grains. Pens were checked daily for sick and dead birds, and cause of death was determined by necropsy and supporting diagnostic laboratory studies at the U.S. Fish and Wildlife Service, National Wildlife Health Research Center (NWHRC) Madison, Wisconsin.

Blood samples were similarly collected from 73 mallards of both sexes trapped from July through early September, 1987. These birds were banded with U.S. Fish and Wildlife Service leg bands, to insure that recaptured birds would not be bled a second time and were immediately released.

Using 21 gauge needles and plastic syringes, blood samples (1.0–2.0 ml) were drawn from the jugular vein of all birds, placed in heparinized glass tubes and frozen for later analysis at NWHRC. Blood lead concentrations were determined with a Perkin-Elmer HGA-400 graphite furnace coupled to a Perkin-Elmer Model 2380 atomic absorption spectrophotometer set at a wavelength of 283.3 nm (Fernandes and Hilligoss 1982). Blood lead concentrations of 0.2–0.5 ppm were considered to be elevated and  $\geq 0.5$  ppm were considered within the range known to be toxic to waterfowl (Friend 1985). Lead poisoning diagnoses in sick and dead birds was based on pathology and a toxic concentration of lead in liver ( $\geq 8.0$  ppm, wet weight) (Friend 1985).

For summer trapped birds, Chi square analysis was used to test for differences in the numbers of males and females with elevated blood lead concentrations ( $>0.2$  ppm; age groupings were consolidated to yield cell expected values of at least 5).

## RESULTS

Of the 108 wild drake mallards captured in February, 15 (14%) had blood lead concentrations greater than 0.5 ppm, 16 (15%) had concentrations ranging from 0.2 to 0.5 ppm, and 77 (71%) were below 0.2 ppm (background concentrations). Within 20 days of capture, 12 birds had died, including 8 of 15

birds (53%) that had blood lead concentrations exceeding 0.5 ppm when initially captured; 2 of 16 birds (12%) with concentrations ranging from 0.2 to 0.5 ppm; and 2 of 77 birds (3%) below 0.2 ppm. Lead poisoning was diagnosed as the cause of death in all 12 birds. Ten of the 12 lead poisoned birds had lead pellets in their gizzards at the time of death (ranging from 1 to 52 pellets). Two of the 12 also had lesions associated with avian cholera, and *Pasteurella multocida* was isolated from their livers. One additional death occurred from among the 77 birds with background lead concentrations. Cause of death was diagnosed as emaciation suspected as a result of parasitism by *Echinuria uncinata*.

Of the 73 birds trapped during the summer, 12 (16%) had elevated or toxic blood lead concentrations. Elevated concentrations were detected in 15% (2 of 13) of birds trapped in July, 11% (5 of 44) in August and 31% (5 of 16) in September (Table 1). A significantly greater proportion of males than females contained elevated concentrations of lead ( $X^2 = 6.62, p < 0.05$ ) (Table 2).

TABLE 1. Distribution of Blood Lead Concentrations of 73 Mallards from Colusa and Delevan National Wildlife Refuges Captured During July, August, and September 1987.

Blood Lead Concentration	Percentage of Total		
	July	August	September
Background ( $\leq 0.2$ ppm).....	85 (11)	89 (39)	69 (11)
Elevated (0.2-0.5 ppm).....	15 (2)	9 (4)	12 (2)
Toxic ( $\geq 0.5$ ppm).....		2 (1)	19 (3)

TABLE 2. Blood Lead Concentrations by Sex for 71 Mallards Captured During July, August and September 1987 on Colusa and Delevan NWRs

Blood Lead Concentration	Percentage of Total	
	Males	Females
Elevated ( $> 0.2$ ppm).....	26 (8)	7 (3)
Background ( $< 0.2$ ppm).....	70 (19)	93 (41)

A significantly greater proportion of males had elevated lead concentrations than females ( $X^2 = 6.62, p < 0.05$ ).

## DISCUSSION

The most sensitive method of determining lead exposure in live waterfowl is the measurement of blood lead concentrations (Anderson and Havera 1985). Dieter (1979) demonstrated that signs of lead poisoning in canvasbacks, *Aythya valisineria*, appeared at blood lead concentrations of 0.2 ppm. At lead concentrations above 0.5 ppm, 12% of canvasbacks exhibited reduced activity of delta-aminolevulinic acid dehydratase (ALAD), a key enzyme in the hemoglobin biosynthetic pathway. Reduced ALAD activity in the brain causes severe biochemical lesions and cerebellar damage (Dieter and Finley 1979). The resulting motor disfunction coupled with other pathologic effects of lead poisoning such as anemia, impaction, and tissue degeneration can eventually lead to death.

Two male mallards trapped in late winter with background lead concentrations ( $< 0.2$  ppm) that later died of lead poisoning, could have ingested lead pellets shortly before or on the day of capture. One lead pellet was found in the gizzard of each bird at necropsy, and thus the lead was not yet detectable in the



blood. The mortality rates of the birds held in captivity do not necessarily reflect rates of lead poisoning mortally in free-ranging populations. Nonetheless, the high lead exposure rate is indicative that lead poisoning is a problem to waterfowl in these areas.

The percentage of adult male mallards trapped in September with elevated or toxic blood lead concentrations (27%) was nearly as high as that of males trapped in February (29%). This was unexpected because most cases of lead poisoning are reported during the winter (Bellrose 1959; NWHRC, unpubl. data). The availability of lead shot is thought to be correlated with the amount of shot deposited on an area during the fall hunting season. However, the heavy clay soils of the Sacramento NWR complex prevent lead pellets from settling into the sediments. Thus, pellets are available to birds on these wetlands year-round. High lead shot ingestion rates have been reported prior to hunting season in other studies. Zwank et al. (1985) found that lead ingestion rates of mallards and northern pintails, *Anas acuta*, were higher before, rather than after, the hunting season on 2 waterfowl wintering areas in Louisiana. Lead pellet ingestion and lead poisoning mortality rates were also higher in the fall rather than in the winter in a study conducted at the Sacramento NWR using sentinel mallards confined to a heavily hunted wetland (NWHRC, unpubl. data). Moreover, lead poisoning in the summer and fall may not be easily observed because summer resident populations are sparse, and sick birds or carcasses do not persist for long in the environment.

The difference in proportions of males and females with elevated blood lead concentrations could be due to differences in feeding habits. Male and female mallards are known to molt at different times (Bellrose 1976). During August and September, males have generally finished molting flight feathers, whereas females are just entering the molt (adults only). These differences in physiologic condition associated with molt might affect their diet (Heitmeyer 1985). Diets high in calcium and protein have been found to mitigate the effects of lead shot ingestion (Sanderson and Bellrose 1986). In addition, females have been shown to be less susceptible to lead poisoning during the breeding season. Increased mobilization of calcium from bones for egg laying and a high metabolic rate apparently decrease the absorption of lead (Finley and Dieter 1978).

Recent conversion to non-toxic shot will not result in immediate major reductions of lead poisoning in certain habitats. The heavy clay soils of the Sacramento Valley apparently reduce settling of lead shot into sediment beyond the reach of feeding waterfowl. Surveys for pellets in wetland sediments at the Sacramento NWR in 1987 revealed densities of up to 900,000 pellets per acre in the top 10 cm (NWHRC, unpubl. data). Although the use of steel shot has been enforced on the National Wildlife Refuges of the Sacramento Valley since the fall of 1986, lead poisoning in waterfowl continues to be documented (NWHRC, unpubl. data).

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# VIRULENCE OF FOUR ISOLATES OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS IN SALMONID FISHES AND COMPARATIVE REPLICATION IN SALMONID FISH CELL LINES<sup>1</sup>

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The virulence of low-passage isolates of infectious hematopoietic necrosis virus (IHNV) obtained from diverse geographic locations was compared in juvenile chinook, *Oncorhynchus tshawytscha*, sockeye (kokanee), *O. nerka*, and coho, *O. kisutch*, salmon, and rainbow (steelhead) trout, *O. mykiss*. All isolates tested were pathogenic for sockeye salmon and trout, but two isolates were comparatively avirulent for chinook salmon. Hybrids of IHNV-resistant coho salmon and susceptible trout appeared to be resistant; however, infection of coho salmon with IHNV was demonstrated. The infectivity and replication of the IHNV isolates were compared in cell lines derived from chinook, coho, and sockeye salmon. Infective dose assays, growth curves, and efficiency of plaquing comparisons showed that the host preference of IHNV isolates could be demonstrated at the cellular level.

## INTRODUCTION

Infectious hematopoietic necrosis virus (IHNV) is a highly destructive pathogen of juvenile salmonid fishes, while adult fish act as asymptomatic carriers (Wingfield and Chan 1970). The virus affects different host species throughout its range on the Pacific Coast of North America. In Alaska, hatchery-reared sockeye salmon, *Oncorhynchus nerka*, have experienced severe outbreaks (Grischowsky and Amend 1976). In California, chinook salmon, *O. tshawytscha*, have been killed by the virus. (Wingfield and Chan 1970). Resident and anadromous (steelhead) rainbow trout, *O. mykiss*, are also susceptible to IHNV, but coho salmon, *O. kisutch*, are considered resistant (Pilcher and Fryer 1980).

In Oregon, epizootics of IHNV have occurred in juvenile steelhead trout at the Round Butte Hatchery on the Deschutes River. Juvenile chinook salmon reared at the same facility have not been affected. However, outbreaks of IHNV have occurred in juvenile chinook salmon reared at the Elk River Hatchery on the Oregon coast (Mulcahy et al. 1980). We compared the virulence of virus isolates from the Round Butte and the Elk River hatcheries, Oregon, and one each from an Alaska and California hatchery to determine if the Elk River and California isolates were unique in their ability to kill juvenile chinook salmon.

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We also wished to determine if Round Butte chinook salmon were resistant to IHNV from the four sample locations. The virulence of the four isolates was also compared in sockeye salmon and rainbow trout. We found the difference in virus epizootics at the Elk River and the Round Butte hatcheries was shown to result from a difference in host preference of the two virus isolates found at these two hatcheries. Round Butte chinook salmon were not more resistant to the virus than the other chinook salmon stocks. Rainbow trout and sockeye salmon were susceptible to all virus isolates tested. Juvenile coho salmon, although demonstrating resistance, could be experimentally infected by IHNV. Resistance to IHNV was also shown in  $F_1$  hybrids of coho salmon  $\times$  rainbow trout.

Differences have been found in the infectivity of IHNV isolates for salmonid cell lines (Phillipon-Fried 1980, Fendrick, Groberg, and Leong 1982). To develop *in vitro* models of host-specific virulence, the replication of the four IHNV isolates was compared in cell lines derived from chinook, coho, and sockeye salmon. Differences among the isolates shown by the *in vivo* experiments were also demonstrated *in vitro* by infective dose assays, growth curves, and efficiency of plaquing.

## MATERIALS AND METHODS

### Cell Lines

Chinook, sockeye, and coho salmon embryo cells (CHSE-214, SSE-5, and CSE-119, respectively, Lannan, Winton, and Fryer 1984) were used in this study. Cells were grown in Eagle's minimum essential medium (MEM) buffered with 10 mM  $\text{NaHCO}_3$ , pH 7.8, supplemented with penicillin (100 IU/ml), streptomycin (100  $\mu\text{g}/\text{ml}$ ), and 5% fetal bovine serum (MEM-5). Growth of cells and all incubations of infected cells were carried out at 16°C.

### Viral Infectivity Assays

Endpoint dilution assays were performed as previously described (Hedrick, Leong, and Fryer 1978). Titers were read after 14 d and expressed as the number of tissue culture infective doses sufficient to infect 50% of inoculated cultures per ml ( $\text{TCID}_{50}/\text{ml}$ ).

Plaque assays were performed using methylcellulose overlays as previously described (Hedrick and Fryer 1981). Titers were expressed as the average of triplicate titrations.

### Growth Curves

Duplicate, 2-d-old monolayers of CHSE-214 and CSE-119 cells in 25  $\text{cm}^2$  flasks were infected with  $10^4$   $\text{TCID}_{50}$  virus in 0.2 ml. After absorption for 1 h, unattached virus was removed with three washes of MEM, and 6 ml of MEM-5 was added to each flask. At various intervals, 0.1 ml of fluid was removed from each flask, diluted in 1 ml MEM, centrifuged at 2000  $g$  for 10 min and frozen at  $-70^\circ\text{C}$ . Finally, samples were simultaneously titered by endpoint dilution assay in CHSE-214 cells.

### Viruses

Viruses were received as primary isolates. The Trinity River (TR), California, chinook salmon isolate was obtained from W. H. Wingfield, California

Department of Fish and Game, Rancho Cordova, CA. The Elk River (ER) chinook salmon isolate and the Round Butte (RB) steelhead trout isolate were provided by W. J. Groberg, Jr., Oregon Department of Fish and Game and Wildlife (ODFW), Corvallis, OR. An isolate from adult chinook salmon at Mendenhall Ponds (MP), Alaska, was received from R. Saft, Alaska Department of Fish and Game, Anchorage, AK. Second-passage virus produced in CHSE-214 cells were used to infect fish. Cell line infectivity assays and growth curves were performed using third-passage virus stocks grown in CHSE-214 cells. Virus harvests were centrifuged at 2000 *g*, pooled, aliquoted and frozen at  $-70^{\circ}\text{C}$  until used. Each pool was titered after thawing by endpoint dilution assay in CHSE-214 cells. Low-passage viruses were used in this study because attenuation and host range adaptation of IHNV has occurred after prolonged serial passage in cell culture (Fryer et al. 1976, Nims et al. 1970).

### Fish

Juvenile fish, eggs, and semen of the four study species of salmonid fishes were provided by the ODFW: chinook salmon from the Elk, Trask and Round Butte hatcheries, sockeye salmon from the Wizard Falls Hatchery, steelhead trout from the Round Butte Hatchery, coho salmon from the Sandy River Hatchery, and rainbow trout from the Oak Springs Hatchery. The juvenile fish, eggs, and semen were from adults found to be negative for IHNV at time of spawning. Juvenile fish were obtained from large lots present in hatching troughs or ponds. Fish were tested immediately after resorption of the yolk-sac, because susceptibility to IHNV under experimental conditions declines rapidly with age (Wingfield and Chan 1970). Coho salmon  $\times$  rainbow trout hybrids were produced by fertilizing eggs from a single rainbow trout with pooled coho salmon sperm collected from 10 fish. Eggs from the same rainbow trout were fertilized with pooled rainbow trout sperm from 10 fish and the pooled coho salmon sperm was used with coho salmon eggs pooled from three fish to produce normal rainbow trout and coho salmon, respectively. Production of true coho salmon  $\times$  rainbow trout hybrids was verified by liver isoenzyme assay performed by G.A.E. Gall, Department of Animal Science, University of California at Davis. All experiments involving live fish were conducted at  $12^{\circ}\text{C}$  in pathogen-free well water.

### Exposure of Fish to Virus

Dilutions of virus were prepared in MEM-5 to achieve concentrations of  $10^1$ – $10^5$  TCID<sub>50</sub> ml when virus was added to containers with 750 ml aerated water. Each container held 20 juvenile chinook salmon or 30 of the smaller steelhead trout or sockeye salmon. In each experiment, control fish were exposed to MEM-5 without virus. After 12-h exposure, the contents of each jar were placed into a separate 68-l aquarium receiving single-pass water flowing at a rate of 1 l/min. Fish were fed twice daily and observed for signs of IHNV infection for 14 d. Dead or moribund fish were removed daily. The 50% lethal concentration (LC<sub>50</sub>) was calculated from the accumulated mortality according to the method of Reed and Muench (1938); however, in cases where mortality was insufficient to determine the LC<sub>50</sub>, an LC<sub>25</sub> was similarly calculated. At the conclusion of each experiment at least 10 control fish were divided into pools of five fish each and examined for virus as previously described (Hetrick, Fryer,

and Knittel 1979) to ensure that the fish were originally free of virus. Five dead fish were pooled from each dilution series and examined for virus to verify that virus was present in dead fish exposed to each isolate.

## RESULTS

### Virulence Testing

Juvenile chinook and sockeye salmon and steelhead trout were exposed to second-passage TR, ER, RB and MP isolates to determine virulence of the isolates in each species (Table 1). In all three chinook salmon stocks tested, the TR and ER isolates were more virulent (lower  $LC_{25}$ ) than the RB and MP isolates, while the TR, RB and MP isolates were more virulent in the sockeye salmon and steelhead trout groups. For the latter two species, the  $LC_{50}$ 's of TR, RB, and MP isolates were similar, while the ER isolate was less virulent. Although RB and MP isolates were virulent for sockeye salmon and steelhead trout, they were comparatively avirulent for all three chinook salmon stocks. Virus was recovered from dead or dying fish in each combination of virus isolate and fish stock, except in the case of Elk River chinook salmon. No Elk River chinook salmon died after exposure to RB or MP. Virus was not found in control fish and mortality did not exceed 5% in any control fish group.

**TABLE 1.** Virulence of Four Isolates of Infectious Hematopoietic Necrosis Virus in Five Oregon Salmonid Stocks. Virulence is Measured by the  $Log_{10}$  Virus Concentration (LC) Required to Produce 25% or 50% Mortality Following a 12-h Exposure.

Fish tested (size)*	Virus Source			
	Trinity River (TR)	Elk River (ER)	Round Butte (RB)	Mendenhall Ponds (MP)
	$Log LC_{25}$ in TCID <sub>50</sub> /ml			
Round Butte chinook (0.42).....	3.3	4.0	5.0	4.8
Trask River chinook (0.54).....	2.3	3.2	4.8	4.3
Elk River chinook (0.57).....	2.1	4.2	> 5.0	> 5.0
	$Log LC_{50}$ in TCID <sub>50</sub> /ml			
Round Butte steelhead (0.23).....	2.8	3.5	2.7	2.7
Wizard Falls sockeye (0.18).....	2.4	2.9	2.2	2.0

\* Average weight of fish in grams at time of exposure. Variation in weight stemmed from average size differences at hatching.

### Resistance of Coho Salmon-Rainbow Trout Hybrids

Groups of 10 each of coho salmon, rainbow trout, and coho salmon rainbow trout hybrids were exposed to the TR isolate at concentrations of  $10^3$ – $10^5$  TCID<sub>50</sub>/ml. The small number of fish used in this experiment was due to the high prehatching and posthatching mortality (98%) of diploid coho salmon rainbow trout hybrids. Coho salmon and coho salmon rainbow trout hybrids were more resistant than rainbow trout (Table 2). Virus was recovered from coho salmon, coho salmon × rainbow trout hybrids, and rainbow trout which died during the 14-d exposure, and also from surviving coho and hybrid salmon.

**TABLE 2. Mortality of Rainbow Trout, Coho Salmon, and Rainbow Trout x Coho Salmon Hybrids Exposed to the Trinity River Isolate of Hematopoietic Necrosis Virus.**

Stock	Mean weight (g)	Mortalities/10 fish Concentration				
		0 <sup>a</sup>	10 <sup>1b</sup>	10 <sup>4</sup>	10 <sup>5</sup>	LC <sub>50</sub> <sup>c</sup>
Oak Springs rainbow trout.....	0.21	0	0	6	8	10 <sup>4.0</sup>
Sandy River coho salmon .....	0.35	0	0	0	6	10 <sup>4.8</sup>
Coho x rainbow hybrid .....	0.19	1	1	0	3	10 <sup>5.0</sup>

<sup>a</sup> Control fish were exposed to the virus diluent, MEM-5.

<sup>b</sup> Concentration of Trinity River IHNV used in 12-h exposure, expressed in TCID<sub>50</sub>/ml.

<sup>c</sup> Median lethal concentration expressed in TCID<sub>50</sub>/ml.

### Infective Dose Determination in Cell Lines

Third-passage virus isolates grown in CHSE-214 cells were titered by the endpoint dilution assay in three salmonid cell lines to determine if the titer of an individual isolate varied according to the cell line used (Table 3). The titer of TR and ER isolates in SSE-5 cells was equal to or less than the titer in CHSE-214 cells. In contrast, the RB and MP isolates produced titers three-to twelve-fold higher in SSE-5 cells than in CHSE-214 cells. Titers of TR and MP isolates were much lower in CSE-119 cells than in the other cell lines. The virus-induced cytopathic effect (CPE) in coho salmon cells was similar to that in the other cell lines, but appeared 2–4 d later and often did not proceed to completion.

**TABLE 3. Titer of Four Virus Isolates Determined by Endpoint Dilution Assay in Cell lines Derived from Chinook (CHSE), Sockeye (SSE), and Coho Salmon (CSE) Embryos.**

Cell line	Virus isolates by chinook salmon stock			
	Trinity River (TR)	Elk River (ER)	Round Butte (RB)	Mendenhall Ponds (MP)
CHSE-214 .....	10 <sup>6.8</sup> <sup>a</sup>	10 <sup>6.7</sup>	10 <sup>5.6</sup>	10 <sup>6.5</sup>
SSE-5 .....	10 <sup>6.6</sup>	10 <sup>6.6</sup>	10 <sup>6.1</sup>	10 <sup>7.6</sup>
CSE-119 .....	10 <sup>3.6</sup>	nd <sup>b</sup>	nd	10 <sup>4.4</sup>

<sup>a</sup> Virus titer expressed in TCID<sub>50</sub> units/ml of undiluted stock virus.

<sup>b</sup> nd = not done.

### Growth Curves

Replication of IHNV in CSE-119 cells was indicated by the appearance of CPE in the TCID<sub>50</sub> assays. To confirm replication, the virus titer was determined after infection of CSE-119 and CHSE-214 cells (Figure 1). An increased titer of virus was detectable 1 d after infection in both flasks of CHSE-214 cells. Release of virus from infected CSE-119 cells was demonstrated 2–4 d after inoculation of virus. Virus titers increased to 10<sup>7</sup> TCID<sub>50</sub>/ml in both CHSE-214 cultures, but did not reach 10<sup>4</sup> TCID<sub>50</sub>/ml in CSE-119 cells. The CPE in the CSE-119 cells did not reach completion 12 d after inoculation of the virus. The TR isolate appeared to be capable of only limited replication in coho salmon cells.

### Efficiency of Plaquing in Cell Lines

The ratio of the titer in CHSE-214 and SSE-5 cells was determined as an index of the relative plaquing efficiency of each isolate (Table 4). The CHSE-214:SSE-5 ratio was 1:1.3 and 1:0.96 for TR and ER, respectively. Ratios for RB and MP isolates were 1:7.4 and 1:2.8, respectively. Thus, the two isolates which were

comparatively avirulent in chinook salmon, RB and MP, plaqued less efficiently in chinook than in sockeye salmon cells, as compared to TR and ER. No clearly definable plaques were observed when the four isolates were plaqued in coho salmon cells (CSE-119).

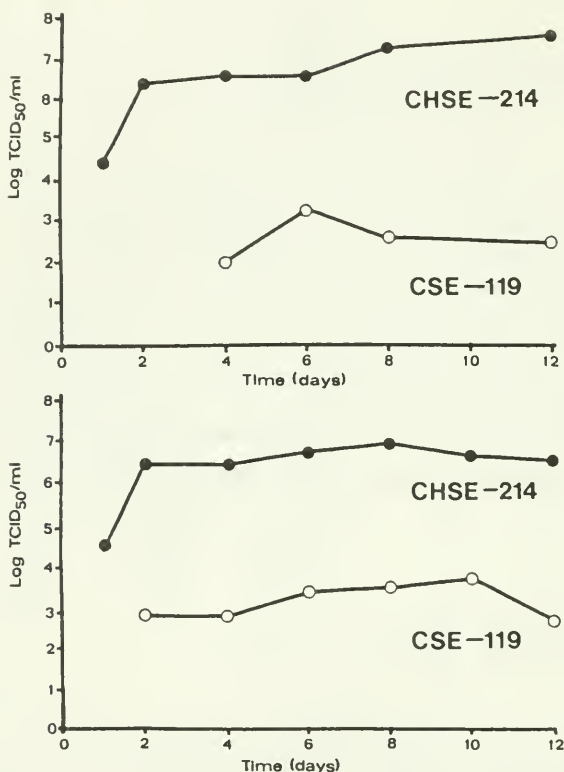


FIGURE 1. Concentration in Log<sub>10</sub> of infectious virus detected in the supernatant fluids of CHSE-214 and CSE-119 cell lines after infection with Trinity River infectious hematopoietic necrosis virus. Each curve represents the titers determined in one of a replicate pair of cell cultures.

TABLE 4. Efficiency of Plaquing of Four Virus Isolates in Cell Lines Derived from Chinook (CHSE) and Sockeye Salmon (SSE) Embryos.

Cell line	Virus isolate by source			
	Trinity River	Elk River	Round Butte	Mendenhall Ponds
CHSE-214	6.2 × 10 <sup>6,a</sup>	5.3 × 10 <sup>6</sup>	8.0 × 10 <sup>5</sup>	1.2 × 10 <sup>7</sup>
SSE-5	8.2 × 10 <sup>6</sup>	5.1 × 10 <sup>6</sup>	5.9 × 10 <sup>6</sup>	3.3 × 10 <sup>7</sup>
	1:1.3 <sup>b</sup>	1:0.96	1:7.4	1:2.8

<sup>a</sup> Virus titer in plaque forming units/ml averaged from three replicate flasks.

<sup>b</sup> Ratio of titer in CHSE-214 cells:titer in SSE-5 cells.

## DISCUSSION

The IHNV isolates examined in this study could be divided into two types: those comparatively avirulent in chinook salmon (RB and MP), and those virulent in chinook salmon, sockeye salmon, and steelhead trout (TR and ER).



This grouping agrees with the natural occurrence of the disease in chinook salmon at the sites of isolation. Although the Alaskan isolate (MP) was isolated from chinook salmon and grown in chinook salmon cells, this isolate possessed far less virulence for chinook salmon than TR or ER. Isolation of IHNV in Alaskan chinook salmon is comparatively rare and may result from exposure to heavily infected sockeye or chum salmon, *O. keta* (Follett, Thomas, and Hauk 1987). Species-specific virulence of IHNV has been previously reported. Virus associated with naturally occurring sockeye salmon epizootics possessed little virulence for chinook salmon experimentally exposed (Rucker et al. 1953, Wingfield et al. 1970). In contrast, IHNV isolated from Sacramento River chinook salmon (SRCV) was found to be pathogenic in chinook and sockeye salmon and steelhead trout (Wingfield and Chan 1970). The TR virus may be similar to SRCV because of its virulence for all species of fish tested. The ER isolate was less virulent in sockeye salmon and steelhead trout than the TR. Strains of IHNV with differing host specificities might act as different agents if present in the same watershed. For this reason, transfer of fish even between two areas where IHNV is present should be regarded with caution.

Wertheimer and Winton (1982) reported variation in susceptibility of chinook salmon stocks to IHNV. Although some variation was found among the three stocks of chinook salmon tested, the results indicated that the lack of IHNV-caused mortality in chinook salmon reared at Round Butte Hatchery is a result of host preference of that strain of virus, rather than an exceptional resistance of Round Butte chinook salmon to IHNV.

Although coho salmon are considered resistant to IHNV, the virus has been found in asymptomatic adult coho salmon at the Trinity River Hatchery (LaPatra et al. 1987). Mortality has not been previously observed following waterborne exposure of juvenile coho salmon to IHNV (Wingfield and Chan 1970, Wingfield et al. 1970). Injection of juvenile coho salmon with virus from the Trinity River resulted in a 3% mortality in one study (Hedrick et al. 1987), but no mortality was observed in other injection experiments (Watson, Guenther, and Rucker 1954; Parisot and Pelnar 1962). The results here may differ from previous studies because of different experimental conditions. Susceptibility to IHNV decreases as the fish grow or the temperature rises (Pilcher and Fryer 1980). We tested coho salmon at a temperature (12°C) conducive to IHNV pathogenesis (Amend 1970). Although some mortality was observed when coho salmon were exposed to very high virus concentrations ( $10^5$  TCID<sub>50</sub>/ml), the coho salmon were more resistant than the rainbow trout. Further studies using larger sample sizes should be done to confirm that IHNV can cause disease or mortality in young coho salmon. The TR isolate should be included in such a study since all reports of virus isolation and mortality in coho salmon have involved virus from the Trinity River system.

Resistance to IHNV was apparently transferred to rainbow trout eggs by coho salmon sperm. The use of pooled rainbow trout sperm, pooled coho salmon sperm, and eggs from one rainbow trout female in this study indicates that the difference in mortality between rainbow trout and coho salmon x rainbow trout hybrids was not due to between-family variation. The transfer of IHNV resistance by coho salmon sperm was also demonstrated by Parsons et al. (1986) using triploid coho salmon x rainbow trout hybrids. Ord et al. (1976) conferred resistance to viral hemorrhagic septicemia by fertilizing eggs of

susceptible rainbow trout with coho salmon sperm. Similarly, the susceptibility of sockeye salmon to IHNV was decreased by fertilization with sperm from more resistant sockeye salmon (McIntyre and Amend 1978). In contrast, coho salmon sperm did not confer resistance to injected IHNV in coho salmon x chinook salmon hybrids, nor were chinook salmon male x coho salmon female hybrids resistant to injected IHNV (Hedrick et al. 1987). Further studies are needed to better define the nature of the resistance factor, and the effects of waterborne or injected virus exposure on hybrid salmonids. Also, future studies with hybrids should use multiple family groups (Amend and Nelson 1977). Another possible problem in our hybrid study was that different sizes of fish were used in the exposure experiments. The fish hatched at different sizes due to the smaller size of the rainbow trout eggs. Smaller fish are more susceptible to IHNV than larger ones, as previously noted. We chose to test all species at a similar stage of maturity: the time of initial feeding.

Evidence for two virulence groups was also found when the four virus isolates were used to infect salmonid cell lines. The titer of RB and MP was lower in chinook than sockeye salmon cells in both plaque and endpoint dilution assays, but TR and ER showed similar titers in cell lines from the two species. Species specificity was also reported by Nims, Fryer and Pilcher (1970), who found that a sockeye salmon cell line produced  $10^2 - 10^3$  times more virus than a chinook salmon cell line after infection by an IHNV isolate from sockeye salmon. Host specificity of IHNV should be considered in the selection of cell lines for diagnostic purposes. In areas where the virus is endemic in sockeye salmon or steelhead trout, the use of CHSE-214 cells may result in reduced chances of detection of virus. Still, the SSE-5 cell line was highly susceptible to the four isolates studied.

Wingfield et al. (1970) found no increase in IHNV titer in CSE-119 cells exposed to an isolate from sockeye salmon, but we found virus titer increased after CSE-119 cells were inoculated with TR. Some of this increase may have been due to absorbed virus releasing from the cell surface, but the appearance of CPE indicates true replication did occur. Possible reasons for the contrasting results may be in the use of the virulent TR isolate, or that the age of cell monolayers and temperature of incubation were lower in the present study. No IHNV plaques were observed by Phillipon-Fried (1980) in CSE-119 cells. We obtained similar results when the four IHNV isolates were inoculated in CSE-119 cells. The limited replication and incomplete CPE seen in infected coho salmon cells may explain the low mortality in coho salmon experimentally exposed to IHNV. The relative resistance of CSE-119 cells suggests that the resistance of coho salmon is in part determined at the cellular level.

### ACKNOWLEDGMENTS

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## THE EFFECTS OF DIFFERENT OTOLITH AGEING TECHNIQUES ON ESTIMATES OF GROWTH AND MORTALITY FOR THE SPLITNOSE ROCKFISH, *SEBASTES DIPLOPROA*, AND CANARY ROCKFISH, *S. PINNIGER*

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Different ageing techniques affect not only the estimates of length at age but also estimates of population growth and mortality rates. This study considers these effects for the splitnose rockfish, *Sebastes diploproa*, and canary rockfish, *S. pinniger*, based on ages determined from the surfaces and sections of otoliths collected during a trawl survey off the west coast of North America in 1980. Estimates of growth based on surface rather than section ages were nearly identical for *S. diploproa* but were higher for *S. pinniger*; slightly different whole otolith ageing techniques are suspected of producing these interspecific differences. For both species, however, estimates of mortality were reduced by more than half when section rather than surface ages were used.

### INTRODUCTION

Ages of fish are needed to estimate two vital parameters of exploited fish populations, namely growth and mortality rates. Many fish species typically are aged by interpreting rings on the otolith, as is the case for rockfishes (*Sebastes*) for which the otolith is the preferred ageing structure (Six and Horton 1977, Chilton and Beamish 1982). Various techniques have been developed to facilitate the detection and the interpretation of otolith patterns used in age determination (Chilton and Beamish 1982). Two methods to determine ages are counting the number of rings or annuli viewed on the exterior of the whole otolith (surface ages) or on a lateral cross section of the otolith (section ages). Section ages are often greater than surface ages for older specimens of many long-lived, slow-growing species (Beamish 1979a, 1979b, Boehlert and Yoklavich 1984), and recent evidence demonstrates that the section ages represent the true ages of fish (Bennett et al. 1982, Leaman and Nagtegaal 1987, Campana et al. in press).

Because present evidence supports the validity of section ages for older fish, important biological and management implications could result if many demersal fish stocks are underaged due to the more common surface ageing

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technique. Archibald et al. (1981) found that estimates of instantaneous mortality ( $Z$ ) were reduced by as much as 50% when the otolith sections were used to age 10 species of rockfishes. Even for fishes that are not long lived, age length data without older fish affect the estimation of von Bertalanffy growth parameters (Hirschhorn 1974). Because population size, growth, and mortality are the basic data required for most production modeling, systematic bias in age determination may lead to serious errors in determining stock production estimates (Le Cren 1974). In the present study, we determine whether significant differences in the estimates of growth and mortality rates are affected by the method of otolith age determination for two species of *Sebastes*—the splitnose rockfish, *S. diploproa*, and canary rockfish, *S. pinniger*.

## MATERIALS AND METHODS

Otoliths from *S. diploproa* and *S. pinniger* were collected during a 1980 trawl survey conducted off the west coast of North America (lat 36° 49' to 50° 00'N). Gear, sampling design, and catch processing generally followed the procedures in Dark et al. (1983). Several differences in the collection and care of otoliths have been described by Boehlert and Yoklavich (1984). Otoliths used in the present study were initially collected for other work; therefore, fish comprising the age subsample were systematically chosen until a predetermined number for each size class was attained. When mortality rates were estimated in the present study, the original age subsample was applied to a simple random sample of length-frequency data to remove any potential sampling bias (Dark 1975) which may have been introduced during sample collection.

The length-frequency data were chosen for each species in the following manner. For *S. pinniger*, the limited length-frequency data from the 1980 survey were combined with length-frequency data collected in 1980 by the Washington Department of Fisheries and the Oregon Department of Fish and Wildlife. For *S. diploproa*, the length-frequency sample was restricted to fish from lat 40° 26' to 48° 47' N, where 76% of the fish in the age sample were collected. This was done because Boehlert and Kappenman (1980) detected increases in growth rate with latitude for *S. diploproa* when specimens were compared from this and two other geographic regions (i.e., lat 34°–37°, 37°–40°, 40°–48° N). Thus, all *S. diploproa* otoliths were analyzed as a single stock, even though 24% were collected south of 40° N, in order that the effects of using surface versus section ages could be evaluated with as large an age sample as possible. Resulting growth curves would most likely represent fish from the northernmost strata (40°–48° N) described by Boehlert and Kappenman (1980).

## Ageing

Ages were determined from the same whole and sectioned left sagittal otolith of *S. diploproa* and *S. pinniger* by one of three readers from the same laboratory. The variability in ageing the otolith data set within and between readers is presented in Boehlert and Yoklavich (1984). Surface ages for both species were derived from whole otoliths by the method of Boehlert and Yoklavich (1984). The otolith was read from the focus to the dorsal edge or, in the case of older individuals, from the focus to the posterodorsal region (Figure 1A, 1B). Otoliths from many older specimens, particularly *S. diploproa*, possess posterior projections. Pairs of translucent and opaque bands in these regions

were included in the counts for *S. diploproa* but not for *S. pinniger*; methodology for the latter species follows the general technique in Six and Horton (1977).

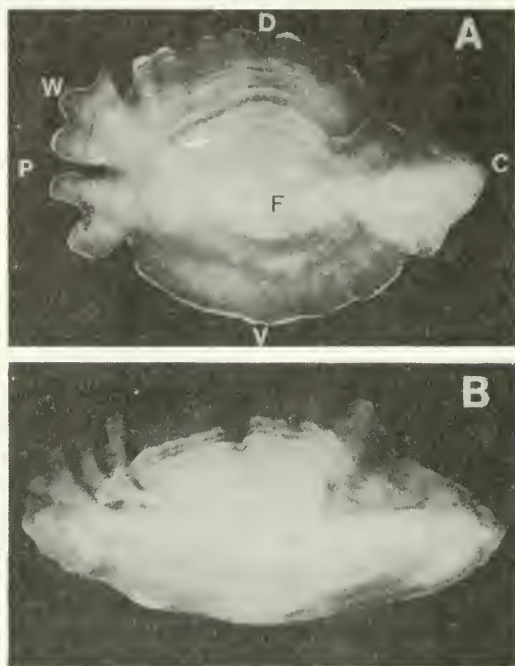


FIGURE 1. External surface of the whole otolith from (A) a 32 cm fork length (FL) female *Sebastes diploproa* with a surface age of 22 years and a section age of 25 years, and (B) a 52 cm FL male *S. pinniger* with a surface age of 18 years and a section age of 19 years. (C) anterior, (D) dorsal, (F) focus, (P) posterior, (V) ventral, (W) posterior projection.

Dorsoventral otolith sections (0.4 mm thick; Figure 2) were obtained with a double-bladed diamond saw; each section was mounted on a microscope slide and polished to remove surface artifacts (Nichy 1977, Boehlert 1985). The otolith section was read (30X or 100X) from the focus to the dorsal edge. For older individuals, the reader began counting toward the dorsal tip, then followed a distinct ring from the dorsal region of the section into the internal dorsal quadrant and continued counting towards the section edge (Figure 2).

In many species of rockfishes, surface and section ages tend to agree for younger individuals (Beamish 1979b, Shaw and Archibald 1981, Boehlert and Yoklavich 1984). For example, G. Boehlert and M. Yoklavich (unpub. data) systematically subsampled every fourth otolith pair from *S. diploproa* and every third otolith pair from *S. pinniger* used in this study and found mean differences between surface and section ages of  $\leq 2$  years for *S. diploproa* females aged  $\leq 12$  and males  $\leq 17$  years and *S. pinniger* females  $\leq 7$  and males  $\leq 8$  years. For this reason, we assigned a section age equal to the surface age for all remaining otoliths with surface ages less than or equal to the values listed above. Thus, an otolith from an *S. pinniger* male with a surface age of 6 years was given a section age of 6 years if the otolith had not been included earlier in the subsample. This procedure allowed an increased sample size.

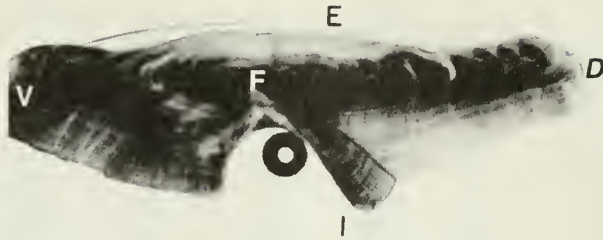


FIGURE 2. Dorsoventral cross section of the left otolith from a 31 cm fork length (FL) *Sebastes diploproa* with a surface age of 28 years and a section age of 39 years. (D) dorsal, (F) focus, (E) external surface, (I) internal surface, (V) ventral. Surface ages were determined by counting rings on the external side of the otolith from the focus towards the dorsal edge.

### Mortality and Growth Rates

Total instantaneous mortality rates ( $Z$ ) were estimated by calculating the slope of the descending right side of the age-frequency distribution (catch curve) by simple linear regression (Ricker 1975). Total instantaneous mortality rates were compared by using an  $F$ -test (Neter and Wasserman 1974).

Individual length at age data were fit to the von Bertalanffy growth model (Ricker 1975) by a nonlinear regression routine (Dixon 1981). Von Bertalanffy growth curves were compared by using the chi-square test for homogeneity of individual parameters (Rao 1973) and by the method developed by Gallucci and Quinn (1979) in which the von Bertalanffy growth function is reparameterized with the introduction of a new parameter ( $\omega$ ). Results of all statistical tests were considered significant at  $P \leq 0.05$ .

## RESULTS

### *Sebastes pinniger*

Otoliths were collected from a total of 363 female and 516 male *S. pinniger* specimens between latitudes  $43^{\circ} 11'$  and  $49^{\circ} 16' N$ . Females had maximum surface ages to 22 years and section ages to 34 years (Table 1). Differences between the two methods were greater for males; maximum surface and section ages were 25 and 60 years, respectively (Table 1).

Fitted growth curves for males based on each ageing method are more similar than growth curves for females. Estimates of  $L_{\infty}$  for both sexes based on surface ages significantly exceeded those based on section ages (by nearly 6 cm fork length (FL) for females and 4 cm FL for males, Figure 3). The difference in growth estimates between treatments apparently occurs beyond the region of the curve where statistical tests on  $\omega$  are most powerful (Gulland 1983).

TABLE 1. Mean Fork Lengths at Age (FL in Centimeters) and Standard Deviation (SD) for Female and Male *Sebastes pinniger* Based on Surface and Section Ages. *N* Indicates the Number of Otoliths.

Age	Surface			Section		
	<i>N</i>	FL	SD	<i>N</i>	FL	SD
	Females					
1.....	—	—	—	—	—	—
2.....	4	15.50	0.58	3	15.67	0.58
3.....	—	—	—	1	15.00	—
4.....	3	30.00	4.36	3	30.00	4.36
5.....	4	31.75	0.50	2	32.00	—
6.....	11	37.27	3.55	8	36.38	3.50
7.....	6	37.67	1.86	5	36.80	3.70
8.....	18	41.28	2.70	9	41.00	7.63
9.....	13	44.08	4.52	16	43.50	3.20
10.....	18	46.11	3.43	20	46.10	3.65
11.....	37	49.27	2.91	28	46.71	4.47
12.....	64	49.92	2.69	50	49.50	3.70
13.....	57	52.23	2.49	47	50.64	3.59
14.....	38	53.97	2.83	34	52.53	2.26
15.....	33	54.48	2.58	18	52.22	3.14
16.....	23	55.52	2.13	21	53.90	3.06
17.....	14	56.00	1.84	16	54.06	2.64
18.....	15	57.00	1.60	13	55.46	2.96
19.....	2	59.50	2.12	15	54.80	2.51
20.....	2	61.50	3.54	11	55.00	1.73
21.....	—	—	—	6	53.83	2.64
22.....	1	59.00	—	4	54.75	1.71
23.....	—	—	—	8	56.38	2.39
24.....	—	—	—	5	57.20	2.49
25.....	—	—	—	5	56.60	2.19
26.....	—	—	—	5	57.40	6.07
27.....	—	—	—	4	57.00	4.83
28.....	—	—	—	1	50.00	—
29.....	—	—	—	3	58.33	1.15
30.....	—	—	—	1	58.00	—
31.....	—	—	—	—	—	—
32.....	—	—	—	—	—	—
33.....	—	—	—	—	—	—
34.....	—	—	—	1	50.00	—
	Males					
1.....	—	—	—	—	—	—
2.....	3	16.00	1.73	2	15.50	2.12
3.....	5	21.80	1.30	4	20.25	2.50
4.....	3	25.00	3.61	4	24.25	3.30
5.....	4	35.25	4.92	3	33.00	8.72
6.....	3	35.33	1.53	6	34.17	3.31
7.....	20	38.70	3.63	13	39.31	3.86
8.....	24	40.42	2.47	22	40.36	2.61
9.....	18	43.61	3.45	9	43.78	3.03
10.....	29	45.24	2.89	18	43.06	4.08
11.....	42	47.00	2.85	20	45.05	3.75
12.....	70	48.49	1.85	35	47.09	2.73
13.....	69	49.59	2.02	28	47.79	2.77
14.....	55	50.22	1.90	22	47.45	3.69
15.....	47	51.55	1.77	23	47.83	2.37
16.....	52	51.79	2.05	13	50.46	2.26
17.....	25	52.84	1.65	23	49.13	1.89
18.....	24	53.46	1.82	24	50.71	1.90
19.....	9	53.33	1.32	27	49.89	1.97
20.....	6	53.67	2.25	27	50.48	2.59
21.....	3	53.33	0.58	27	51.33	2.11
22.....	4	54.25	0.96	22	50.32	2.06
23.....	—	—	—	12	51.50	1.83
24.....	—	—	—	12	50.42	1.83
25.....	1	57.00	—	12	50.58	3.68

(Continued)



TABLE 1.—Continued

26.....	—	—	—	14	52.07	2.23
27.....	—	—	—	8	52.50	2.14
28.....	—	—	—	7	52.14	2.34
29.....	—	—	—	2	51.00	1.41
30.....	—	—	—	5	52.60	1.82
31.....	—	—	—	10	52.30	2.63
32.....	—	—	—	3	51.67	0.58
33.....	—	—	—	8	52.50	1.85
34.....	—	—	—	3	54.00	1.00
35.....	—	—	—	6	52.83	1.17
36.....	—	—	—	3	54.33	4.62
37.....	—	—	—	2	52.50	2.12
38.....	—	—	—	10	52.80	1.99
39.....	—	—	—	1	52.00	—
40.....	—	—	—	6	52.67	1.86
41.....	—	—	—	2	54.00	1.41
42.....	—	—	—	2	53.50	0.71
43.....	—	—	—	1	53.00	—
44.....	—	—	—	1	52.00	—
45.....	—	—	—	4	53.25	1.89
46.....	—	—	—	1	50.00	—
47.....	—	—	—	1	48.00	—
48.....	—	—	—	1	52.00	—
49.....	—	—	—	—	—	—
50.....	—	—	—	2	52.00	1.41
51.....	—	—	—	—	—	—
52.....	—	—	—	1	53.00	—
53.....	—	—	—	1	53.00	—
54.....	—	—	—	—	—	—
55.....	—	—	—	—	—	—
56.....	—	—	—	1	56.00	—
57.....	—	—	—	—	—	—
58.....	—	—	—	—	—	—
59.....	—	—	—	1	55.00	—
60.....	—	—	—	1	56.00	—

Catch curves constructed from surface and section ages of *S. pinniger* clearly differed (Figure 4). Regardless of the ageing technique used, however, both sexes were fully recruited to the fishery by age 12. Estimates of  $Z$  derived from surface and section ages significantly differed for both sexes:  $Z$  values estimated from section ages were 61% less for females and 78% less for males than those calculated from surface ages (Table 2).

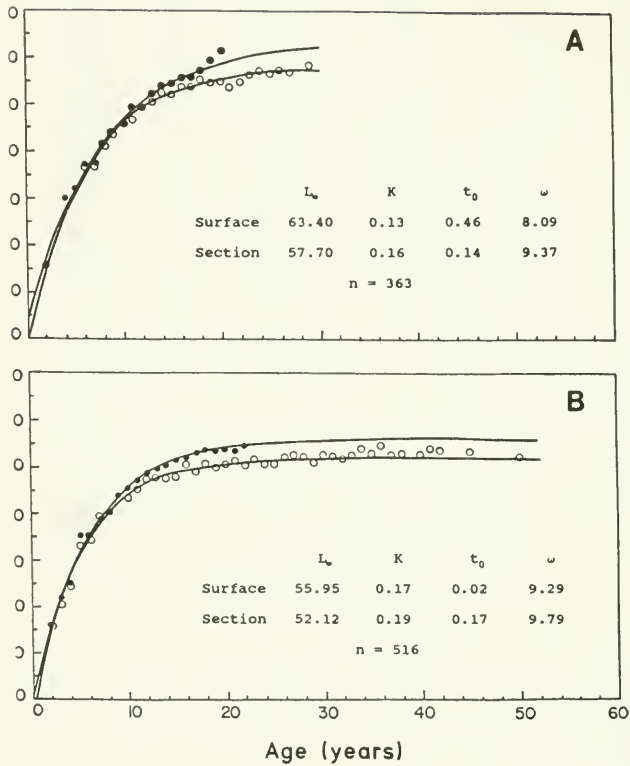


FIGURE 3. Fitted von Bertalanffy growth curves, parameter estimates (see text for explanation), and mean lengths at age based on surface (closed circles) and section ages (open circles) of otoliths from (A) female and (B) male *Sebastes pinniger*. Points representing single individuals are not shown.  $N$  indicates sample size.

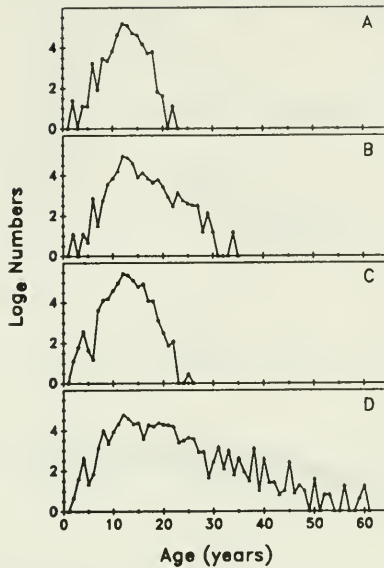


FIGURE 4. Catch curves based on (A, C) surface and (B, D) section ages of otoliths from (A, B) female and (C, D) male *Sebastes pinniger*.

### *Sebastes diploproa*

Otoliths from 1,131 female and 922 male *S. diploproa* were collected between lat 36° 49' and 48° 47' N. Both sexes of *S. diploproa* reached much greater ages than those attained by *S. pinniger*. Female *S. diploproa* attained ages similar to males and had surface ages to 55 years and section ages to 81 years compared with 46 and 84 years, respectively, for males (Table 3). Growth curves of either sex based on surface and section ages were essentially identical, and parameter estimates and  $\omega$  were not significantly different (Figure 5).

Catch curves constructed from surface and section ages of *S. diploproa* clearly differed (Figure 6). In all cases, particularly among females, substantial variation occurred between year classes, with a succession of weak year classes between ages 10 and 20. Therefore,  $Z$  was determined for fish older than 21 years. As with *S. pinniger*, significant differences were found between estimates of  $Z$  derived from surface and section ages for both sexes; estimates based on section ages were 55% less for females and 76% less for males than those based on surface ages (Table 2).

TABLE 2. Estimates of Total Instantaneous Mortality ( $Z$ ) for *Sebastes pinniger* and *S. diploproa*. Range Indicates Ages Over Which  $Z$  Was Determined.

Sex	Surface		Section	
	$Z$	Range	$Z$	Range
	<i>Sebastes pinniger</i>			
Female.....	0.452	12-22	0.178	12-34
Male.....	0.405	12-25	0.089	12-60
	<i>S. diploproa</i>			
Female.....	0.109	25-48	0.049	22-71
Male.....	0.130	23-40	0.031	26-75

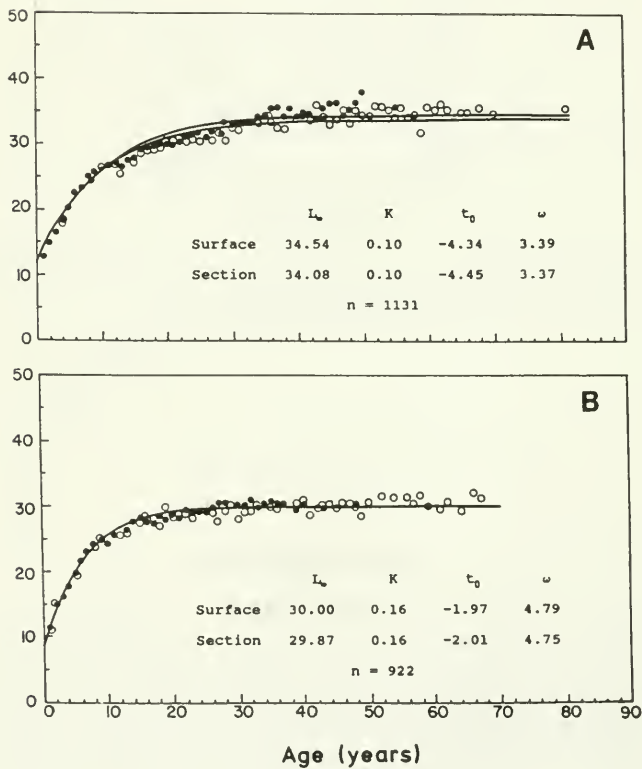


FIGURE 5. Fitted von Bertalanffy growth curves, parameter estimates (see text for explanation), and mean lengths at age based on surface (closed circles) and section ages (open circles) of otoliths from (A) female and (B) male *Sebastes diploproa*. Points representing single individuals are not shown.  $N$  indicates sample size.

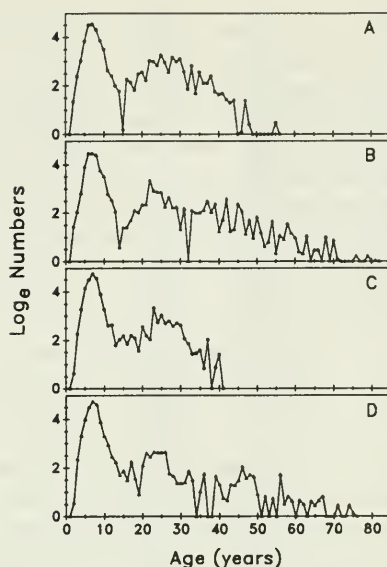


FIGURE 6. Catch curves based on (A, C) surface and (B, D) section ages of otoliths from (A, B) female and (C, D) male *Sebastes diploproa*.

## DISCUSSION

### Differences in Growth

The effects of changing the methodology of fish age determination can vary from a minor error to major differences. Estimates of maximum age in the genus *Sebastes* have changed markedly in recent years (Beamish 1979b; Bennett et al. 1982; Leaman and Nagtegaal 1987); thus, one would expect similarly remarkable differences in estimates of growth within a species. For *S. pinniger*, estimates of the  $L_{\infty}$  were significantly lower when section rather than surface ages were used although the increase in the growth completion rate (von Bertalanffy's  $k$ ) for both sexes (Figure 3) was not significant. Therefore, when surface ages are used, errors in ageing incorrectly place older, larger individuals into younger age groups, thus inflating their mean lengths at age (Wilson 1985). This results in lower mean lengths at age for many of these section age groups relative to surface age groups, and a corresponding decrease in  $L_{\infty}$  based on section ages.

Another explanation to account for the differences between growth curves constructed from surface and section ages in *S. pinniger* is that a curve fitting problem occurs, particularly for females. Thus,  $L_{\infty}$  may be overestimated when data are available for only the ascending limb of the von Bertalanffy growth function (Knight 1968, Gallucci and Quinn 1979, Vaughan and Kanciruk 1982). Note that the estimate of  $L_{\infty}$  is typically greater than the maximum observed length in our study (Table 1, Figure 3). Because surface ages have a lower range than section ages and are more concentrated on the ascending limb of the growth curve, they may fail to adequately estimate the upper portion of the

curve, which represents  $L_{\infty}$ . For example, the large differences between estimates of  $L_{\infty}$  for female *S. pinniger* may occur because their surface ages are more concentrated on the ascending portion of the curve than are those of males (Table 1). This situation is analogous to fitting growth curves with incomplete representation of the range of ages (Hirschhorn 1974).

Unexpected results of our study were the similarities in estimated growth parameters for *S. diploproa* based upon section and surface ages (Figure 5) despite the much higher maximum section ages (Table 3). This difference from *S. pinniger* is likely due to our surface ageing methodology. We suggest that the surface ageing technique for *S. diploproa* has changed over several years to provide older age estimates. Boehlert (1980), for example, used surface ages and observed only 0.5% of all specimens in excess of 25 years of age compared with 18.9% in the present study. Earlier surface ages for *S. diploproa* were assigned without rolling or tilting the otoliths, thus preventing the enumeration of additional annuli on the posterior "winglike" projections (Figure 1A). More recently, annuli located on posterior projection (present in older individuals of many species of *Sebastes*) have been included in surface age counts in order that surface ages more closely represent section ages (Chilton and Beamish 1982, Boehlert and Yoklavich 1984). In the present study, surface ages for *S. pinniger* were assigned using standard criteria for this species (Six and Horton 1977) without including these counts (which are typically less well developed on otoliths of *S. pinniger*); the counts were included in assigning surface ages to *S. diploproa*. Thus, while maximum ages increased, counts including the posterior projection resulted in surface ages closer to section ages and minimized the higher mean length estimates for ages on the ascending arm of the growth curve as seen in the surface ages of *S. pinniger* (Figures 3, 5). Had the age estimates from Boehlert (1980) or the "other agency" readers in Boehlert and Yoklavich (1984) been used, it is likely that differences in growth parameters similar to those for *S. pinniger* would have been observed for *S. diploproa*.

TABLE 3. Mean Fork Lengths at Age (FL in Centimeters) and Standard Deviation (SD) for Female and Male *Sebastes diploproa* Based on Surface and Section Ages. *N* Indicates the Number of Otoliths.

Age	Surface			Section		
	<i>N</i>	<i>FL</i>	<i>SD</i>	<i>N</i>	<i>FL</i>	<i>SD</i>
1.....	8	13.00	1.31	7	12.86	1.35
2.....	12	14.83	1.53	14	14.93	1.54
3.....	24	16.54	1.38	18	16.50	1.54
4.....	36	18.47	2.09	32	17.72	2.04
5.....	68	20.40	2.31	76	20.38	2.23
6.....	119	22.59	2.02	117	22.60	2.03
7.....	125	23.22	2.02	117	23.15	2.04
8.....	119	24.97	2.21	130	24.97	2.14
9.....	82	25.71	1.92	75	25.73	2.05
10.....	61	26.48	2.47	60	26.30	2.68
11.....	25	26.56	1.45	30	26.63	1.43
12.....	20	27.15	3.28	21	27.00	2.66
13.....	13	26.46	2.50	12	25.50	3.53
14.....	8	27.50	1.69	4	27.50	1.73
15.....	2	28.00	1.41	6	27.17	2.64
16.....	11	29.36	2.42	5	28.60	2.07
17.....	10	29.30	1.77	7	29.14	1.57
18.....	7	29.71	1.60	11	29.45	0.93

(Continued)

TABLE 3—Continued

19	13	30.15	1.68	7	29.57	2.15
20	15	30.13	1.06	11	30.36	1.80
21	10	30.00	2.45	11	30.36	1.75
22	21	30.71	1.55	27	30.81	1.49
23	18	31.33	1.88	19	30.37	1.67
24	17	31.53	2.37	15	30.80	0.94
25	25	31.80	1.91	17	30.59	1.37
26	18	31.33	1.97	9	31.22	1.99
27	14	32.21	2.46	14	30.79	2.12
28	23	32.22	2.19	9	31.89	2.15
29	20	33.30	1.81	9	30.56	2.01
30	21	33.19	1.57	3	32.67	1.53
31	14	33.07	2.40	8	32.25	1.75
32	9	33.33	3.16	1	37.00	—
33	17	33.47	2.15	9	33.44	2.30
34	7	34.29	2.56	7	33.43	1.72
35	17	34.53	2.53	9	34.22	2.17
36	15	35.53	1.60	9	33.56	1.51
37	12	35.58	1.73	12	32.58	1.73
38	15	34.47	2.50	7	32.57	1.90
39	10	35.50	1.90	13	33.77	2.86
40	6	34.33	1.86	4	34.25	2.36
41	8	35.00	2.14	8	34.38	2.39
42	5	34.60	2.51	5	33.87	2.00
43	4	34.00	1.41	7	36.00	1.15
44	7	35.57	1.13	4	34.50	1.29
45	2	36.50	0.71	11	33.09	1.97
46	4	36.50	1.00	11	35.33	3.30
47	5	34.80	1.64	6	33.29	2.73
48	2	35.50	0.71	7	33.29	2.43
49	2	36.50	0.71	3	35.33	0.58
50	2	38.00	1.41	10	34.50	2.68
51	—	—	—	4	34.50	1.91
52	—	—	—	4	36.00	1.41
53	—	—	—	5	36.00	1.22
54	—	—	—	8	35.37	2.45
55	3	36.00	1.00	2	34.50	2.12
56	—	—	—	6	35.83	1.94
57	—	—	—	4	34.25	3.10
58	—	—	—	6	34.67	2.34
59	—	—	—	3	32.00	2.65
60	—	—	—	5	35.80	1.92
61	—	—	—	2	35.50	0.71
62	—	—	—	3	36.33	2.08
63	—	—	—	4	35.50	1.29
64	—	—	—	—	—	—
65	—	—	—	2	35.50	1.41
66	—	—	—	2	35.00	1.41
67	—	—	—	—	—	—
68	—	—	—	4	35.75	1.71
69	—	—	—	1	38.00	—
70	—	—	—	3	34.67	2.08
71	—	—	—	1	35.00	—
72	—	—	—	1	30.00	—
73	—	—	—	—	—	—
74	—	—	—	—	—	—
75	—	—	—	1	39.00	—
76	—	—	—	1	34.00	—
77	—	—	—	—	—	—
78	—	—	—	1	36.00	—
79	—	—	—	1	34.00	—
80	—	—	—	1	36.00	—
81	—	—	—	2	35.50	3.54
				Males		
1	12	11.33	1.61	11	11.09	1.45
2	8	14.87	0.99	6	15.00	1.10

(Continued)

TABLE 3—Continued

Age	<i>Surface</i>			<i>Section</i>		
	<i>N</i>	<i>FL</i>	<i>SD</i>	<i>N</i>	<i>FL</i>	<i>SD</i>
3	27	16.15	2.16	27	16.48	2.08
4	47	17.70	1.92	48	17.63	2.05
5	86	19.98	2.02	79	19.77	2.25
6	100	21.87	1.86	101	21.71	1.96
7	128	23.23	1.82	124	23.16	1.83
8	114	24.32	1.91	119	24.12	2.11
9	62	24.69	2.05	61	24.90	1.79
10	30	24.23	2.16	32	24.28	2.25
11	15	25.53	3.20	21	25.52	3.12
12	16	25.81	1.83	14	25.57	1.70
13	7	26.43	1.72	11	25.82	2.96
14	8	27.63	2.07	6	27.67	2.42
15	9	28.22	0.83	8	27.63	1.30
16	7	27.57	1.27	5	28.40	1.67
17	11	27.64	1.43	10	28.50	1.27
18	9	28.56	1.51	6	27.17	1.17
19	5	28.00	1.22	3	29.67	1.15
20	13	28.92	1.12	9	28.67	1.41
21	9	28.44	1.67	14	28.64	1.08
22	9	29.56	2.13	12	28.92	1.24
23	29	28.93	2.20	14	28.32	1.50
24	16	29.44	1.71	13	29.31	1.18
25	20	29.15	1.31	14	29.43	1.40
26	15	29.73	1.10	15	29.13	0.99
27	17	30.41	1.84	6	27.67	3.88
28	13	30.46	1.39	6	29.50	0.84
29	16	29.94	1.18	5	30.20	1.10
30	16	30.19	2.48	4	28.25	2.50
31	9	30.11	1.90	4	29.25	0.96
32	6	31.00	0.89	6	29.33	1.75
33	4	29.75	2.22	5	30.00	0.71
34	5	30.40	0.55	1	30.00	—
35	5	30.80	0.84	2	30.00	2.83
36	3	30.33	1.15	6	29.83	0.98
37	7	30.43	1.27	1	30.00	—
38	1	30.00	—	1	26.00	—
39	2	29.50	3.54	4	30.25	2.63
40	4	30.50	1.29	4	30.75	1.71
41	1	31.00	—	2	28.50	0.71
42	—	—	—	2	29.50	2.12
43	—	—	—	3	30.00	2.00
44	—	—	—	4	30.25	0.50
45	—	—	—	5	29.80	0.84
46	1	31.00	—	8	30.50	1.51
47	—	—	—	5	30.20	1.92
48	—	—	—	6	29.83	2.86
49	—	—	—	6	28.67	1.63
50	—	—	—	2	30.50	2.12
51	—	—	—	1	31.00	—
52	—	—	—	2	31.50	0.71
53	—	—	—	1	30.00	—
54	—	—	—	3	31.33	2.52
55	—	—	—	1	30.00	—
56	—	—	—	6	31.50	0.84
57	—	—	—	2	30.50	0.71
58	—	—	—	2	31.50	0.71
59	—	—	—	2	30.00	—
60	—	—	—	1	30.00	—
61	—	—	—	2	29.50	2.12
62	—	—	—	2	30.50	0.71
63	—	—	—	1	29.00	—
64	—	—	—	2	29.50	0.71
65	—	—	—	1	32.00	—

(Continued)



TABLE 3—Continued

66	—	—	—	3	32.00	1.73
67	—	—	—	2	31.50	0.71
68	—	—	—	1	36.00	—
69	—	—	—	—	—	—
70	—	—	—	—	—	—
71	—	—	—	1	32.00	—
72	—	—	—	—	—	—
73	—	—	—	1	31.00	—
74	—	—	—	1	32.00	—
75	—	—	—	1	33.00	—
76	—	—	—	—	—	—
77	—	—	—	—	—	—
78	—	—	—	—	—	—
79	—	—	—	—	—	—
80	—	—	—	1	35.00	—
81	—	—	—	—	—	—
82	—	—	—	—	—	—
83	—	—	—	—	—	—
84	—	—	—	1	30.00	—

### Mortality

The results of this study demonstrate that total mortality ( $Z$ ) is significantly less when section rather than surface ages are used for slow-growing, long-lived fishes such as *S. pinniger* and *S. diploproa* (Table 2). As evident in the larger reduction in  $Z$  for males versus females, the problem is more pronounced in males, possibly because their slower growth increases the difficulty of assigning accurate surface ages. This may explain why  $Z$  is higher in male *S. diploproa* than in females when surface ages are used and lower when section ages are used. The absence of older *S. pinniger* females also contributed to the smaller decrease in  $Z$  when section ages were used.

This study has documented important differences in estimates of growth and mortality which result solely from differences in otolith ageing methodology. It is hoped that subsequent research will incorporate these estimates into various stock assessment models to evaluate the effect that these different otolith ageing techniques have on models designed to evaluate yield and production characteristics of long-lived, slow-growing fish stocks.

### ACKNOWLEDGMENTS

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# GROWTH AND LONGEVITY OF GOLDEN TROUT, *ONCORHYNCHUS AGUABONITA*, IN THEIR NATIVE STREAMS<sup>1</sup>

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Golden trout, *Oncorhynchus aguabonita*, from 17 streams in the Kern Plateau region of the Sierra Nevada, California, were aged using otoliths, and growth rates were determined using length-age and weight-age relationships. Growth rates, condition factors, and densities of trout were correlated with site-specific biological and physical factors using stepwise multiple regression techniques. These stream populations were highly stunted, and individuals attained quite old ages (9 years). Densities were usually low and high density had a significant negative effect on growth ( $P < 0.001$ ). In addition, growth was positively affected by amount of aquatic vegetation, amount of bank vegetation, stream channel stability, and elevation. While site-specific factors such as trout density may influence trout growth, the low growth rates throughout the study area were probably due to the low productivity of these unstable montane streams and the short growing period at high elevations.

## INTRODUCTION

Golden trout, *Oncorhynchus aguabonita*, formerly *Salmo aguabonita*, have been widely introduced throughout the United States of America and other parts of the world, but are endemic to only two watersheds, both in the southern Sierra Nevada mountains of California (Fisk 1983). There are two subspecies of the golden trout; *O. a. aguabonita* is native to the headwaters of the South Fork Kern River and Golden Trout Creek and *O. a. whitei* is native to the Little Kern River, Tulare County. Golden trout were initially thought to be most closely related to cutthroat trout (Jordan 1892), but are now understood to belong to the rainbow trout series (Berg 1987).

Although the golden trout is the official California state fish, its natural history in native habitats is poorly understood. What is known of golden trout natural history is primarily based on introduced populations of lake-dwelling fish (Fisk 1983). In these populations, spawning is typically initiated in June and continues through July. Eggs hatch in 29 to 50 days, and after 2-3 weeks in the gravel the fry emerge and grow rapidly during the first summer. They reach approximately 4.5 cm by age one year, 12 cm at two, and 19 cm at three years of age (Needham and Vestal 1938, Fisk 1983). Lake fish reach reproductive maturity at three years of age and may survive to spawn as many as three times. The maximum recorded age is six years. Golden trout from streams rarely achieve lengths greater than 18 cm and ages of such populations are unknown (Fisk 1983). Factors which influence growth rates of stream populations are also unknown.

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Growth rates of trout can vary markedly in relation to temperature, food ration, and density (Elliott 1982). In many cases, the differences within a species may be greater than those between species. Under good conditions, non-anadromous trout in streams may achieve a length greater than 40 cm after only three years (Carlander 1969). Growth is often retarded at higher elevations, due to lower temperatures and a shorter growing season. Purkett (1951) reported that three-year old rainbow trout, *Oncorhynchus mykiss*, formerly *Salmo gairdneri*, averaged 29 cm at 1,600 m and 24 cm at 1,850 m, and a 24-year old brook trout, *Salvelinus fontinalis*, reached only 24 cm at 3,322 m in a low productivity Sierra Nevada lake (Reimers 1979). If higher elevation habitats have lower fish densities than lower sites, however, reduced competition at higher sites may result in faster growth rates (McAfee 1966).

In this paper, we report on ages, growth, and population densities of golden trout, *O. a. aguabonita*, from 12 of its native streams in the Golden Trout Wilderness, Inyo National Forest. Five additional study streams contained populations introduced from nearby native populations around the turn of the century. These included three sites on the eastern edge of the Golden Trout Wilderness and two sites in Kings Canyon. Several populations in the Golden Trout Wilderness have been the subject of an intensive management project to preserve the native habitat and gene pool of the golden trout (Fisk 1983). Brown trout, *Salmo trutta*, had been introduced to the South Fork Kern River drainage, and these predators and competitors were removed by California Department of Fish and Game biologists from 1976 to 1982. Golden trout were transplanted extensively during this operation, though trout were not transplanted into any of our study streams. Our objective was to provide information on golden trout ages and growth rates from stream populations and to determine what biological and physical factors affected trout growth rate, condition, and density.

## STUDY SITES

The 17 study streams all originate on or near the Kern Plateau of the southern Sierra Nevada (Inyo and Tulare counties, California; Figure 1). Most are tributaries of the South Fork Kern River or Golden Trout Creek, both within the Golden Trout Wilderness. Three streams flow eastward into the Owens Valley and two are tributaries of the Kings River in the southern portion of Kings Canyon National Park and drain into the Central Valley.

The southern portion of the Sierra Nevada was largely unaffected by the Pleistocene glaciation which shaped the valleys north of the Kern Plateau (Jahns 1954). Consequently, most of the stream valleys in this region consist of broad alluvial flats separated by low granitic ridges sparsely vegetated with lodgepole, *Pinus contorta*, and foxtail, *P. balfouriana*, pines. The meadows range in elevation from 2,300 to 3,200 m, and are composed of relatively unconsolidated granitic sands and fine sediments. They are more subject to erosion and degradation than meadows in the glaciated Sierra Nevada (Albert 1982).

Characteristics of the individual streams are provided in Table 1. The streams of this region are typically of low gradient and stream bottoms consist of unstable sand and occasional gravels and cobble. Meanders are common in the unconfined meadow flats, and most streams are relatively wide and shallow.

Discrete riffle-pool sequences are largely absent. The banks are generally steep-sided, rarely densely vegetated, and active erosion sites are common. True riparian vegetation is absent, except for mesic herbs and occasional willow shrubs, *Salix* spp. In-stream cover which can be used by trout is rare. As a consequence of stream openness, summer stream temperatures fluctuate greatly, ranging daily from 3° to 22°C. Golden trout are the only fish species inhabiting the study streams.

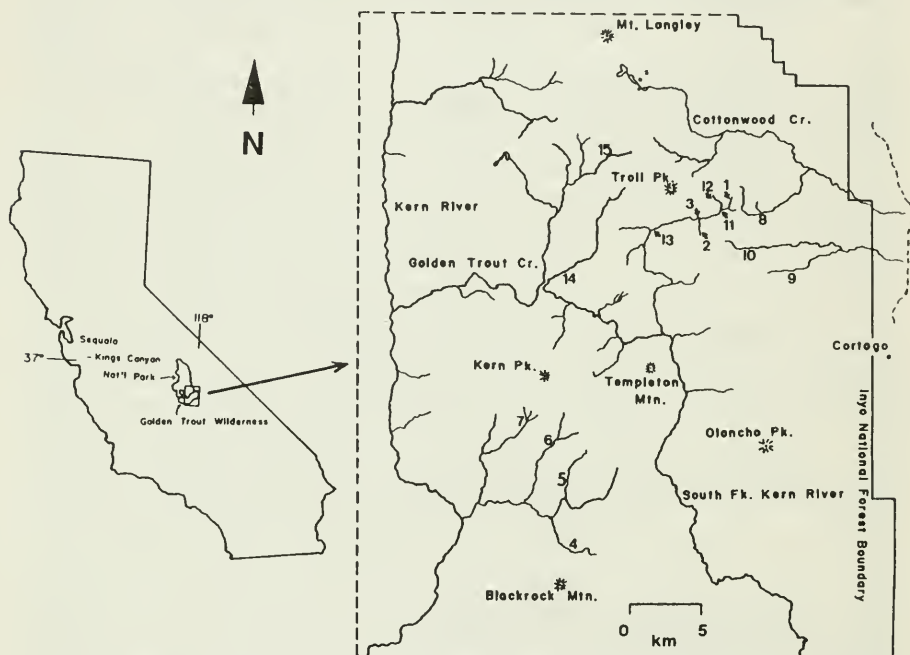


FIGURE 1. Map of California showing study sites on the Kern Plateau. Study sites are numbered and correspond to numbers in Table 1. The two study sites in Kings Canyon National Park (16, 17) are not shown.

## MATERIALS AND METHODS

Sampling occurred during the summers of 1983 and 1984. At each site, a 100 m linear transect was established along a representative stream section, and all samples were taken from this section. The section was mapped three-dimensionally to assess geomorphological characteristics such as meander patterns, width/depth relationships, and pool/riffle ratios. Two bottom substrate cores (15 cm deep x 8 cm diam) were taken and partitioned into five sediment size classes with sieves (mesh sizes = 2.0, 1.0, 0.5, and 0.1 mm—particles larger than 2 cm were hand separated from the 2 mm sieve). The three largest size classes were weighed in the field and the two smallest size classes were returned to the laboratory for more accurate weighing.

Invertebrates were collected with a 30 cm x 30 cm modified Hess sampler. The sampler was pushed approximately 10 cm into the substrate and the substrate within the sampler was vigorously stirred by hand. Any suspended

TABLE 1. Stream Characteristics for Study Sites shown in Figure 1. The Sample Sizes Used to Calculate Condition (K) and Density ( $\#/m^2$ ) are Given as  $\#/100\ m$ .

SITE NO.	SITE	ELEVATION (m)	WIDTH/DEPTH	POOL/RIFLE	STABILITY RATING	% BANK VEGETATED	% STREAM VEGETATED	# FISH/100 M	# FISH/ $M^2$	CONDITION (K)
1	NE MULKEY	2950	10.8	0.18	80	61	64	13	0.09	1.40
2	BEAR	2930	20.8	0.06	102	90	92	13	0.14	1.16
3	MULKEY/BEAR	2880	11.5	0.30	135	85	71	58	0.11	1.32
4	CASA VIEJA	2560	4.2	0.21	59	100	56	27	0.11	1.45
5	LONG	2850	6.9	0.40	72	76	41	27	0.05	1.47
6	RED ROCK	2650	3.3	0.26	81	77	22	8	0.04	1.32
7	COLD	2780	3.3	0.65	94	90	44	21	0.07	1.44
8	ROUND	3000	10.6	0.26	126	70	53	52	0.08	1.29
9	DIAZ	3050	32.8	0.07	141	9	0	29	0.06	1.45
10	ASH	3050	9.0	0.59	78	90	81	16	0.03	1.32
11	UPPER MULKEY	2930	3.4	0.59	84	89	48	48	0.17	1.36
12	MULKEY CS	2950	7.5	0.31	83	90	28	16	0.06	1.25
13	LOWER MULKEY	2850	28.6	0.12	132	55	52	—	—	1.39
14	TUNNEL	2730	12.9	0.25	123	80	65	11	0.02	1.24
15	STOKES	2970	10.1	0.42	92	82	15	40	0.08	1.40
16	WF FERGLUSON	2730	6.5	0.61	69	73	11	21	0.02	1.40
17	PARADISE	2780	6.3	0.19	57	89	42	12	0.07	1.32

organisms and other organic material was washed into a 330  $\mu\text{m}$  mesh net attached to the downstream end of the sampler. Samples were preserved in 70% ethanol for size partitioning and species identification in the laboratory. Four samples were collected from riffles at each site.

Bank condition was characterized every meter along one randomly chosen 100 m section of stream bank within the study site. At each point, we noted the type of vegetation, whether or not the stream edge was bare, the presence or absence of aquatic vegetation (macrophytes and algae), and whether or not the bank was undercut. If the bank was undercut, the extent of undercutting was measured.

The Pfankuch stream stability rating was calculated for each site (Pfankuch 1975). This index is based on a visual assessment of subjective measures related to stream channel stability, including bank condition, substrate type, vegetative cover, width-depth ratio, and pool-riffle ratio. Stream channel stability is negatively correlated with the index value. For example, narrow, deep streams with overhanging banks and substrates composed mostly of cobble and gravel receive a lower score than wide, shallow streams with highly eroded banks and substrates composed mostly of sand and silt.

Fish were collected by electroshocking (Smith-Root Model 11 Electrofisher). Three passes were conducted at each site in order to obtain a regression estimate for population size (Seber and LeCren 1967). However, this method was not appropriate for the study streams, since the first pass often produced fewer fish than the second and third passes. Because of the limitations of population estimates based on the regressions, the data were used to estimate minimum densities within the stream sections. Fish were retained in buckets until all passes were completed and then measured.

The standard lengths (SL) of all captured fish were measured to the nearest mm on a measuring board. We estimated weights by immersing the fish in a graduated cylinder containing a known volume of water and recording the volume of water displaced. We assumed, and verified in the laboratory, that the specific density of fish tissue was similar to that of water (1.0 g/ml). Therefore, a fish that displaced 50 ml of water was recorded as weighing 50 g. Ten fish from each site, representing a range of sizes, were sacrificed and preserved in 95% ethanol and returned to the laboratory for age determinations. Smaller samples were preserved from three streams which contained few fish. In addition, 10 juveniles (<1 year of age) were collected from each site where they were present. Since electroshocking did not effectively capture juveniles, they were instead collected using handnets.

Both scales and otoliths (sagittae) were used for age determination; annuli and presumed daily growth increments of otoliths were examined at 100–400X in oil immersion under a light microscope (Campana and Neilson 1985). Otoliths from fish >3 years old were first ground with carborundum 600 grit to enhance transparency, while otoliths from younger fish were viewed directly.

We investigated the importance of 24 biological and physical site characteristics in determining golden trout growth, condition (K), and density. Site characteristics included riparian stream cover, substrate size composition, bank condition, gradient, elevation, suspended particulates, stream surface area, stream width/depth ratios, pool/riffle ratios, watershed area, abundance of bank and instream vegetation, fish density, stream channel stability, and aquatic

insect abundance. Data were analyzed using stepwise multiple regression procedures. The effects of stream channel stability on trout growth, condition, and density were analyzed separately since the stability index incorporated many of the other independent variables.

In stepwise multiple regression procedures in which fish growth (sl/age, weight/age) or condition were included as dependent variables, each fish was a separate observation. When fish density was entered as the dependent variable, each stream was a separate observation. Proportional data were arc-sine square root transformed. The required significance level for inclusion in the regression model was  $p < 0.15$ . Only those fish more than one year old were used in the analyses since young of the year were present in only five of the study streams when collections were made in 1983.

## RESULTS

A total of 376 fish from 17 streams were weighed and measured during 1983. Of these, 176 fish from 15 streams were preserved for age determinations. In 1984, an additional 20 fish were preserved from two streams from which no samples had been collected in 1983.

Scales were unsuitable for age determination since annuli were non-existent. In contrast, annuli were easily counted on nearly all otoliths, possibly due to the large and discrete differences in seasonal temperature cycles (Campana and Neilson 1985). Based on annuli counts, golden trout frequently lived more than five years; the oldest was nine years old. We did not validate annulus formation, but are confident that our age estimates are accurate, since otoliths accurately reflected ages of fish up to 23 years old in an extremely stunted brook trout population (Reimers 1979).

Inter-annular increments were present on all otoliths and were assumed to be produced daily. Daily increment production has been verified in several fish species closely related to golden trout including steelhead trout (Campana 1983), chinook salmon (Neilson and Geen 1982), and sockeye salmon (Marshall and Parker 1982). The number of increments between successive annuli ranged from 90–120. Although it is not known for golden trout whether growth ceases when increment formation stops, the increment counts suggest a period of rapid growth of between three and four months per year (June–September). Growth at other times of the year is probably very slow.

Otoliths from young-of-year trout always showed a distinct discontinuity, after which increments were clearly reduced in width (Figure 2). We believe this represents the date at which alevins emerged from the gravel after depletion of the yolk sac. Similar discontinuities were reported from otoliths of sockeye salmon (Marshall and Parker 1982) and correspond to the date of first feeding.

Lengths of golden trout for all age classes are shown in Figure 3. First year growth was rapid, after which fish grew at a slower rate, especially after the fifth year. The length of golden trout for ages 1–9 was fitted to an equation of the form  $y_{sl} = 7.30 + 4.02 \ln x_{age}$  ( $R^2 = 0.51$ ,  $p < 0.0001$ ,  $n = 138$ ; Table 2). Weight followed a pattern similar to length, but was more variable (Figure 4). The weight of trout of ages 1–9 was fitted to an equation of the form  $y_{wt} = 2.85 + 19.90 \ln x_{age}$  ( $R^2 = 0.30$ ,  $p < 0.0001$ ,  $n = 138$ ; Table 2). When fit



to a logarithmic equation, the length-weight relationship for all trout was [ $\ln w = -3.59 + 2.70 \ln l$ ] ( $R^2 = 0.90$ ,  $p < 0.0001$ ,  $n = 343$ ).

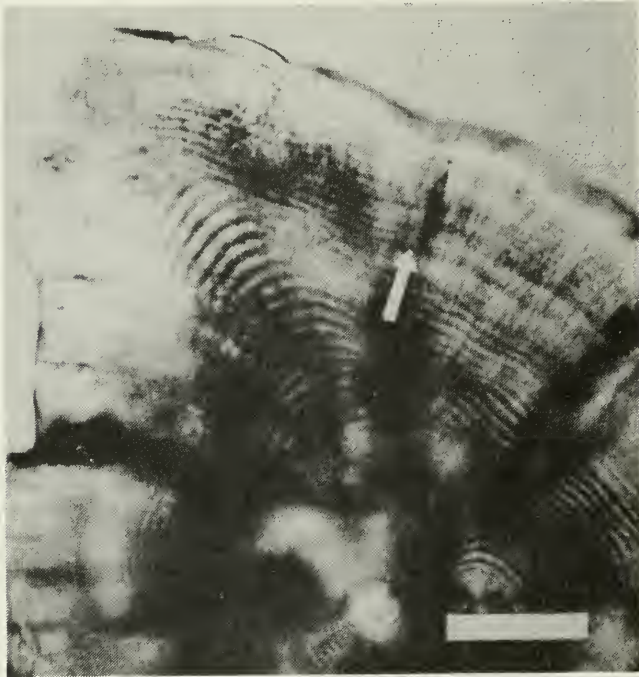


FIGURE 2. Otolith from a 2.5 cm golden trout. Arrow points to suggested emergence mark. Scale bar = 100  $\mu\text{m}$ .

Age-length data was also fitted to a Von Bertalanffy growth function (FISHPARM computer software—Prager et. al. 1987). The Von Bertalanffy equation for all collected fish was  $l_t = 15.5(1 - \exp(-0.42[t + 0.54]))$ . The asymptotic length for this sample of golden trout was 15.5 cm.

The effects of site characteristics on trout growth (measured as SL/age) were evaluated for 95 fish from 12 streams using stepwise multiple regression. The remaining 43 fish were eliminated because their associated stream variables contained missing values. Fish age accounted for most (62%) of the variation in trout growth explained by the model (Table 3). The percent of stream length covered with aquatic vegetation also added significantly to the model and explained 2% of the variation in trout growth. Fish density (number of fish/ $\text{m}^2$ ) did not vary much between sites (Table 1) and did not explain a significant amount of the variation in trout growth.

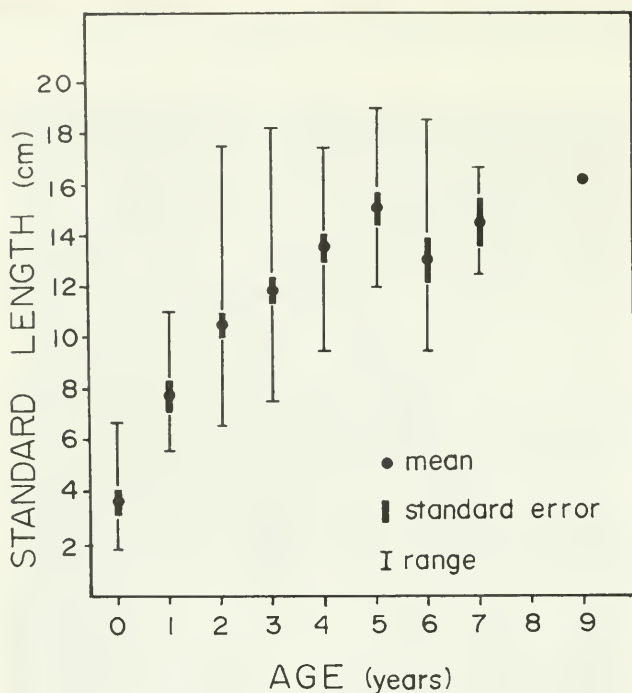


FIGURE 3. Golden trout lengths by age class. Sample sizes for age classes 1-9 are given in Table 2. N = 58 for age 0.

TABLE 2. Actual and Predicted Standard Lengths and Weights of Trout of Ages 1-9. See Text for Equations.

	AGE CLASS (YEARS)								
	1	2	3	4	5	6	7	8	9
n	10	27	47	28	10	10	5	0	1
predicted length (cm).....	7.3	10.1	11.7	12.9	13.8	14.5	15.1	15.6	16.1
actual length (cm).....	7.1	9.8	11.8	13.2	15.0	13.1	14.2	—	16.1
predicted weight (g).....	2.9	16.6	24.7	30.4	34.9	38.5	41.6	44.2	46.6
actual weight (g).....	6.4	14.0	23.9	31.7	47.3	33.2	33.2	—	55.0

TABLE 3. Results of Stepwise Multiple Regression Analysis of Trout Growth Measured As SL/age (n = 95). Variables Are Listed As They Entered The Model. See Text for Definition of Independent Variables.

Variable	Slope	P	R <sup>2</sup>
fish age.....	-0.76	0.0001	0.62
% stream vegetated.....	0.95	0.005	0.02
fish density (#/m <sup>3</sup> ).....	-3.53	>0.10	0.01

When growth was measured as weight/age, regression analysis for 87 fish from 11 streams indicated that golden trout density explained the largest amount of the variation in growth (21%; Table 4). Elevation did not vary much between sites (Table 1), but still explained 8% of the variation in trout growth. The percent of the bank covered by vegetation explained an additional 7% of the variation.

**TABLE 4.** Results of Stepwise Multiple Regression Analysis of Trout Growth Measured as Weight/Age ( $n = 87$ ). Variables are Listed as they Entered the Model. See Text for Definition of Independent Variables.

Variable	Slope	P	R <sup>2</sup>
fish density (#/100 m).....	-0.19	0.0001	0.21
elevation (m).....	0.01	0.0001	0.08
% bank vegetated.....	7.99	0.004	0.07

Condition factors ranged from 1.16 to 1.47, with a mean of  $1.34 \pm 0.11$  S.E. (Table 1). Although 106 fish were used in the model, none of the site-specific stream variables satisfied the minimum significance level for entry into the regression model.

Multiple regression analyses using fish density (measured as the number of fish per 100 m of stream and as the number of fish per  $m^2$ ) as the dependent variable resulted in none of the site variables satisfying the minimum required significance level for entry into the regression model. Four streams were eliminated from the analysis because of missing values.

The Pfankuch stream stability rating was significantly correlated with fish growth measured as weight/age ( $r = -0.35$ ,  $p = 0.0001$ ,  $n = 138$ ), but not as SL/age ( $r = -0.09$ ,  $p > 0.30$ ,  $n = 138$ ). The correlation between the stream stability rating and fish density measured as the number of fish/100 m was marginally significant ( $r = 0.44$ ,  $p = 0.08$ ,  $n = 16$ ), but the stream stability rating and fish density measured as the number of fish/ $m^2$  were not significantly correlated ( $r = 0.05$ ,  $p > 0.50$ ,  $n = 16$ ). Trout condition was also not significantly correlated with the stream stability rating ( $r = -0.07$ ,  $p > .15$ ,  $n = 343$ ).

## DISCUSSION

Scales proved unsatisfactory for aging golden trout in this study. Reimers (1958) experienced similar difficulties with scales taken from brook trout from an alpine Sierra Nevada lake. Otoliths, however, proved highly suitable for age determinations. The oldest fish sampled was 16.1 cm SL and was in its tenth year of growth, making it the oldest golden trout on record. Six and seven year old fish were common. Other species of trout occasionally attain similar ages in streams (*O. mykiss*—7 years, Greeley 1933; *O. clarkii*—10 years, Oregon State Game Comm. 1950; *S. trutta*—8 years, Sigler 1952). Most other records of salmonids near 10 years of age or greater are from lake populations (Fenderson 1954, Sumner 1948) or are sea-run individuals (Sumner 1962).

The exceptionally low productivity of streams occupied by golden trout may be a factor in their longevity as well as their retarded growth. In one remarkable case, Reimers (1979) found that introduced brook trout, *Salvelinus fontinalis*, survived for up to 24 years of age in a high altitude, low productivity Sierra Nevada lake. Reimers accounted for their exceptional age by their minimal energetic costs. Activity was reduced by low temperatures in conjunction with extreme food depletion. These fish became highly stunted and did not reproduce until age 16, when population densities declined and allowed food levels to increase and make reproduction possible. Considerable evidence correlates stunting due to reduced food ration and low temperature with enhanced longevity (McCay et al. 1956, Comfort 1963). Golden trout were clearly stunted, suggesting that their increased longevity may be the result of an analogous situation.

In streams, golden trout growth rates are much lower than the rates reported from lake populations (Needham and Vestal 1938, Curtis 1934, McAfee 1966). This agrees with considerable information regarding other species of trout (Carlander 1969), and may be due to several factors. In lakes, fish have access to zooplankton and large prey items not available in streams. This is particularly true following introduction of new populations (including *O. aguabonita*) to fishless lakes, before the prey community composition has been altered (Needham and Vestal 1938). Fish feeding behavior is different in lakes and streams; in streams, fish must expend energy maintaining position and feeding in fast currents (Jenkins 1969, Smith and Li 1983), reducing energy available for growth.

Regression analyses suggested that several factors may influence the growth of golden trout in our study streams. When growth rate was measured as standard length/age, most of the variation was explained by age (Table 3); younger age classes grew more rapidly than older age classes. The amount of the stream covered with aquatic vegetation was positively correlated with trout growth. Aquatic vegetation often provides important habitat for invertebrates and may promote higher invertebrate abundance and diversity (Dudley et al. 1986). Thus, aquatic vegetation may increase the amount of food available to golden trout.

Weight at each age class showed much more variation than size at each age class (Figures 3 and 4). Since weight can be modified over a shorter time period than length, growth rates based on weight may have been more sensitive to the effects of the site variables used in the analyses than growth rates based on length. Growth rates measured as weight/age were affected by numerous site variables (Table 4). Sites with higher fish density (number of fish/m<sup>2</sup>) contained fish with significantly slower growth rates. Increased fish density may result in decreased growth rates by decreasing per capita food availability or by increasing competition for foraging sites or cover (Chapman 1966, Chapman and Bjornn 1969, Elliot 1984). Such competition could force fish to expend energy on agonistic interactions instead of on growth.

Density measured as the number of fish/100 m may be a better predictor of growth rates than density measured as the number of fish/m<sup>2</sup> if these trout populations are limited more by the availability of cover than by foraging sites. Nearly all cover in the study streams occurred along the banks and was heavily utilized by fish. Foraging sites were generally in mid-stream and may not have been in short supply in the study streams, since individuals often changed sites without interactions from conspecifics.

Fish from higher elevation streams had higher growth rates than those from lower elevations. McAfee (1966) suggested that if higher elevation habitats have lower fish densities than lower sites, reduced competition at higher sites may result in faster growth. However, our analyses should have removed any density effect and we are thus unable to provide a satisfactory explanation for this relationship.

There was a positive relationship between the amount of streambank vegetation and trout growth. Overhanging vegetation provides cover for fish and may increase the number of terrestrial insects available to trout.

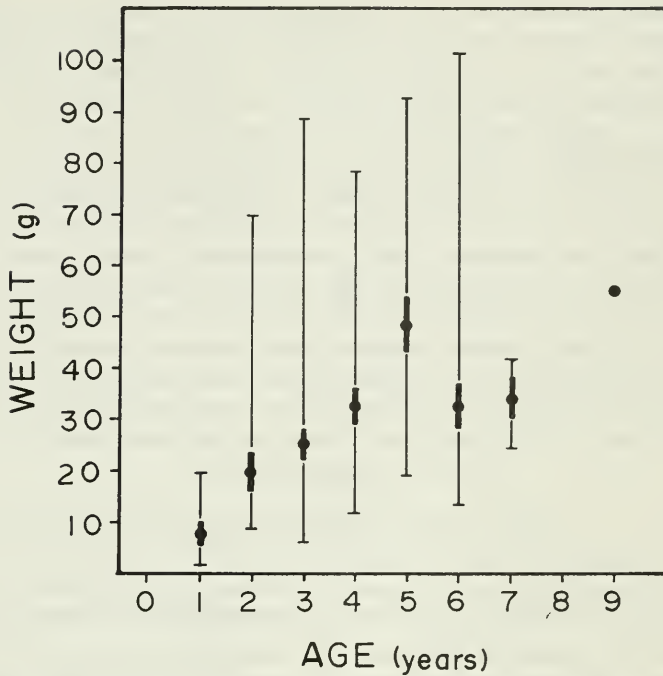


FIGURE 4. Golden trout weights by age class. Samples sizes for each age class are given in Table 2.

The negative correlation between the Pfankuch stream stability rating and trout growth (weight/age) suggests that trout from streams with higher channel stability gained weight faster than those from streams with lower channel stability. Similar effects of channel stability on fish growth have been found for rainbow trout (Van Velson 1979) and brown trout (Dahlem 1979) and were suggested to result from increased food and cover.

Condition factors for 343 trout ranged from 0.90 to 2.16. Despite this variability, our calculated mean  $K$  was 1.34, which is similar to  $K$  values estimated for lake populations of golden trout ( $K = 1.315$  in Needham and Vestal 1938, 1.34 in Curtis 1934). Apparently, populations of golden trout achieve quite similar condition relationships, even in very different habitats.  $K$  values of other trout species vary considerably among habitats, but it appears that trout in montane streams have  $K$  values similar to those of golden trout, ranging from 1.15 to 1.63 (Carlander 1969). This uniformity in condition even among different trout species may explain why none of the measured site characteristics affected trout condition in our analysis.

Trout density was not affected by any of the measured site characteristics used in the regression analysis. This analysis should be interpreted with caution, however, as each stream was considered as a separate observation and only 13 streams were used in the analysis. Thus, even if any site characteristics (e.g. amount of suitable spawning gravel or aquatic insect biomass) did affect trout density, the analysis may have lacked the power to detect such effects. The marginally significant correlation between trout density (number of fish/100m)

and the Pfankuch stream stability rating suggests that less stable stream channels support higher densities of fish. Although we are unable to provide an explanation for this relationship, it may suggest that fish grew more slowly in streams with lower channel stability because of higher trout densities.

In summary, several site variables affected trout growth. Trout density, trout age, and the Pfankuch stream stability rating were negatively correlated with trout growth while the amount of aquatic vegetation, the amount of bank vegetation, and elevation were positively correlated with trout growth. Differences between sites in stream stability, trout density, bank vegetation, and aquatic vegetation may all influence trout growth by increasing or decreasing the amount of available food or cover. Small increases or decreases in the availability of food or cover may be important in these low productivity streams. However, the low growth rates of golden trout throughout the study area were probably a result of the low productivity of these unstable montane streams and the short period of time available each year for rapid trout growth.

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# COMPARISON OF EFFICIENCY AND SELECTIVITY OF THREE GEARS USED TO SAMPLE WHITE STURGEON IN A COLUMBIA RIVER RESERVOIR <sup>1</sup>

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We compared the efficiency and size selectivity of setlines, gillnets, and angling to select a cost-effective means of capturing a large number of adult and subadult white sturgeon, *Acipenser transmontanus*, unharmed while minimizing size selectivity and catch of non-target game fish. Setlines provided the greatest catch rate per sampling week (61.4), followed by gillnets (49.4) and angling (34.4). Setlines also captured a wider size-range of sturgeon than gillnets or angling. No other game fish were caught with setlines or angling while gillnets caught other game fish including salmon and steelhead, *Oncorhynchus spp.*

## INTRODUCTION

White sturgeon, *Acipenser transmontanus*, is a valuable resource along the Pacific Coast of North America (Pycha 1956, Semakula and Larkin 1968, Kohlhorst 1980, Cochnauer 1983, Oregon Department of Fish and Wildlife 1988). In the Columbia River, white sturgeon support recreational, commercial, and tribal fisheries (Galbreath 1985). With the decline of anadromous salmonid fisheries (Raymond 1988), the white sturgeon fishery has rapidly increased in importance. Total effort and landings have increased several-fold since 1970 (Oregon Department of Fish Wildlife 1988), and effort by recreational white sturgeon anglers now exceeds effort by recreational salmon anglers downstream from Bonneville Dam (Hess and King 1988).

The status of white sturgeon varies within the Columbia River basin. Although the population below Bonneville Dam has supported a harvest of over 50,000 fish annually in recent years, populations in the Snake and Kootenai rivers (Columbia River tributaries) have diminished to the point where no harvest is allowed. Populations have declined in tributaries, possibly for several reasons: (i) migration of white sturgeon into the upper basin has been blocked by the construction and operation of hydroelectric dams (Bajkov 1951, Lukens 1981); (ii) habitat, including food availability, flow, and temperature has been altered by the creation of reservoirs formed by these dams (Bajkov 1951, Coon et al. 1977, Haynes et al. 1978, Lukens 1981); (iii) other biological and physical factors such as predation and the level of pesticides also may have changed (Bosley and Gately 1981).

We needed a gear that would collect a large sample of white sturgeon unharmed to evaluate effects of dam construction and operation on white sturgeon and to design management strategies to optimize yield (Rieman et al. 1987). Other considerations included sampling efficiently to minimize the cost

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of sampling hundreds of kilometers of river, gear size selection which might bias representation of the population (Beamesderfer and Rieman 1988), and incidental catch of other game fish. Hence, the objective of this report is to describe the most effective gear for capturing subadult and adult white sturgeon unharmed while minimizing gear selectivity and catch of other game fish.

## STUDY AREA

We selected The Dalles Reservoir, a mainstem impoundment of the Columbia River (Figure 1), for our gear analysis because this reservoir is relatively small compared with other impoundments in the Columbia River and access to all parts of the reservoir is good. The Dalles Reservoir is located between The Dalles and John Day dams (river kilometer 308 to 347). It was formed in 1957 with the closure of The Dalles Dam, a U.S. Army Corps of Engineers hydroelectric, navigation, and flood control project. At mean operating level, the reservoir has a surface area of 3,800 hectares, water elevation of 48.2 m above mean sea level, and depth as great as 61 m. The upper reservoir is riverine, and measurable current exists throughout the reservoir. Average daily inflow and outflow ranges from 3,000 to over 12,000 m<sup>3</sup>/s.

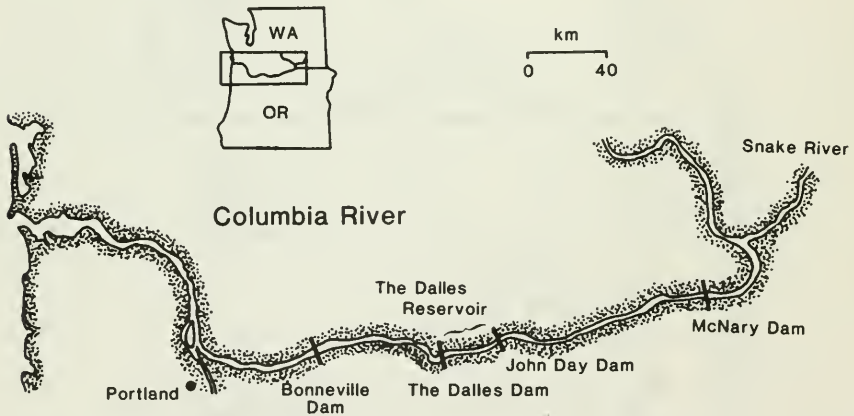


FIGURE 1. Location of The Dalles Reservoir.

## METHODS

We chose setlines, gillnets, and angling as potential ways to collect white sturgeon based on review of the literature and discussions with commercial fishermen. We sampled from March through September, 1987. Effort of each gear was evenly distributed throughout the reservoir and the period of sampling.

Setlines consisted of a 182-m long mainline of 6.4 mm diameter nylon rope, along which 40 hook lines (gangions) were equally spaced. Each gangion consisted of a removable, spring-loaded snap attached to a 0.5-m length of parachute cord by a swivel, with a hook attached to the other end of the cord. Each setline included 10 each of size 10/0, 12/0, 14/0 and 16/0 circle hooks. Circle hooks have the point bent at a 90° angle to the shaft to better retain hooked fish for long periods of time. Each end of a setline was held in place by

a 15–20 kg anchor. Each anchor was also attached to a buoy with rope identical to the mainline. Lines were set for 4–48 h in depths from 3 to 50 m. Hooks were baited with 2.5- to 5-cm long cross-section slices of adult pacific lamprey, *Entosphenus tridentatus*, or 2.5- to 10-cm<sup>2</sup> pieces of adult coho salmon, *Oncorhynchus kisutch*, with skin attached. Only one bait type was used on each line. Setlines were deployed and retrieved from a 7 m fiberglass skiff equipped with a hydraulic pot hauler.

Gillnets were sinking type and set stationary and perpendicular to the current. Each net was 45.6 m long and consisted of six, equal length, alternating panels of 5.1 cm, 8.3 cm and 11.4 cm bar mesh. Net panels were constructed from multifilament and cable nylon. Each panel was hung with 4.6 m deep mesh on a framework with a 3-m long vertical slacker lines attached to the float and leadline at 3.8 m intervals along the length of the net. Gillnets were held in place by 4.5–20 kg anchors depending on current. A buoy marked each anchor. Gillnets were fished for 1–4 h in depths from 3 to 35 m. Nets were deployed and retrieved by hand from the boat.

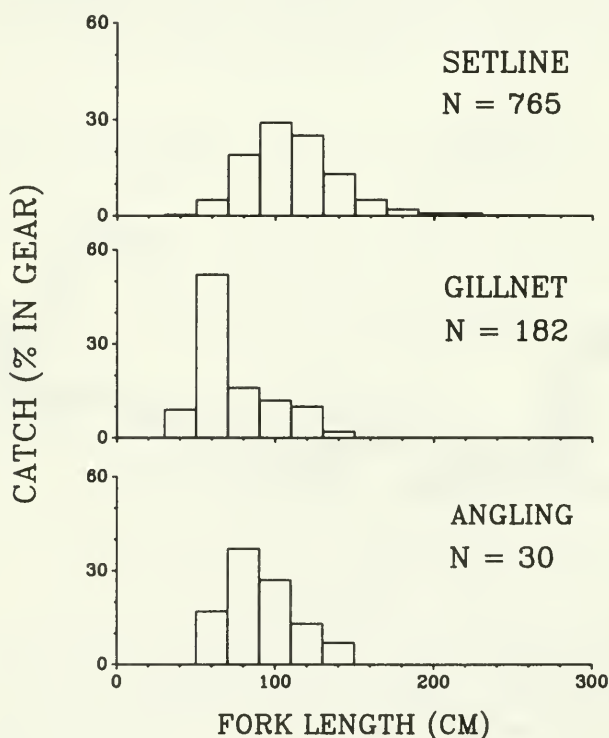


FIGURE 2. Length-frequency distributions of white sturgeon collected with various gear in The Dalles Reservoir of the Columbia River, 1987.

Angling gear consisted of a medium heavy action rod with a sensitive tip, a bait casting reel with 18 kg breaking strength monofilament line and 7/0 or 9/0 J-type hooks. Rods were closely attended. Hooks were baited with Pacific lamprey pieces, coho salmon pieces, whole eulachon, *Thaleichthys pacificus*,

whole juvenile coho salmon or pickled herring, *Clupea* sp. We fished from an anchored boat for durations of 1/2 to 3 h in depths from 15 to 45 m.

We measured fork length of captured white sturgeon to the nearest centimeter. Hook size and bait type were recorded for all sturgeon caught by setlines and angling. We also identified and counted the catch of other fish species.

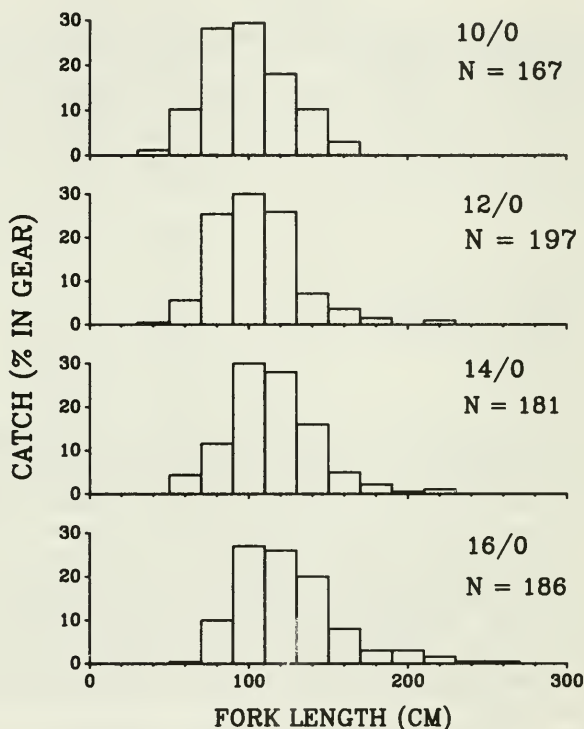


FIGURE 3. Length-frequency distributions of white sturgeon collected with various hook sizes on setlines in The Dalles Reservoir of the Columbia River, 1987.

We evaluated gear based on harm caused to white sturgeon, sampling efficiency, size selectivity, and catch of other game fish. Harm was evaluated by the number of dead white sturgeon in the catch by gear. Sampling efficiency was evaluated by comparing catch-per-unit-effort (CPUE) among gears. We standardize CPUE of gear by calculating mean catch per crew week (40 hours of sampling by a crew using the gear) based on 13.4 crew weeks of setlining, 3.7 crew weeks of gillnetting and 0.9 crew weeks of angling. We evaluated size selectivity by comparing length-frequency distributions of catch among gears. We assumed size selectivity was least where the range of lengths sampled was greatest. Statistical differences in length frequencies of fish captured among gears were identified with chi-square tests. We also used chi-square analysis to test for the selectivity associated with hook size and bait type used while setlining.

TABLE 1. Summary of white sturgeon effort and catch in The Dalles Reservoir, 1987.

Gear	Number of Observations	Crew hours	Catch	Catch per 40 crew hours
Setline .....	233	538	826	61.4
Gillnet .....	87	149	184	49.4
Hook and line .....	25	36	31	34.4

## RESULTS

All three gear sampled white sturgeon essentially unharmed. Direct mortality was only one fish for each gear.

Setlines were the most productive gear (Table 1). Catch per crew week with setlines was 1.24 times catch with gillnets and 1.78 times catch by angling.

TABLE 2. Incidental catch of fish other than sturgeon in The Dalles Reservoir, 1987.

Species	Setline	Gillnet	Hook and Line
Carp, <i>Cyprinus carpio</i> .....	0	4	0
Channel catfish, <i>Ictalurus punctatus</i> .....	0	1	0
Chinook salmon, <i>Oncorhynchus tshawytscha</i> .....	0	1	0
Largescale sucker, <i>Catostomus macrocheilus</i> .....	0	6	0
Northern squawfish, <i>Ptychocheilus oregonensis</i> .....	19	14	0
Sockeye salmon, <i>Oncorhynchus nerka</i> .....	0	3	0
Steelhead, <i>Oncorhynchus mykiss</i> .....	0	10	0
Walleye, <i>Stizostedion vitreum</i> .....	0	2	0

Different gear caught different sizes of fish (Figure 2) and differences were significant between setlines and gillnets ( $X^2 = 340.7$ ;  $df = 7$ ;  $p < 0.01$ ). Setlines captured white sturgeon over a much wider range of lengths and fish of a greater length than gillnets. Sample sizes from angling were inadequate to statistically compare length distribution differences with other gears.

Differences in length-frequency distributions were significant for white sturgeon captured by various hook sizes ( $X^2 = 88.3$ ;  $df = 18$ ;  $p < 0.01$ ) (Figure 3). Larger hooks took larger fish and fish over a wider range of fork lengths. White sturgeon greater than 90 cm fork length appeared fully recruited to all setline hook sizes.

No significant difference in length-frequency distributions was detected between bait types ( $X^2 = 5.2$ ;  $df = 5$ ;  $p = 0.389$ ).

Gillnets frequently caught fish other than sturgeon including several game species (Table 2). Setlines caught only the nongame northern squawfish. No other fish were caught while angling for sturgeon.

## DISCUSSION

We concluded that setlines were the best available gear for our study. Setlines caught more white sturgeon per crew hour than the other gears and did not catch any other game fish. Setlines also appeared to take the most representative sample of white sturgeon over 90 cm based on length-frequency distributions of catches. We were primarily concerned with white sturgeon 90 cm and larger, corresponding to lengths harvested in the fisheries, and the reproductive stock (fish longer than 183 cm, the maximum legal length limit).

The gillnets had many drawbacks and few advantages over setlines. Whereas white sturgeon mortality was low in gillnets, the nets captured fish other than sturgeon, particularly adult salmon and steelhead, often with a substantial mortality rate. This restricted use of gillnets to areas and times where salmon and steelhead were absent. Gillnets also captured white sturgeon from a narrower range of lengths than did setlines, and much of the catch consisted of fish under 90 cm fork length.

Angling was also inferior to setlines. Although mortality was low, the effort per fish was high. The length range of white sturgeon captured with hook and line fell within that captured by setlines, although a similar distribution might be expected with a greater sample size.

We will only sample with setlines for the remainder of the study. However, we have made a few modifications to address the efficiency and selectivity of our setlines. We are discontinuing using 10/0 hooks for the following reasons: (i) they required more crew hours to use because they were harder to sharpen and bait and required more frequent replacement; (ii) the white sturgeon they captured were within the range of those captured with 12/0 hooks; and (iii) these hooks were often straightened out or snapped off, apparently unable to hold larger fish. We will only use 136-kg test gangions because of the number of broken 68-kg test gangions observed.

Finally, we will only use Pacific lamprey for bait. Pacific lamprey slices appear to be an attractive bait for white sturgeon for more than one day. Coho salmon pieces often fell apart within 24 hours. Further, Pacific lamprey is relatively easy to obtain and minimizes preparation and gear deployment time.

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We thank Doug Engle and Ray Hartlerode for assistance with field sampling. Bruce Rieman guided this project through its early stages. Wayne Burck, Mary Buckman, and Tony Nigro provided constructive editorial comments on early drafts of this manuscript. The Bonneville Power Administration provided funding for this work under Contract DE-AI79-86BP63584.

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## NOTES

**GIANT BLUEFIN TUNA OFF SOUTHERN CALIFORNIA,  
WITH A NEW CALIFORNIA SIZE RECORD**

Most of the Pacific bluefin tuna, *Thunnus thynnus orientalis* (Temminck and Schlegel, 1844), landed by purse seiners in the eastern Pacific weigh 5 to 25 kg, but fish weighing over 50 kg are not uncommon. Exceptionally large bluefin tuna were caught, mostly in November and December, 1988, by a fleet of small purse seiners based in San Pedro, California. During the period of 28 October 1988 to 4 January 1989, 15 boats made 56 trips to areas just south of the east end of Santa Rosa Island, northwest of San Nicolas Island, and to Tanner and Cortes banks (Figure 1). They captured an estimated 987 fish weighing from about 50 to 458 kg; the total weight was about 139 mt and the average size was about 141 kg. These fish are of special interest because bluefin tuna of this size are of great value on the Japanese sashimi market, no bluefin of this size had been caught previously in such quantities in the eastern Pacific, and many of these were larger than any bluefin caught previously in California.

Meristic characters and morphology were used to verify identification. Gill raker count on a 242-cm specimen was  $13 + 23 = 36$ , and the ventral surface of the liver was striated in several specimens, which, coupled with the extreme size, indicate these fish were not yellowfin tuna, *T. albacares*. The caudal keels were usually black or dark grey, indicating that these were not southern bluefin, *T. maccoyii*. The pectoral length, as a percentage of fork length, was 16.8% for the record fish, indicating it was a Pacific bluefin tuna (Gibbs and Collette 1967).

Yukinawa and Yabuta (1967) fitted a von Bertalanffy growth curve to age data derived from scales of bluefin up to 7 years old from the western Pacific and extrapolated lengths-at-age to 20 years. From their equation, bluefin of this size are estimated to be from 5 to 17 years old. Bluefin of the same size from the Atlantic are estimated by analysis of otoliths (Hurley and Iles 1983) to be 7 to 24 years old, although significant variation in age exists among individuals of the same length and between sexes.

The largest fish (Figure 2) was caught at ca. 0200 h on 18 December 1988, at San Nicolas Island, by the 21-m purse seiner SEA QUEEN. It weighed 457.7 kg and measured 271.2 cm fork length (FL). It exceeded the previous California record (Dotson and Graves 1984) by 220.7 kg, although there is an unsubstantiated record of a 408-kg fish taken in a net in Monterey Bay early in the 20th century (Holder 1913). The fish was male, appeared robust, and its position relative to the length-weight curve was well above the rest of the values used for fitting it (Figure 3).

Large bluefin were initially sighted around 15 October, but were over deep water and no successful sets were made. On 27–30 October several vessels caught ca. 63 tons of smaller bluefin (50–70 kg), mostly around Tanner and Cortes banks. On 30 October, a favorable report from spotter aircraft sent the

boats north to the relatively shallow (less than 50 fm) area south of East Point on Santa Rosa Island. As the fish were wild and not catchable during the day, the boats, assisted by the spotter aircraft, set at night on visible trails caused by movement of the fish through bioluminescent plankton. The nets of most vessels in the fleet normally fish at 45–50 fm maximum depth, and unless the schools were in shallower water, the fish usually escaped underneath or otherwise evaded the net. Sets were made in water as shallow as 18 fm and as deep as 100 fm. Catches of the large fish were first made on 31 October and continued sporadically through December with the last catch occurring on 3 January 1989. Most of the sightings were made between Santa Rosa Island and Begg Rock.



FIGURE 1. Areas of catches (crosshatched) of large bluefin tuna captured during November and December 1988.

Schools such as these are conspicuous during both night and day when near the surface, and with the aid of fairly constant spotter aircraft activity in previous years, were seen sporadically in 1986 and 1987, although less frequently than in 1988. Schools were reported from the Sixtymile Bank northwest to the Rodriguez Seamount but few, if any, were caught. Prior to this season (1988), spotter aircraft may have ignored large fish in small schools (1–5 fish) because they were either misidentified as pods of marine mammals or judged unprofitable in tonnage. As prices rose dramatically due to interest shown by Japanese buyers, the spotters and vessels also became interested in the small schools of larger fish.





FIGURE 2. The California record bluefin tuna.

Drift gill-net vessels also reported the large fish, although offshore and over deeper water (greater than 1000 fm), during October–December of 1986, 1987, and 1988, but caught no bluefin greater than about 50 kg, as the larger fish tore holes in the nets. During this period of 1988, a gill-net vessel set its gear in the Santa Rosa Flats area, and the pilot of a spotter aircraft observed a school of bluefin swim through the net. Subsequent examination of the net revealed the large holes similar to those evident after fishing in the offshore areas.

The mean weight of the fish caught mostly at Santa Rosa Island during November (194 kg) was much greater than that of those caught during December (100 kg), when fishing shifted to San Nicolas Island and Tanner and Cortes banks. The greatest mean size of fish and smallest number of fish per successful set (Table 1) were captured during the latter half of November, when a total of 79 fish (6.6 per set) averaged 260 kg for the 2-week period of 18 November–1 December. Calkins (1982) presented length-frequency data by year for surface-caught bluefin in the eastern Pacific Ocean which showed few instances of fish approaching these sizes.

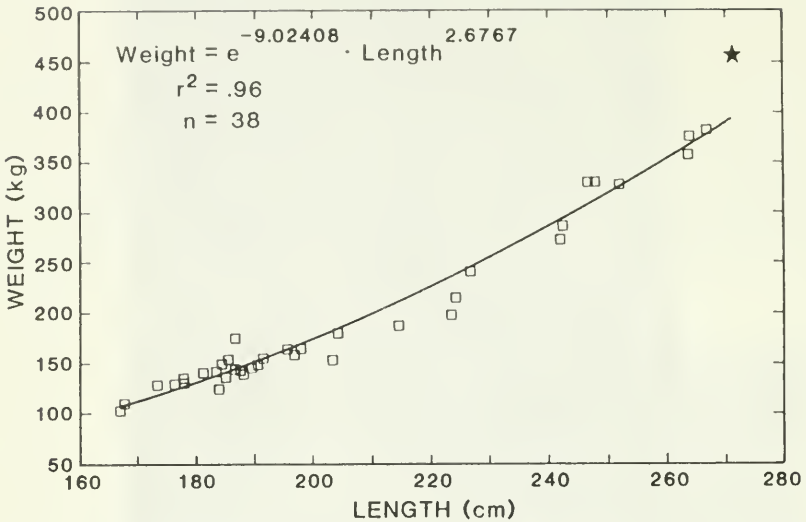


FIGURE 3. The length-weight relationship for 38 bluefin tuna captured during November and December 1988 off Southern California. Record fish indicated by star.

TABLE 1. Summary of Southern California Landings (in Kg) of Large Bluefin Tuna Captured Between 28 October 1988 and 3 January 1989.

Week ending	Area	Trips	Total no. of fish	Mean no. of fish	Mean wt. per fish	Total landings	Mean catch
Nov 3	Tanner, Cortes banks, Santa Rosa I.	6	56	9.3	150	8,404	1,401
Nov 10	Santa Rosa I.	10	189	18.9	178	33,744	3,374
Nov 17	"	5	103	20.6	197	20,284	4,057
Nov 24	"	5	34	6.8	272	9,230	1,846
Dec 1	"	7	45	6.4	251	11,318	1,617
Dec 8	Tanner Bank	5	84	16.8	52	4,385	877
Dec 15	Tanner Bank, San Nicolas I.	9	287	31.9	126	36,208	4,023
Dec 22	San Nicolas, Sta. Barbara I.	5	36	7.2	170	6,107	1,222
Dec 29	—	0	0	—	—	—	—
Jan 5	Tanner Bank	4	153	38.3	59	9,072*	2,268*
TOTAL		56	987	17.6	141	138,753	2,478

\* estimated

According to records of the Inter-American Tropical Tuna Commission (IATTC), catches of large bluefin in the eastern Pacific are rare. During August–December 1977, fish of about 50 to 110 kg were caught off northern Baja California by purse seiners. In October 1986, one vessel caught 45 mt of bluefin in shallow water off Santa Rosa Island, about half of which were exceptionally large. Fifty of these were measured by the IATTC; their lengths ranged from 180 to 200 cm (ca. 109 to 145 kg). On 7 December 1981, a single fish weighing 237 kg was caught in a drift gill-net 12 mi south of Anacapa Island (Dotson and Graves 1984). The record for bluefin tuna captured in California waters by a sport angler stood at 113.9 kg from 1899 to 4 October 1983, when a 164.9-kg fish was captured at Osborn Bank (S.J. Crooke, Calif. Dept. of Fish

and Game, Long Beach; pers. comm.). Large bluefin tuna are known to occur in Baja California waters around Guadalupe Island (lat 29°11'N, long 118°17'W). On 21 July 1982, an angler captured a 126.5-kg bluefin and on 22 September, of that year, a diver speared a 180.5-kg fish at this locale.

Bluefin weighing more than 225 kg occur frequently in Japanese longline catches in the central and western Pacific. The largest bluefin recorded by the Far Seas Fisheries Research Laboratory of Shimizu, Japan, weighed about 555 kg (ca. 3 m). It was caught during April 1986, about 300 mi south of Kyushu Island, Japan. Large Pacific bluefin also occur occasionally in the southwest Pacific (Collette and Smith 1981). Bluefin between 225 and 450 kg are caught regularly in the Atlantic, and fish between 450 and 680 kg are sporadically encountered there (Hisada and Suzuki 1982).

IATTC employees collected biological samples from 62 of the fish and made abstracts of the logbook records of all the vessels. The mean sea-surface temperature (for those boats recording it) was 14.1°C. Gross examination of stomach contents during processing revealed that when captured the fish had been feeding primarily on chub mackerel, *Scomber japonicus*, and small (10 cm) market squid, *Loligo opalescens*, indicating feeding near the surface.

The gonads of 45 fish were examined; all were males. No studies of sex ratios in large (> 100 cm) bluefin tuna in the eastern Pacific appear in the literature, but Yamanaka et al. (1963) note that females predominated in fish sampled by longline during May and June in the area east of Formosa (Taiwan), and males in fish sampled around Sanriku during June through August. Rivas (1975) found a preponderance of females in Atlantic bluefin tuna caught in the Straits of Florida and near the Bahama Islands during April and May, and a preponderance of males in the Gulf of Maine and off Nova Scotia and Newfoundland in July through October. Although there is an indication of segregation by sex in these large fish in the eastern Pacific, lack of data from other geographical areas precludes further speculation.

The reason for the appearance of these exceptionally large fish in the eastern Pacific is unknown. Immature Pacific bluefin are trans-Pacific migrators (Orange and Fink 1963, Clemens and Flittner 1969, Bayliff 1980), with a portion of the population migrating from the western Pacific after the first or second year of life and staying in the eastern Pacific for up to 2 years or more, before returning to the western Pacific. Bluefin tagged in the eastern Pacific and recaptured in the western Pacific were at liberty for a minimum of 22 months (Clemens and Flittner 1969, Bayliff 1988). There is no evidence of spawning, e.g. presence of larvae or juveniles or reproductively-active adults, in the eastern Pacific (Bell 1963). First maturity occurs at 3 to 4 years (Hirota, et al. 1976), and fish older than that (50 to 150 kg) occur in the eastern Pacific at irregular intervals, especially late in the year around offshore banks, but not nearly as frequently as do the younger ones. It is not known whether these large fish were recent arrivals from the western Pacific or had spent several years in the eastern Pacific.

Ex-vessel price for the vessel owners who sold their catches outright was \$15.40 to \$26.40 per kg, although other owners opted to receive a share of the profits from the sale of the fish in Japan, where prices ranged from \$35.20 to over \$77 per kg.

## ACKNOWLEDGMENTS

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## BOBCAT ELECTROCUTIONS ON POWERLINES

Powerline electrocution of birds has been well documented in the scientific literature (Anderson 1933, Dilger 1954, Harrison 1963, Boeker and Nickerson 1975, Kothman and Litton 1975, Switzer 1977, Pomeroy 1978, Benson 1981, Olendorff et al. 1981, Dedon and Colson 1987, Williams and Colson 1989). However, published accounts of electrocutions of other vertebrate taxa are extremely rare. Edison Electric Institute (1980) noted that snakes, mice, tree squirrels, *Sciurus* spp.; flying squirrels *Glaucomys* spp.; raccoons; *Procyon lotor*; and black bears, *Ursus americanus*; occasionally come in contact with energized electrical equipment and, therefore, are subject to potential electrocution. Among wild mammals, squirrels are the most frequent victims of electrocution because of their penchant for chewing on energized wires (Commonwealth Edison 1975, Pacific Gas and Electric Company [PG&E] unpubl. data). There is some evidence that interactions between black bears and power lines are increasing in number as civilization encroaches on the species' habitat. In Albuquerque, New Mexico, for example, a tranquilized black bear that had climbed a power pole to escape capture suffered a non-fatal electrical burn as it fell into a 7,200-volt powerline (W. R. Pilz, Public Service Company of New Mexico, pers. comm.). To my knowledge, powerline electrocutions have not been reported in the literature for any other mammalian species. This note documents the electrocution of two bobcats, *Felis rufus*, on PG&E power poles in north-coastal California.

The carcass of an adult female bobcat was discovered by a PG&E line crew on 19 September 1988, approximately 8.5 km northwest of Inverness, at Point Reyes National Seashore. The animal was draped over a transformer attached to a pole supporting a 12,000-volt powerline (Figure 1). Maggots had infested the carcass, suggesting that the animal had died several days prior to being discovered. PG&E linemen reported that a similar bobcat electrocution occurred in the late-1970s, approximately 16 km southwest of Inverness, near the Point Reyes Light Station. However, no additional details are available on this earlier mortality.

Bobcat electrocution appears to be an extremely unusual and random event, as evidenced by the absence of published accounts of powerline interactions. These animals often climb trees when threatened, or occasionally while hunting (Chapman and Feldhamer 1982). Where trees are absent, as in the coastal scrub habitat covering much of Point Reyes National Seashore, it is reasonable to assume that bobcats will climb utility poles to escape danger or pursue prey. I speculate that the animals in question may have climbed power poles to escape the pursuit of dogs originating from the local ranches. This behavior would be consistent with the well-documented "treeing" response displayed by bobcats and other felids when pursued by trained hunting dogs. After an animal climbs a power pole, simultaneous contact with two energized wires or a wire and a grounded object (e.g., a transformer) would normally be sufficient to cause a "flashover" of electricity and electrocution of the animal.

I gratefully acknowledge W. Crawley, T. J. McMorrow, and B. E. Perron for providing background information on the bobcat electrocutions. E. W. Colson, M. F. Dedon, T. Silver, and B. F. Waters reviewed the manuscript.

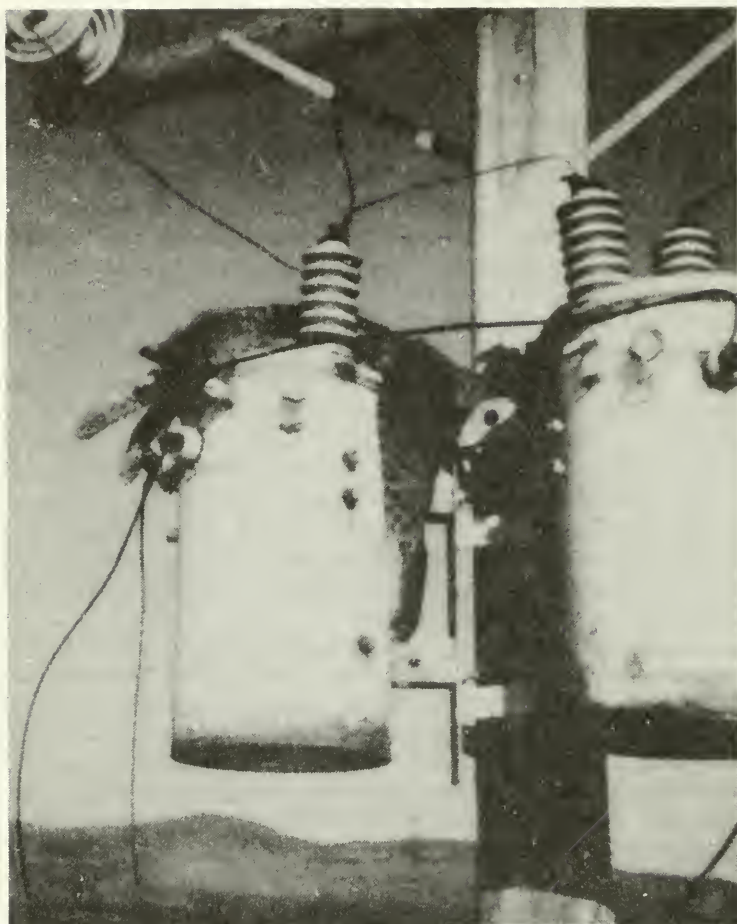


FIGURE 1. Carcass of an adult female bobcat on a 12,000-volt powerline transformer.

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## BOOK REVIEWS

**MARINE POPULATIONS: AN ESSAY ON POPULATION REGULATION AND SPECIATION**  
by Michael Sinclair. 1988. University of Washington Press, Seattle, WA. 260 p. \$25.00 cloth, \$15.00 paper.

The oceans are a highly dynamic environment. Anyone who has studied planktonic life forms sooner or later is confronted with the question of "How do these organisms maintain populations in regions that are conducive to their life history?" Plankton are subjected to currents transporting them from areas optimal for growth and reproduction to areas that may preclude growth, increase mortality rates, and not allow for recruitment of these organisms or their progeny back to the originating population. This question not only relates to phytoplankton and invertebrate zooplankton, but to fishes that have a planktonic life stage. It is this question and its implications that the author examines in this book.

We know a good amount about the life history of Atlantic herring and the author uses these populations to show that self-sustaining populations exist in areas of the ocean in which ocean dynamics can be used to ensure that some portion of the planktonic larval stages will recruit back to the originating population. In response to ocean dynamics, populations of herring have spawning periods timed to take advantage of seasonal changes in currents, spawning in the proximity of bays and estuaries, or the migration of spawning adults to areas in which larval stages will have maximal survival to a size at which they are capable of returning to the spawning areas. Those that are carried to regions from which they cannot contribute to the population are lost from the gene pool.

Following this analysis, the author defines the "member/vagrant hypothesis". This hypothesis leads to several tenants. Populations (which have a planktonic life stage) are found in "geographical settings . . . within which the species' life cycle is capable of closure". Population size for the most part is limited by the geographical area that allows for closure—those animals spawned outside of the area (or time period) are lost to the population. For such organisms food may seldom be a limiting factor. Likewise intraspecific competition may seldom come into effect. Recruitment success or failure becomes primarily a function of ocean dynamics. A weakening of currents may result in high recruitment despite lower productivity because more larvae remain in the geographical area that allows closure. Conversely stronger currents with higher net productivity may increase larval survival but if most of these are carried away never to recruit back to the population we have recruitment failure. Once populations become limited to specific geographical areas by ocean dynamics, a mechanism for speciation becomes apparent. We now have isolated spawning groups.

The author goes on with further examples of populations, both fishes and invertebrates, in which the hypothesis is apparently working. The more I read, the more excited I became. In the last several chapters, the author questions a number of ecological generalizations (food is a limiting factor, etc). Likewise certain aspects of evolutionary theory, primarily that populations are self-regulating and therefore competition is a strong factor in natural selection, are not applicable to animals whose populations are controlled by ocean dynamics.

If any of these statements pique your curiosity, by all means read this book. I hope that the arguments put forth here are given serious thought. All too often we tend to sit back and assume an understanding of phenomena because a theory exists or a simple explanation is given, i.e., that factor is density dependent, when in fact we do not really know the underlying relationships. Perhaps this is why a number of fisheries today are in trouble. If many of our pelagic fisheries are limited by ocean dynamics, then it comes as no surprise that our population dynamics models have not served us well.

The next question then becomes "What do we do now?" Hopefully this work will lead to advances in fishery management as well as a re-examining of ecological and evolutionary mechanisms when the old explanations fail. I would hope that anyone interested in population dynamics and certainly students now taking such courses would read this book.

—*John J. Geibel*

**BATTLING THE INLAND SEA: American Culture, Public Policy, & the Sacramento Valley, 1850–1986**

By Robert Kelley. 1989. University of California Press, Berkeley, CA, 416 p., \$35.00

This book presents an excellent history of flood control efforts in the Sacramento valley primarily from the 1850's through 1920, when the present flood control system had been adopted and its



implementation was well underway. Events from 1920 through the flood of 1986 are described briefly. The author describes the interrelationships between flood control, swampland reclamation and hydraulic mining. It is a story of repeated failures but eventually largely successful conclusion, as the largest flood of record was contained with minimal harm.

The author is a historian, and an important feature of the book is how he has interwoven flood control activities with the underlying social and political events. Among the latter is the evolution from a constitutional requirement that governmental actions be prescribed in detail in law to the acceptance of laws delegating considerable discretion to the executive branch to act within broad policy. Also of interest, are the shifts back and forth between populist driven local control and centralized professional management, depending primarily on whether the Democrats or Republicans controlled government.

In the Preface the author acknowledged that reclamation of the valley "ended in the destroying of a large natural environment". He, however, makes no attempt to describe the resources which were lost. Nevertheless, those interested in the evolution of our present society in the Sacramento valley will find the book worth reading.

—*Harold K. Chadwick*

#### ARIZONA GAME BIRDS

By David E. Brown. 1989. The University of Arizona Press, Tucson, AZ. 307 p., illustrated. \$19.95 cloth.

I have been an avid consumer of information about upland game birds for over twenty years, as both a hunter and researcher. Unfortunately, good books about western upland game birds have been few and far between. I am most pleased to report that this is a good one and will no doubt become a standard for personal libraries.

The author uses his experience as a wildlife manager with the Arizona Department of Game and Fish, a naturalist, and a hunter to provide the reader with an informed and insightful treatment of Arizona game birds. Each chapter covers the distribution, habitat, life history, management history, population dynamics, and hunting of one of the thirteen game birds. The material is detailed enough to be of value to the professional, yet not so detailed as to be laborious to read. The text is well referenced, although the literature is only effectively cited up to 1985. However, it still provides an efficient and easy way to enter the scientific literature. The book was interesting to read and I recommend it highly.

—*Sonke Mastrup*

## MISCELLANEA

The following excerpts are from early issues of *California Fish and Game*:

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Forests, water power, and wild game are three of California's greatest resources. They are ours to use but not destroy.

—*from cover of Volume 1, Number 1—October 1914*

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The writer has been observing the fisheries of southern California for nearly thirty years. In that time the supply has dropped off to a menacing extent, due to lack of laws, lack of protection, and over-fishing where fishes should be protected.

—*Charles Frederick Holder, Volume 1, Number 1—October 1914*

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Students of natural history have become fully aware that as the country is settled marked changes take place in its bird life. A few of our species, such as the linnnet and mockingbird, have become more numerous than they were in the early days. But many more have become noticeably scarcer; some have disappeared altogether. Bird life as a whole has diminished in quantity to an alarming degree.

—*Joseph Grinnell, Volume 1, Number 1—October 1914*

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Everyone owns a share in the natural resources of this state. The protection and conservation of game is, therefore, to the interest of every citizen.

—*H. C. Bryant, Volume 1, Number 3—April 1915*

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Only a little study of the conservation situation in America is sufficient to show we have allowed certain parties at interest to take more than their rightful share of the resources of wild nature which as a matter of simple justice belong, not only to all the people now living, but also to the generations of the future indefinitely.

—*W. P. Taylor, Volume 1, Number 4—July 1915*

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Two sea otters were seen basking in the sun in the kelp beds off Del Monte wharf on October 22, 1916. They were apparently an old and young one, and the theory is that the old one came back to look for one of her young which was caught in a sea bass net last year.

—*P. H. Oyer, Volume 3, Number 2—April 1917*

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The sea urchin, which is quite abundant on our coast, will some day be an article of economic importance. A few are gathered and the meat eaten by Japanese in California and by natives in Alaska.

—*John N. Cobb, Volume 3, Number 3—July 1917*

## EDITORIAL POLICY

*California Fish and Game* is a technical, professional, and educational journal devoted to the conservation and understanding of fish and wildlife. Original manuscripts submitted for consideration should deal with the California flora and fauna or provide information of direct interest and benefit to California researchers and managers. Authors should submit the original manuscript plus two copies, including tables and figures.

**MANUSCRIPTS:** Authors should refer to the *CBE Style Manual* (Fifth Edition) and a recent issue of *California Fish and Game* for general guidance in preparing their manuscripts. Some major points are given below.

1. *Typing*—All text submitted, including headings, footnotes, and literature cited must be typewritten doublespaced, on white paper. Papers shorter than 10 typewritten pages, including tables, should follow the format for notes. Letter quality computer print-out is acceptable.
2. *Citations*—All citations should follow the name-and-year system. The "library style" is used in listing literature cited.
3. *Abstracts*—Every article must be introduced by a concise abstract. Indent the abstract at each margin to identify it.
4. *Abbreviations and numerals*—Use approved abbreviations as listed in the *CBE Style Manual*. In all other cases spell out the entire word.

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**FIGURES:** Consider proportions of figures in relation to the page size of *California Fish and Game*. The usable printed page is 117 by 191 mm (4.6 by 7.5 in.). This must be considered in planning a full page figure, for the figure with its caption cannot exceed these limits. Photographs should be submitted on glossy paper with strong contrasts. All figures should be identified with the author's name in the upper left corner and the figure numbers in the upper right corner. Markings on figures should be made with a blue china marking pencil. Figure captions must be typed on a separate sheet headed by the title of the paper and the author's name.

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