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Title: REARING CONDITIONS AND THEIR EFFECTS ON GROWTH, FOOD CONVERSION, AND SURVIVAL OF ENGLISH SOLE (Parophrys vetulus Girard)

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Richard S. Caldwell

This study consisted of determining some of the conditions for culture of young English sole (Parophrys vetulus Girard) in the laboratory. Rearing of the larvae to the juvenile stage was attempted unsuccessfully; techniques used and problems encountered during culture are discussed. Growth of juveniles was studied at combinations of temperatures and daily rations of the diet Oregon Moist Pellet ranging from 9.5 to 21° C and from zero to 16% of dry body weight. The effects of diet (artificial versus natural), population density, and dominance behavior on growth were also studied. Acutely tested tolerance (96 hour bioassay) to salinities in the range of 0 to 34‰ was determined.

In the temperature-ration experiment, fastest growth of the juveniles (0.95%/day) occurred at the lowest temperature and highest ration. Growth declined with decreasing ration and increasing temperature. At 21° C, juveniles lost weight regardless of ration. For fish fed Oregon

Moist Pellet the maintenance ration was determined to be 3.1% at 9.5° C, increasing to 4.7% at 18° C. Highest food conversion occurred at the lowest temperature and the 8% ration. Food conversion declined with increasing temperature and at rations greater than 8%. At an 8% ration and the temperatures 12, 15, and 18° C, growth rate of fish about 16 to 19 months old was approximately the same as for fish about 4 to 7 months old. However, the approximate maximum daily food consumption rate decreased from about 20% in 5 gram fish to 16% in 9 gram fish to 10% in 33 gram fish.

At the same restricted ration (8%), juveniles grew much faster on a clam-shrimp diet (1.16%/day), which was thought to be a more natural diet, than the Oregon Moist Pellet diet (0.42%/day). However, a greater total ration of the Oregon Moist Pellet (16%) could be consumed than the clam-shrimp diet (10%). Despite the faster growth on the natural diet, mortality during the experiment was higher in groups fed the clam-shrimp diet (32%) than in groups fed the Oregon Moist Pellet diet (3%). The reason for this differential mortality was not determined.

Over the range of initial population densities of 0.5 to 5.3 kg/m^3 no differences in growth rate were observed. In later experiments juveniles were reared at densities up to 15.1 kg/m^3 without mortalities nor slowing of growth.

Aggressive behavior was observed between English sole throughout the experiments. Active fish nipped at the tails of, and grew faster ($p < 0.01$) than, less active fish. When active and less active individuals were separated, both groups grew at the same rate statistically

when fed the same ration, although the active fish continued to grow faster. Active fish (22.7 grams) were significantly smaller ($p < 0.05$) as a group at the beginning of the experiment than less active, and slower growing, fish (24.1 grams).

Salinities down to 3.3‰ were tolerated by laboratory adapted juveniles for 96 hours without dying. At 1.3‰ salinity the time to 50% mortality at 9.5° C (46 hours) was twice that at 16.5° C (23 hours) for 0-group sole. At 1.6‰ salinity and 16.5° C, the time to 50% mortality was 1.5 times greater with 0-group fish (24 hours) compared with I-group fish (16 hours).

Diseases encountered during the experiments included gyrodactyliasis, caused by the monogenetic trematode Gyrodactylus, and three presumed bacterial infections: a fin rot, a coldwater lesion-producing disease, and a systemic bacterial infection, probably vibriosis. Treatments and preventive measures used are given. In addition, the occurrence and consequences of the parasites Philometra americana (Nematoda) and Glugea (Microsporida) and skin tumors are discussed.

Rearing Conditions and Their Effects on Growth, Food Conversion, and Survival of English Sole (*Parophrys vetulus* Girard)

by

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INTRODUCTION

Fish farming, or aquaculture, has attracted increasing attention in recent years because the demand for some desirable species has exceeded the supply. Many economically important fishes are not good candidates for culture because they are too large, too active, or too fragile, but the suitability for culture of most remains to be evaluated (Idyll 1971).

The farming of flatfish (flounders and soles) has been demonstrated to be potentially feasible by British investigators (Shelbourne 1964). My purpose in this study was to explore opportunities for farming a species of flatfish native to Pacific Northwest waters. The study has focused on the English sole, a species which enjoys a favorable market demand (Forrester 1969a) and which is available for collection at all life stages in shallow coastal waters (English 1969).

THE LIFE HISTORY OF ENGLISH SOLE

English sole (Parophrys vetulus Girard) are medium-sized fishes inhabiting the shallow waters of the continental shelf from Baja California to Western Alaska. This small-mouthed sole feeds primarily on polychaete worms, bivalves, and brittle stars, although other invertebrates and small fish are eaten occasionally (Forrester 1969a). Juveniles consume mainly small crustaceans and polychaetes (English 1969).

Adults spawn during the winter, producing small (1 mm in diameter), pelagic eggs that hatch in a few days. On the basis of experi-

mental evidence, Alderdice and Forrester (1968) estimated that 90% of the eggs would hatch into viable larvae over the ranges of 6.5 to 10° C and 20 to 32‰ salinity. Within a few weeks the larvae metamorphose into the adult form and begin a benthic existence (Forrester 1969a). The juveniles appear in Oregon's estuaries in the spring and remain the summer. In the fall most of the year class permanently joins the adults on the continental shelf (Olson and Pratt 1973, Pearcy and Myers 1974, Westrheim 1955). The size at first commercial exploitation, about 30.5 cm, is reached by some individuals by age three (Forrester 1969a).

English sole occur in several relatively distinct populations. Along the coast from Northern California to Washington one population is centered between the Columbia River and Cape Flattery, and another is centered between Cape Mendocino and Cape Blanco (Pacific Marine Fisheries Commission 1961). Considerable seasonal intermingling may take place between adjacent stocks, but long range migrations are rare (Forrester 1969b, Jow 1969).

GENERAL PRINCIPLES OF AQUACULTURE

Aquacultural practices are as varied as the number of species cultured and the location of culture, but economic well-being and eating habits of a country influence the type of culture and species farmed. In developing countries, the product must be inexpensive to produce. This results in the culture of mainly herbivorous fish or plankton feeders, such as carp and mullet, which can obtain the necessities of life from constructed or natural ponds. Such fish may serve

as a main protein source for the local people (Bardach et al. 1972). In developed countries there is an abundance of protein, so fish produced by aquaculture generally are carnivorous species, such as salmon and catfish, which are sold as specialty items or released into natural waters for recreational fisheries. These fish are reared in rather elaborate facilities in which the fish must be fed and the quality of the water controlled within limits imposed by the species (Bardach et al. 1972). The prerequisite information for successful intensive culture, such as in salmon hatcheries, includes knowledge of (1) optimum physical and biotic conditions for growth, reproduction, and survival, (2) basic nutritional requirements, and (3) infectious diseases likely to be encountered and the treatments and prevention methods available. In reality, these are interacting factors, the effects of which cannot be separated from one another. In this thesis they are discussed separately for ease of presentation.

Optimum Physical and Biotic Environment

Many features of the aquatic environment must not exceed certain limits naturally, or must be controlled, in order to culture fish. To maximize growth and reproduction, the limits are even narrower. For marine fish the water should be of the proper temperature and salinity, contain sufficient oxygen, be free of toxicants, and the water flow should be adequate to remove metabolic products; the light should be of the suitable intensity and photoperiod; the fish should not be crowded; and the container should be designed with the fish's needs in mind (Burrows 1972, Klontz 1973).

Fish held in captivity cannot avoid changes in their environment so must adapt or die. Under proper experimental conditions a zone of tolerance bounded by upper and lower lethal limits for many physical factors may be determined. The width and absolute values of the zone of tolerance depend on the genetic background, size, and physiological state of the fish, the interaction with other physical factors, and length of exposure (Brett 1970). Generally, reproductive processes and development of early stages are confined to narrower ranges than adults can tolerate (Kinne 1963). Fishes, especially during the older stages, can make physiological (Fry 1971) and biochemical (Hochachka and Somero 1971) adjustments to compensate for changes in the environment if the changes are not too great and if the fishes are given adequate time before the next stress. However, growth slows in suboptimal environments because compensation for stress generally requires additional energy, and food consumption, activity, and metabolism are modified. Warren and Davis (1967) and Warren (1971) have considered the latter bioenergetic question.

Temperature is one of the most important factors determining the health of fishes because most species are thermal conformers and reaction rates within the organism are temperature dependent (Burrows 1972). Since temperature affects many processes, such as digestion, food consumption, metabolism, and activity, and the effects of temperature may be modified by size and physiological state of the fish and other physical factors, Brett (1970) felt that no simple relationship between growth and temperature could be expected. However, the general relationship of an increase in growth rate with increasing temperature up

to an optimum and then a decrease with a further increase in temperature has been shown in sockeye salmon (Oncorhynchus nerka) (Brett et al. 1969), desert pupfish (Cyprinodon macularius) (Kinne 1960), a cichlid (Cichlasoma bimaculatum) (Warren and Davis 1967), and other fishes (Brett 1970). General optimum temperature ranges are 10-15° C for salmonids and similar cold-water species and 20-25° C for minnows, bluegills, catfish, goldfish, and related warm-water species (Snieszko 1974).

Fishes as a group tend to be tolerant of a wide range of salinity. The eggs and yolk-sac larvae of marine fishes are generally isosmotic with their medium but can tolerate a wide range of salinity (Holliday 1971). As the kidneys, integument, and other organs develop, the older stages are better able to osmoregulate, but the response to salinity also depends on tissue tolerance and physiological state (Holliday 1971). In the few fishes studied, no pattern of the metabolic costs of continued osmoregulation has emerged, but several species have been shown to grow larger in nature and faster in the laboratory in more saline water (Holliday 1971). This may be due to an effect salinity has on appetite. Kinne (1960), studying the effects of salinity on the growth of the euryhaline fish C. macularius, found that food consumption was highest in 35‰, less in 15‰ and 55‰, and least in fresh water.

Fishes require sufficient oxygen for metabolic processes. The oxygen available to a fish depends on the amount dissolved in the water and the species-specific critical level below which the fish cannot extract the oxygen. Fishes may tolerate oxygen concentrations down to

2 ppm or below for indefinite periods of time, but growth may slow at oxygen levels less than air-saturation. Warren et al. (1973) reported that young coho salmon (O. nerka) consumed a smaller unrestricted ration and grew slower as dissolved oxygen decreased from air-saturation (near 9 ppm) at all temperatures tested (8.6 to 21.8° C). However, growth rate of fish fed a restricted ration, which equaled the maximum amount consumed at 4 ppm, was not dependent on oxygen concentration over the range of 4 to 9 ppm. In one instance, coho salmon grew at an oxygen concentration of 2.4 ppm, and it was thought that growth would have occurred at even lower oxygen levels. For largemouth bass (Micropterus salmoides) fed an unrestricted ration, Warren et al. (1973) found no change in food consumption rate and growth rate caused by dissolved oxygen over the range of about 4.2 ppm to air-saturation at temperatures below 15° C but did find that growth rate decreased with decreasing oxygen concentrations above 15° C. Therefore, the minimum tolerable dissolved oxygen levels for fish culture depend in part on the species, the ration, the temperature, and the reduction in growth rate that is acceptable. Burrows (1972) felt that dissolved oxygen levels above 6 ppm are desirable for salmonid species in hatcheries.

Many toxicants may be present in the incoming water or leached out of pipes and fittings. Burrows (1972), Klontz (1973), and Spotte 1970) have listed those commonly encountered in fish culture and have discussed their sources, acceptable levels, and effects on fishes.

Light controls the behavior and physiology of fishes in many ways, but of special interest to the fish culturist is that day-length has

been shown to be a timing device for maturation in fishes (Pickford and Atz 1957). Haydock (1971) demonstrated that long photoperiods, plus warm water and adequate feeding, stimulated gonad maturation in the gulf croaker (Bairdiella icistia) so spawning could occur at any time of the year in captivity. Light can also be a harmful factor. Exposures to ultra-violet radiation from the sun or daylight fluorescent lamps have caused mortality in developing pelagic fish eggs and larvae (Snieszko 1974). Finally, light may affect growth of fishes. Brown (1946b) found that brown trout (Salmo trutta) grew faster at short day-lengths (six hours) than at long day-lengths (12 or 18 hours).

The metabolic products causing greatest harm to fishes are carbon dioxide and ammonia. Carbon dioxide is known to impede the oxygenation of hemoglobin, but critical levels have not been established for fishes because of the lack of information on the response of most species and the interaction with other factors, such as temperature and low dissolved oxygen. Dahlberg et al. (1968) found that swimming performance of juvenile largemouth bass (M. salmoides) was not affected by free carbon dioxide concentrations near 48 ppm over the range of 1.2 to 24.0 ppm dissolved oxygen. For juvenile coho salmon (Oncorhynchus kisutch) free carbon dioxide concentrations of 18 and 61 ppm reduced swimming performance at air-saturation oxygen levels but not at dissolved oxygen levels below saturation (Dahlberg et al. 1968). The National Technical Advisory Committee (1968) based on little experimental data the recommendation that free carbon dioxide level in natural waters not exceed 25 ppm in order to avoid harming aquatic life. Klontz (1973) felt that concentrations above 12 ppm were undesirable in fish hatcheries. Car-

bon dioxide is eliminated at the water's surface, and removal can be accelerated by aerating (Snieszko 1974).

Ammonia, especially in the un-ionized form (NH_3), is highly toxic to fishes. Constant exposure to 0.7 ppm ammonia (NH_3) damages the gills, reducing stamina, growth, and disease resistance (Burrows 1972). Snieszko (1974) considered more than 0.2 ppm undesirable for salmonids. Ammonia is a greater problem in marine fish culture than in freshwater culture because the higher pH of sea water favors the formation of un-ionized ammonia, which exists in a dynamic equilibrium with the ammonium ion (Spotte 1970). Since ammonia is very soluble in water, it can be removed only by providing adequate water flows or by means of biological filtration (Spotte 1970).

Population density affects growth of fish in two ways. First, there is a maximum loading density (weight/volume) beyond which fish must be moved to a larger container or growth will be significantly reduced (Burrows 1972). Maximum loading density depends on the social interactions of the cultured species. Coho salmon (O. kisutch), a schooling species (except as fry), tolerate more crowding than rainbow trout (Salmo gairdneri), a territorial species (Klontz 1973). Older salmonids can be crowded more than younger salmonids (Burrows 1972). Second, below the maximum loading densities there may be an optimum density (number of fish/volume) at which growth is fastest. Brown (1946a) found that growth of brown trout (S. trutta) was fastest at three intermediate densities than at a higher or lower density.

The design of the container in which the fish are reared determines the health of the fish to a great extent (Klontz 1973). Contain-

ers can be designed to facilitate feeding and cleaning, to inhibit the spread of disease, to flush efficiently, and to maximize carrying capacity.

Stress in the physiological sense has been defined by Brett (1958) as "...a state produced by any environmental or other factor which extends the adaptive responses of an animal beyond the normal range or which disturbs the normal functioning to such an extent that, in either case, the chances of survival are significantly reduced." One of the first signs of stress is decreased growth rate (Klontz 1973). If one aspect of the environment is suboptimal, the fishes may be stressed and less tolerant to changes in other factors. Although fish held under intensive culture are undoubtedly stressed to some extent by being crowded together in an unnatural environment, the fish culturist may maximize production by monitoring the fish's environment to prevent undesirable changes.

Nutrition

Good nutrition is necessary to maximize growth, survival, and reproduction. This involves providing a palatable, economical, and nutritionally balanced ration for the cultured species. Malnutrition promotes disease (Ashley 1972, Snieszko 1972). Unfortunately, little is known about the nutrition of marine fishes, but based on recent work with freshwater salmonids and ictalurids, some tentative requirements can be inferred. The ten essential amino acids of terrestrial vertebrates have been found to be needed for normal growth in several salmonids, channel catfish, Japanese eels, and two flatfish (Cowey and

Sargent 1972, Halver 1973). Protein requirements are known to be high for carnivorous fish as compared to birds and mammals (Cowey and Sargent 1972), and have been shown to depend on water temperature, fish size, and fish species (Halver 1973). Cowey et al. (1970) found that plaice (Pleuronectes platessa) grew faster as the proportion of protein in the diet increased up to the level of 50% protein in the dry diet. In addition, plaice fed diets containing leaf-protein concentrate grew at rates inversely related to the amount of plant protein, indicating that quality of protein may affect growth (Cowey et al. 1971). Lipids are important energy sources for fishes, and body lipid composition depends on dietary lipids to a great extent. Fatty acids of different groups may not necessarily be substituted for one another (Cowey and Sargent 1972). Salmonids and catfish are inefficient users of carbohydrates, extracting only about 40% of the total caloric value compared to 96% for mammals, but carbohydrates can replace protein as an energy source if basic protein requirements for growth are met (Halver 1973). This is significant because carbohydrates are much less expensive than proteins. Halver (1973) listed recommended quantities of 14 vitamins needed by trout, salmon, and catfish.

Experimental and foreign commercial aquaculture operations generally feed whole organisms to carnivorous fishes (Bardach et al. 1972). For instance, yellowtail (Seriola quinqueradiata) in Japan are fed trash fish, since a large, guaranteed source is located near the production sites (Harada 1970), but feeding raw fish directly may result in the introduction of diseases (Leitritz 1959). The trend in salmonid hatchery programs and catfish farming in the United States has been

toward pelleted diets. Pellets are more adaptable to mechanization of feeding, and components may be added on the basis of cost as well as nutritional adequacy (Bardach et al. 1972).

Most attempts to rear marine fish larvae have involved feeding wild plankton or cultured organisms (May 1970). When Rollefson (1939, cited by Shelbourne 1964) first discovered that brine shrimp (Artemia salina) were easily cultured and acceptable as food to larval plaice (P. platessa), the outlook for the mass culture of marine larvae was improved substantially. However, brine shrimp are too large to be eaten by many first-feeding marine fish larvae, so it was not until the 1960's when smaller food organisms, such as the rotifer Brachionus plicatilus, were discovered that the rearing of many marine fish larvae was possible (Harada 1970). To rear larvae on a commercial scale economically, however, it may be necessary to develop a pelleted diet. Adron et al. (1974) have recently reared larval plaice (P. platessa) to the juvenile stage on a formulated, micro-encapsulated diet.

Communicable Diseases

Knowledge of the diseases of cultured marine fishes is limited. Most of the serious, known pathogens have been from the bacteria, protozoa, and monogenetic trematodes with viruses of suspected but unknown significance (Sindermann 1970). The most important marine bacterial pathogen is Vibrio, which has caused mortalities in many species, including flatfishes (Amlacher 1970). Cisar and Fryer (1969) described a Vibrio epizootic in chinook salmon (Oncorhynchus tshawytscha) held in Alsea Bay, Oregon. Vibrio has also caused exten-

sive mortalities in chum (Oncorhynchus keta) and pink (Oncorhynchus gorbuscha) salmon during experiments at the Marine Science Center on Yaquina Bay, Oregon (unpublished report, 1974, Use of heated sea water for farming oysters and salmon, Department of Fisheries and Wildlife, Oregon State University). Pathogens associated with mortalities during flatfish aquaculture experiments in Great Britain include the monogenetic trematode Gyrodactylus unicopula and the ciliates Helicostoma buddenbroki and Trichodina sp. (MacKenzie 1970, Pearse 1972, Purdom and Howard 1971).

Based on experience with freshwater species, general procedures have been developed for the prevention and treatment of many infectious diseases (Amlacher 1970, Herman 1972, Rucker 1972). Gyrodactylus, an external parasite, can be treated effectively with dilute formalin solutions (MacKenzie 1970). Systemic bacterial infections, such as vibriosis, can be cured with sulfonamides or antibiotics, which are mixed in the food, or with nitrofurans, which are absorbed directly through the skin (Rucker 1972). Antibiotics have also been used to increase the survival of developing eggs and larvae in the laboratory (Shelbourne 1964, Struksaker et al. 1973). However, no treatment is currently available for many diseases, such as those caused by viruses, so infected fish must be destroyed (Amlacher 1970, Klontz 1973). To avoid slowed growth, prevention of diseases is preferable to treatment. Fryer et al. (1972) have developed an oral vaccine that gives significant protection to chinook salmon (O. tshawytscha) from vibriosis. Environmental stresses, such as excessive handling, low oxygen, and high population densities, have been shown to lower disease resistance

in fishes (Klontz 1973, Wedemeyer 1970). If fish live in a healthy environment, they may harbor pathogens but not suffer from diseases (Wedemeyer 1970).

FLATFISH CULTURE IN GREAT BRITAIN

Flatfish hatcheries were built in the late 1800's in Europe and North America to augment natural recruitment through liberation of yolk-sac larvae, but it was not until 1951 that a systematic effort to rear flatfish from eggs to adults on a mass scale was begun at the Fisheries Laboratory in Lowestoft, England. Shelbourne (1964) has reviewed the early attempts at marine fish farming and has described the long-term research program on flatfish culture at Lowestoft. During the first ten years, the information needed to rear the larvae of the plaice (P. platessa) and the sole (Solea solea) to metamorphosis, such as feeding habits and optimum physical conditions, was slowly accumulated. By 1962 the rearing technique and the design of the culture system had been improved to the point where up to 66% of the fertilized eggs in some groups metamorphosed (Shelbourne 1964).

Since the early 1960's many of the details of culture have been investigated to improve further the culture of plaice and sole. Adron et al. (1974) demonstrated that plaice larvae can be reared successfully on an artificial diet. Cowey et al. (1970, 1971) have studied the protein requirements of juvenile plaice. Kirk and Howell (1972) compared growth of young plaice fed artificial versus natural diets. MacKenzie (1970), Pearse (1972), and Purdom and Howard (1971) have described diseases of cultured plaice and sole.

Recently, attention has turned to more commercially valuable species than plaice and sole, which were chosen initially for their ease of rearing (Jones 1972a). A technique to rear the larvae of turbot (Scophthalmus maximus), worth about 45 cents/pound in the period 1969-1970 (Jones 1972a), has been developed after six years of study (Jones 1972a, 1973), and the juveniles have been reared to a marketable size in cultivation trials (Purdom et al. 1972).

EXPERIMENTAL OBJECTIVES

The objectives of my thesis research were first to develop a basic culture technique to raise English sole through a complete life cycle, and secondly to improve upon the basic culture technique by determining the optima of several environmental parameters. Because of the paucity of information on culturing marine fishes in general and English sole in particular, and the difficulty involved in rearing fishes, the approach was to study in a preliminary way as many of the important factors affecting growth and survival in the laboratory as was possible. The basic culture technique involved (1) providing an environment suitable for growth and survival of the eggs, larvae, and juveniles, (2) providing a diet that promoted growth of the larvae and juveniles, and (3) identifying pathogenic organisms and developing methods of treatment and prevention. Specific experimental objectives were (1) to determine the optimum temperatures for the growth and survival of the larvae, (2) to determine the optimum temperature-ration combinations for fastest growth and best food conversion of the juveniles, (3) to determine if growth slows with age, (4) to evaluate

the effects of crowding on the juveniles in terms of growth, (5) to evaluate behavioral interactions between juveniles, and (6) to determine the tolerance to low salinity of the juveniles.

METHODS AND MATERIALS

SOURCE AND ACCLIMATION OF FISH

Sexually mature (ripe) English sole were collected by otter trawl in 28 to 40 fathoms of water about five to ten miles off Yaquina Bay, Oregon. The fish were transported to Oregon State University's Marine Science Center the same day and usually spawned within 48 hours.

Juveniles were obtained by otter trawl from Yaquina Bay, Oregon, between buoys 20 and 26 in 1972 and between buoys 12 and 15 in 1973 (Fig. 1). The collection site was changed when it was learned that English sole juveniles pick up undesirable parasites in the upper estuary (Olson and Pratt 1973). Juveniles were transported live to the Marine Science Center, selected for size and lack of injury, and randomly distributed about 50 to an aquarium. In the laboratory, the juveniles were held in flowing sea water for one week to allow for adjustment to laboratory conditions and the establishment of feeding. An additional two to three weeks was then allowed for acclimation to experimental conditions. Temperature was changed one degree Celsius per day when required. At the start of each growth experiment the number of fish per tank was reduced to 25, selecting so that the initial biomass and variation in size was approximately equal in all tanks. In later studies juveniles from earlier growth experiments were used.

CULTURE CONDITIONS FOR LARVAE

A dry fertilization technique (Shelbourne 1964) was used, in which one or more females were stripped by hand into a clean beaker which

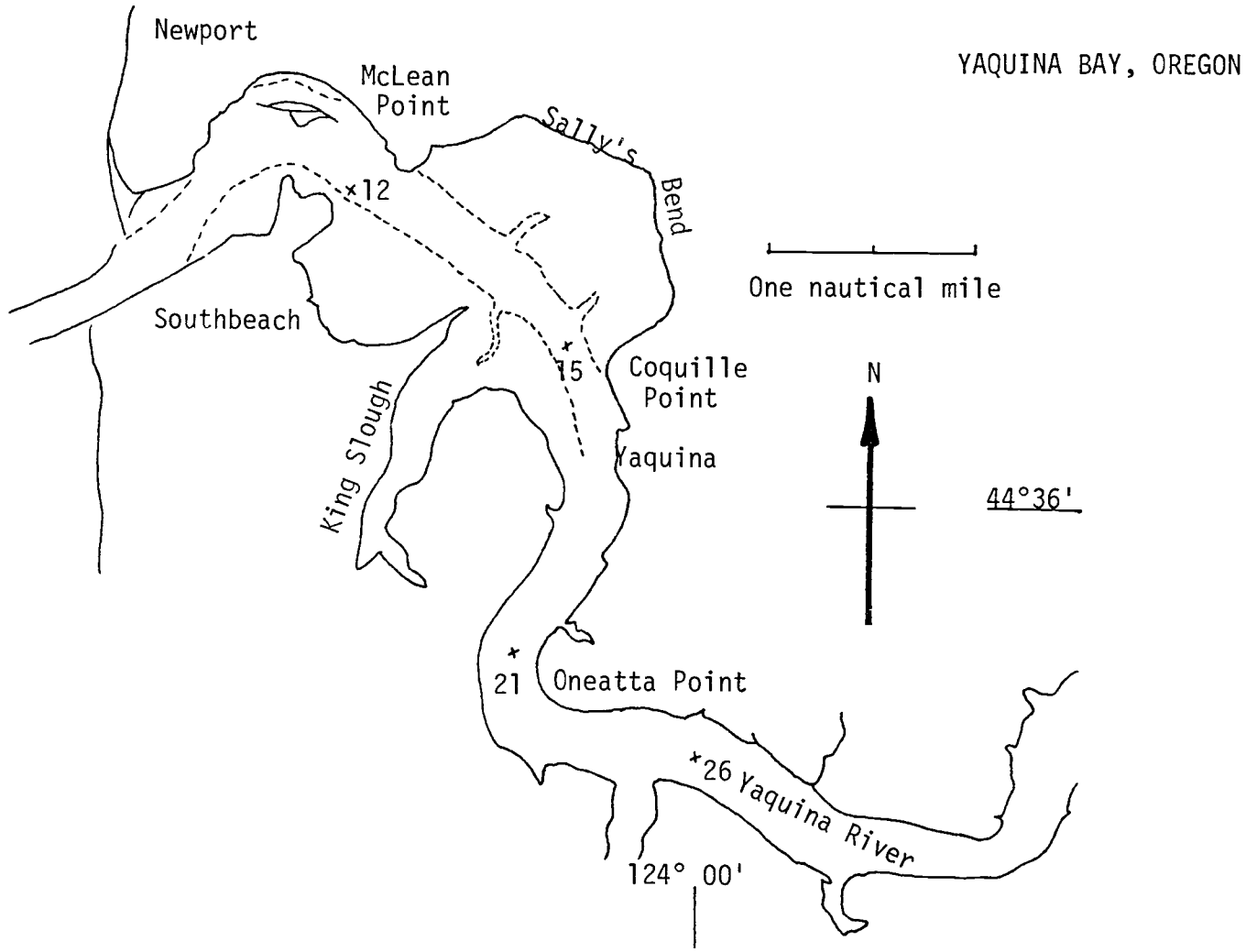

Figure 1. Sites for the collection of 0-group English sole in Yaquina Bay were in the channel between buoys 12 and 15 and between buoys 21 and 26.

contained no sea water, and sperm from several males was added. After a ten minute fertilization period, fresh sea water (8 to 9° C, 30 to 32‰) was added and the fertilized eggs floated to the water's surface, where they were collected by pipette or in a 44μ sieve for transfer to the rearing containers.

A closed system was used to rear the eggs and larvae. The eggs were incubated in one-gallon glass jars. Newly hatched larvae were transferred by pipette to cylindrical, polyethylene rearing containers (19.5 cm X 20.2 cm dia., and six liters capacity) resting in shallow water baths (129.5 cm X 68.4 cm X 15.3 cm, holding 135 liters). The bottoms of the rearing containers were replaced with 180μ "Nitex" screen to allow an exchange of sea water between the rearing containers and baths, which served as reservoirs. The insides of the rearing containers were painted black with epoxy marine finish (Valspar Corp.) to provide a contrasting background for feeding larvae. All plastic utensils and painted containers were soaked for two weeks in flowing sea water to leach out possible toxicants.

Surface sea water was collected as needed approximately ten miles off the Oregon coast. This water contained little suspended material and was generally of 30 to 32‰ salinity. Passing the water through a 5μ polypropylene filter (AFCO Filter Products) removed most of the larger organisms. To inhibit bacterial growth, the sea water was treated with 50 mg/liter of a 1:1 penicillin-streptomycin mixture before adding the eggs, as was recommended by Oppenheimer (1955). Larvae were not treated with antibiotics. During the experiments, the sea water in the baths was aerated using an oil-free diaphragm pump,

and was filtered through two layers of dacron fiber to remove suspended particles from the water, crushed oyster shell to help buffer the water, and granular activated carbon to remove dissolved organics. The water in the rearing containers was not filtered or aerated directly. To help maintain a healthy chemical balance of the sea water, debris was siphoned daily from the bottom of the rearing containers and fresh sea water added to replace that lost by siphoning. The living larvae floated in the water column and did not often pass through the siphon. In addition, sea water was replaced in the water baths at the rate of about eight liters per day.

Most experiments were performed at a temperature of 10 ± 0.5° C, and all were conducted at a salinity of 30 to 32 ± 1‰. Cool white fluorescent bulbs (40 W) provided 46 to 66 foot-candles of illumination at the surface of the rearing containers (Weston Illumination Meter, Model 756, Quartz Filter). The photoperiod approximated natural day-length and was adjusted every two weeks.

The larvae were routinely fed both the dinoflagellate Gymnodinium splendens and the rotifer Branchionus plicatilis within one day after hatching. These food organisms were cultured using the methods of Lasker et al. (1970) and Theilacker and McMaster (1971). When the larvae reached about 4.5 mm in length, newly-hatched brine shrimp nauplii, Artemia salina, were added. Larvae were fed every other day.

CULTURE CONDITIONS FOR JUVENILES

Cream-colored fiberglass tanks (84 cm X 61 cm X 30 cm) with a 10 cm standpipe and containing 52 liters of water served as

environmental containers for juveniles during all but the density and salinity tolerance experiments. The sea water flowed first through head tanks, where some of the particulate matter settled out and the water could be heated or cooled when required and then into the experimental tanks. Although English sole normally are associated with a sandy substrate, sand was not put in the tanks because: hydrogen sulfide levels built up rapidly in the sand due to decomposition of uneaten food and fecal wastes; juveniles brought in from the field and placed in tanks containing sand buried themselves and did not feed; and all young sole had more difficulty seeing the food against the sand background than against the light colored bottom.

The oxygen levels were maintained above 90% saturation in all experiments by the use of aerators, which also served to drive off excess carbon dioxide. However, occasional interruptions of the water and air supplies occured, which caused some fish to be exposed to low dissolved oxygen. This resulted in deaths in four instances.

Cool white fluorescent lighting (40 W) illuminated the tanks with 10 to 36 foot-candles at the water surface (Weston Illumination Meter, Model 756, Quartz Filter). Photoperiod was approximately equal to natural day-length and was adjusted once every two weeks.

Oregon Moist Pellet (OPR-2 formulation; Bioproducts; Warrenton, OR) served as food in most experiments and was always fed half in the morning and the balance in the evening. Formulations of this diet are discussed by Hublou (1963). The daily rations were calculated as percent of body weight (dry weight food/dry weight fish), and were adjusted after each weighing and after fish died. Except at 21° C

the fish almost always consumed the entire ration. The amount of food eaten was recorded daily and the moisture content of the food determined at monthly intervals.

SAMPLING PROCEDURE AND ANALYSIS OF DATA

Growth of juveniles was estimated in terms of wet weight. Fish were weighed after being placed in a tared container of water on a Mettler top-loading balance. The young were anesthetized with MS-222 (tricaine methanesulfonate, Sigma Chemical Co.), allowed to drip dry in a net for ten seconds, weighed individually to the nearest 0.01 g, and immediately returned to their respective tanks. A sample of fish from every experimental group was dried at 70° F for 24 hours at the beginning and end of each experiment to determine moisture content for use in adjusting rations.

Average relative growth was calculated for each growth interval using the formula given in Warren (1971):

$$\text{growth rate} = \frac{W_2 - W_1}{0.5(W_1 + W_2)(t_2 - t_1)}$$

where W_1 is the average weight of fish at the beginning of the growth interval, W_2 is the average weight of fish at the end of the growth interval, and $(t_2 - t_1)$ is the number of days in the growth interval. Growth was expressed as percent increase in wet weight per day. Dead fish were included in the estimate of W_2 in order to offset the effect of the higher mortality rate of smaller fish and were assumed to have died on the last day of the interval for convenience of calculation.

With the exception of one experiment fish were not marked, so individual variations in growth could not be determined.

During most growth experiments, there were between 5 and 11 estimates of growth rate depending on the number of weighings, so the treatments could be analyzed statistically using Analysis of Variance and Least Significant Difference tests for factorial experiments and Student's t-test for paired comparisons (Snedecor and Cochran 1967).

Gross food conversion efficiency was calculated as the increase in dry weight of the fish divided by the dry weight of the food fed over the whole experimental period. In the calculation of food conversion, food fed was considered to be eaten, whether or not it was actually consumed. The quantity of uneaten food rarely amounted to more than 1% of that fed, except at 21° C.

The efficiency of assimilating the Oregon Moist Pellet diet was determined by the method of Conover (1966). If the inorganic component of the food is not significantly altered by the digestive process, assimilation efficiency can be determined as

$$\text{assimilation efficiency} = \frac{F - E}{(1 - E)F} \times 100$$

where F equals the fraction of organic matter in the food and E equals the fraction of organic matter in the feces. Fecal samples were collected about 30 minutes after feeding in the morning and represented the products of two or more fish. Each sample was washed with 0.9% (w:v) ammonium formate (Conover 1966), centrifuged, dried at 70° C for 24 hours, weighed to the nearest 0.01 mg, ashed at 450° C for 24 hours,

and weighed again to the nearest 0.01 mg. Samples of food were treated in the same manner.

EXPERIMENTAL

REARING THE LARVAE

Rearing eggs and larvae in a hatchery is the only practical method of regularly obtaining large numbers of most species of juveniles to fatten to a marketable size. This involves artificially spawning wild adults or maintaining a brood stock, incubating the eggs, and feeding the larvae. Since Alderdice and Forrester (1968) have determined the optimum temperatures and salinities for egg development, the primary objective of my studies was to develop a successful technique to rear the larvae to the juvenile stage. Although I made six attempts, I was unable to meet this goal. The following experiment, which was designed to determine the effects of temperature on growth and survival, is typical of these studies.

Four male (28.2 to 30.1 cm total length) and two female (38.9 and 39.6 cm) English sole were caught off Newport in February and all spawned two days later by stripping into a single container. Rearing temperatures were 5, 7.5, 10, or 12.5° C and were maintained within 0.5° C of the desired levels. There were 200 eggs (0.944 mm diameter) per rearing container and three containers per temperature. At each temperature two groups of larvae were fed; in one group growth was followed and in the other survival was determined. The third group was starved in order to learn what percent of the mortality in the fed groups could have been due to starvation alone. Dead eggs and larvae were removed and counted each morning.

The rate of development increased with increasing temperature.

The hatching of the eggs was complete in 12 days at 5° C as compared to four days at 12.5° C (Fig. 2). In most groups 75% or more of the original eggs hatched into yolk-sac larvae (2.71 mm total length) but even in the most successful trials less than 20% began feeding. The larvae began searching for food within one to four days after hatching, depending on temperature. The stomachs of some fish contained food, but 60 to 75% of the larvae failed to eat enough and soon starved. Unfed larvae starved by the 27th day at 5° C compared to 11 days at 12.5° C. Since fed larvae lived no longer than starved controls at 5° C, it is probable that these larvae did not feed (Fig. 2). The feeding larvae reached a size of 5.0 to 5.5 mm total length before dying compared to 4.3 to 4.6 mm total length for the starved controls.

Toward the end of all rearing trials, a bacterial slime formed on the walls of the rearing containers and sometimes on the water surface. No attempt was made to control this bacterial growth. At the same time, the pH of the water dropped from 8.2 to 7.0 - 7.5. Occasionally, large populations of ciliates, which were introduced with the food organisms and replacement water, appeared near the termination of an experiment. Sick and dead larvae were observed to be covered with mucous and sometimes ciliates.

Casual observation of a number of spawnings revealed a trend toward lower percent fertilization when adults were held for longer periods in the laboratory before spawning. For instance, in one case spawning within 48 hours of capture resulted in about 90% successful fertilization. In another instance, no viable eggs resulted when females were held in the laboratory for seven days. This may be

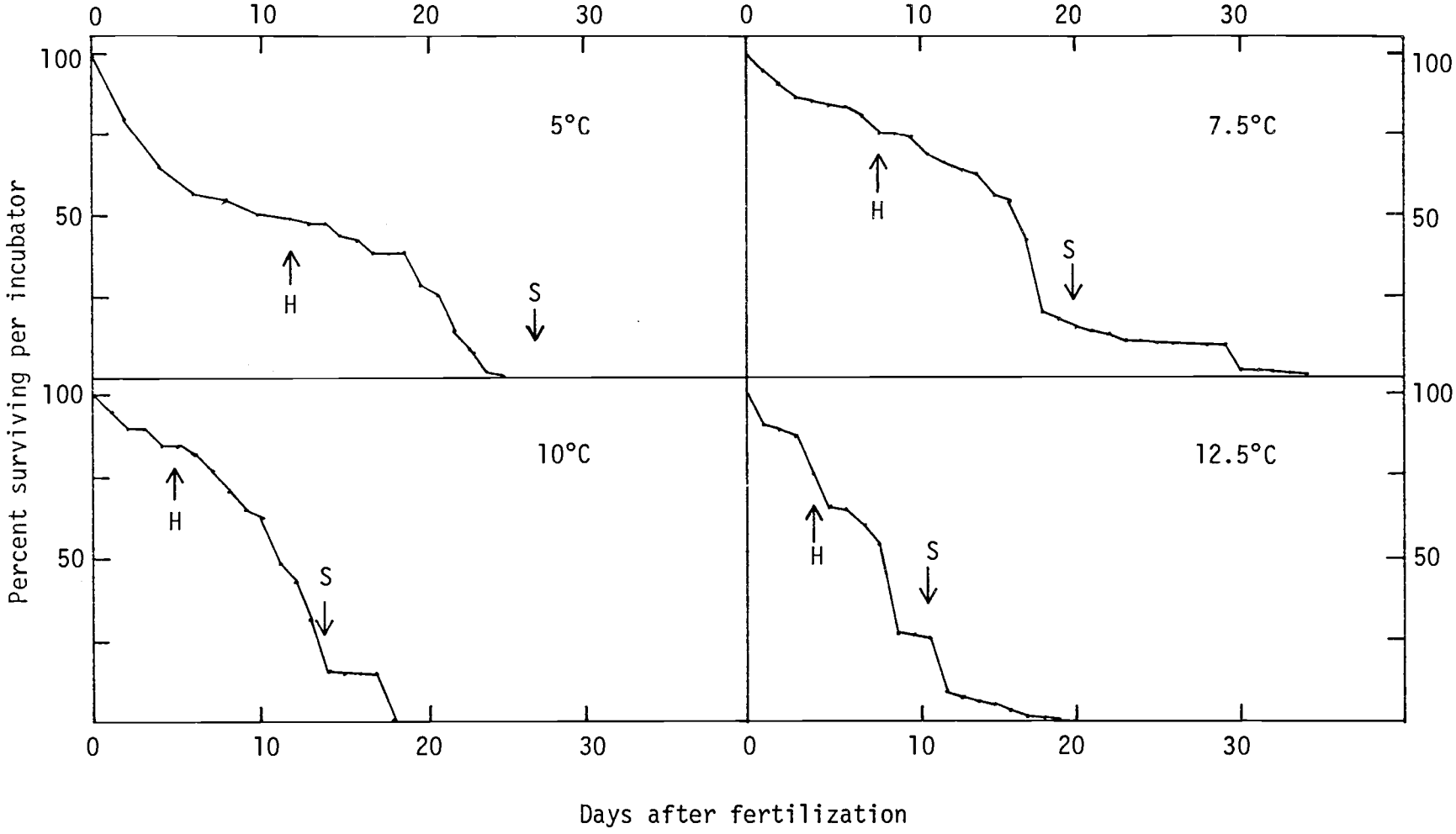

Figure 2. Survival curves of English sole eggs and larvae reared at four temperatures. Experiments began with just-fertilized eggs. H = hatching complete; S = last day starved controls lived.

related to the fact that adult English sole did not feed in the laboratory, and gonad reabsorption may have been initiated.

GROWTH STUDIES WITH THE JUVENILES

Maximizing growth and survival of young fish in captivity requires much information about the physiological, nutritional, and behavioral characteristics of the cultured species. The goal of my studies with the juveniles was to begin studying some of the more important factors influencing growth. Experiments on the effects of temperature, ration, age, diet, population density, and competition are described.

Effects of Temperature and Ration

Food consumption rate and temperature are major determinants of growth in fishes. One reason for studying the effects of temperature and ration on growth lies in the predictive value these factors have for estimating when the fish will reach a marketable size, something one must frequently know months ahead of time (Klontz 1973). Given this information, the fish farmer knows the amount of food to feed to produce a desired growth rate at a given water temperature. In addition, knowing the optimum temperature-ration combinations allows one to determine optimum levels of other factors, resulting in still faster growth and higher food conversion.

Juvenile English sole were caught in Yaquina Bay and allowed to acclimate three to four weeks. Three separate growth experiments were conducted. Methods were similar except that in experiment A the fish were weighed once a week, except for week 11, and were collected

between buoys 21 and 26, while in experiments B and C the fish were weighed once every two weeks and were collected between buoys 12 and 15 (Fig. 1). Experiment A was run at all combinations of the temperatures 12, 15, 18, and 21° C and the daily rations 4, 8, 12, and 16% (dry weight), except for the combination of 21° C and 4% ration. Experiment B was run at all combinations of the temperatures 9.5, 12, and 15° C and the rations 4, 8, 12, and 16%. Fish in experiment C were held at 9.5, 12, 15, and 18° C and were not fed. Temperatures were usually maintained within ± 0.5° C (range) except at 9.5° C where the temperature varied ± 1.0° C almost daily. The heating equipment malfunctioned a few times but in only one case, when fish at 18° C suffered an 8° C drop in mid-experiment, was the drop to ambient more than 3° C. In all cases the heaters were replaced within a few hours. The results within experiments were analyzed statistically by means of analysis of variance, but differences between experiments were not compared because of differences in the number of estimates of growth rate within a given experiment. The efficiency of assimilating the Oregon Moist Pellet diet was determined on a separate group of juveniles (average weight 30 grams) that was held at ambient temperatures (11 to 14° C) and fed once a day somewhat below the maximum ration.

The growth of 0-group (age group 0) English sole juveniles at various temperature and ration combinations is shown in Figure 3 and Table 1. In the range of temperatures tested, growth rate was inversely related to temperature. At 21° C English sole juveniles lost weight regardless of the ration. Over the range of 9.5 to 15° C, growth rate declined gradually with increasing temperature, but the

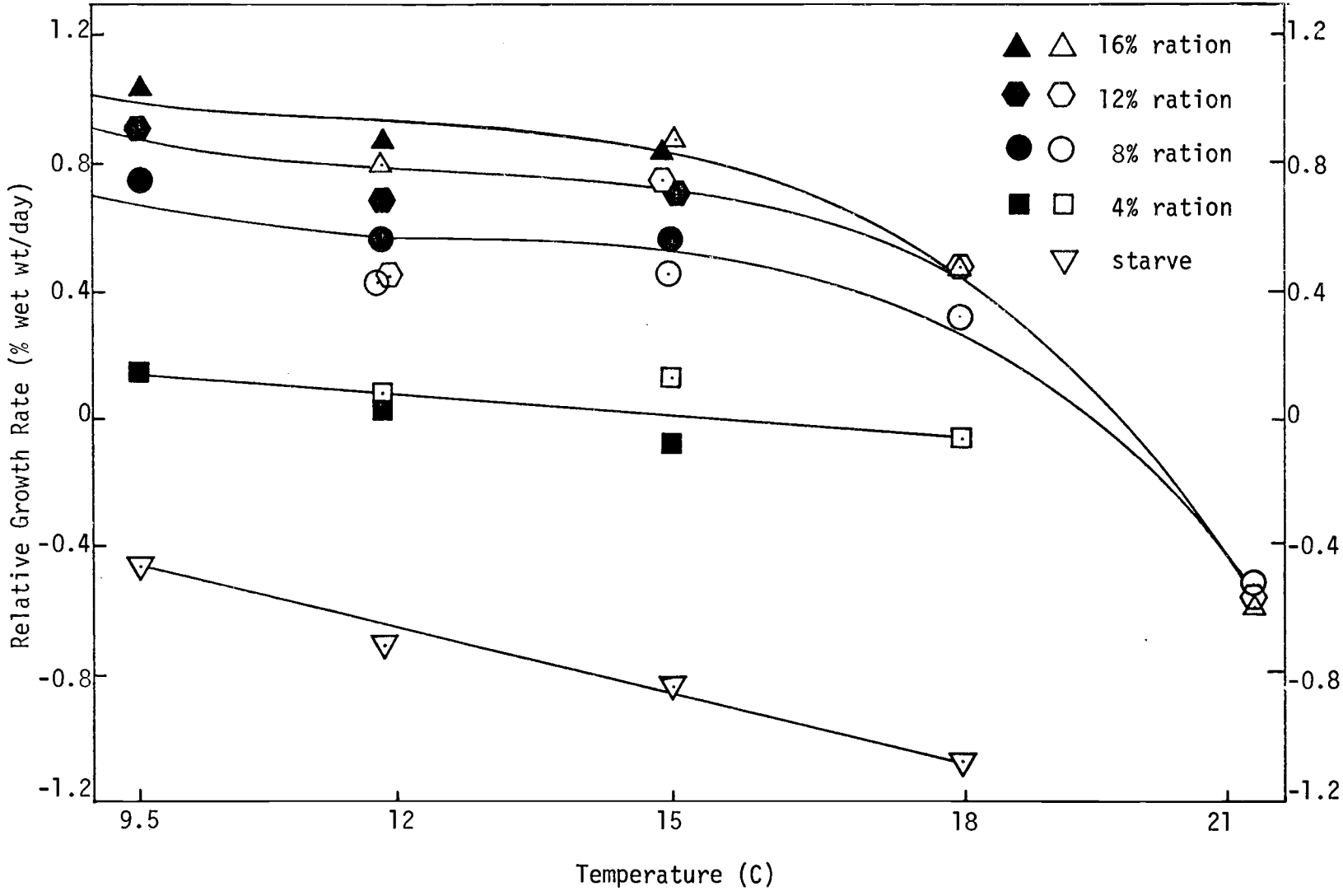

Figure 3. Relation of growth of 0-group English sole to temperature at five rations. Experiment A: open symbols; Experiment B: closed symbols; Experiment C: starved groups. Lines fitted by eye.

TABLE 1. The effect of temperature and ration on the weight, growth rate (± 2SE), food conversion, and survival of 0-group English sole juveniles. Initial numbers were 25 fish per tank.

Exp.	Temp. (C)	Ration (% dry wt)	No. fish surviving (n)	Initial wet wt (g)	Terminal wet wt (g)	Growth rate (%/day)	Gross food conversion (% dry wt)	Duration (days)
A	12	4	20	5.33	6.28	0.08 ± 0.27	2.04	84
		8	19	5.47	9.47	0.44 ± 0.17	6.39	84
		12	19	5.34	8.96	0.45 ± 0.24	4.35	84
		16	21	5.32	11.49	0.80 ± 0.29	5.71	84
A	15	4	19	5.42	6.80	0.13 ± 0.15	2.72	84
		8	17	5.39	10.15	0.46 ± 0.17	7.51	84
		12	18	5.44	12.48	0.75 ± 0.12	7.20	84
		16	22	5.38	12.23	0.88 ± 0.11	6.31	84
A	18	4	17	5.36	6.28	-0.07 ± 0.17	...	84
		8	18	5.39	8.33	0.33 ± 0.16	4.70	84
		12	13	5.35	10.54	0.47 ± 0.27	4.56	84
		16	16	5.29	9.56	0.47 ± 0.28	3.63	84
A	21	8	10	5.46	5.56	-0.51 ± 0.24	...	21
		12	5	5.40	5.32	-0.56 ± 0.55	...	21
		16	12	5.37	5.19	-0.54 ± 0.27	...	21
B	9.5	4	18	3.99	5.11	0.14 ± 0.29	3.97	84
		8	21	4.21	8.07	0.67 ± 0.21	10.47	84
		12	18	3.72	8.71	0.82 ± 0.32	9.02	84
		16	20	3.79	9.56	0.95 ± 0.22	7.66	84
B	12	4	15	4.55	5.54	0.03 ± 0.25	0.02	84
		8	13	4.15	8.93	0.56 ± 0.31	8.99	84
		12	21	3.96	7.79	0.68 ± 0.33	7.56	84
		16	20	3.75	8.76	0.87 ± 0.23	7.07	84
B	15	4	10	4.07	5.30	-0.08 ± 0.28	...	84
		8	19	4.04	7.31	0.57 ± 0.36	9.59	84
		12	18	4.26	7.95	0.71 ± 0.26	7.63	84
		16	17	4.05	10.00	0.85 ± 0.34	7.15	84
C	9.5	0	15	6.18	4.39	-0.45 ± 0.04	...	84
	12	0	16	5.41	3.50	-0.70 ± 0.20	...	70
	15	0	9	5.52	3.57	-0.83 ± 0.22	...	56
	18	0	7	5.67	3.71	-1.07 ± 0.15	...	42

decline was not statistically significant (Tables 2 and 3) except between 15 and 18° C (Table 2) due to a large drop in growth rate at the highest ration (Fig. 3). It was observed in preliminary experiments that English sole held in the laboratory consumed less food when water temperatures dropped below about 7° C and feeding ceased in the range of 2 to 3° C.

Growth rate was found to increase with increasing ration at all temperatures except 21° C (Fig. 4): Juveniles fed the highest three rations (8, 12, and 16%) grew significantly faster than those fed the 4% ration (Tables 2 and 3). Feeding the highest ration (16%) also resulted in significantly faster growth than the 8% ration (Tables 2 and 3). Unfed fish lost weight at a rate directly proportional to the environmental temperature.

Gross food conversion (dry weight basis) decreased with increasing temperature in the same pattern as growth rate decreased with increasing temperature (Table 1). Food conversion was highest at an intermediate ration (8%) at all temperatures and decreased with a further increase in ration (Fig. 5). The highest food conversion, 10.5%, was obtained at the combination of 9.5° C and 8% ration (Fig. 5). Food conversion on a wet weight basis (wet weight growth/wet weight food) for the Oregon Moist Pellet diet was about 3.5 times the food conversion on a dry weight basis.

The mean assimilation efficiency plus or minus two standard errors for sole fed Oregon Moist Pellet was determined to be 65.9 ± 5.3% ($n = 47$). Based on this estimate and the maximum food conversion obtained, I calculated that a maximum of about 15% of the food absorbed

TABLE 2a. Analysis of variance of the effects of temperature over the range of 12 to 18°C and ration on growth of 0-group English sole.

	df	Mean square	F-ratio	significant
Ration means	3	2.476	22.60	at 99%
Temperature means	2	0.537	4.91	at 95%
Interaction	6	0.086	0.78	no
Subtotal	11			
Within groups (error)	108	0.110		
Total	119			

TABLE 2b. A least significant difference test comparing the growth of English sole at different rations.

Ration (% dry wt)	Relative Growth Rate (% wet wt/day)	Significant Ration Comparisons	Difference	LSD Value
4%	0.0630	8% to 4%	0.3531	0.1692
8%	0.4161	12% to 4%	0.5208	"
12%	0.5838	16% to 4%	0.6677	"
16%	0.7307	16% to 8%	0.3146	"

TABLE 2c. A least significant difference test comparing the growth of English sole at different temperatures.

Temperature (C)	Relative Growth Rate (% wet wt/day)	Significant Temperature Comparisons	Difference	LSD Value
12	0.4429	15-18°C	0.2316	0.1465
15	0.5669			
18	0.3353			

TABLE 3a. Analysis of variance of the effects of temperature over the range of 9.5 to 15°C and ration on growth of 0-group English sole.

	df	Mean square	F-ratio	significant
Ration Means	3	2.520376	20.33	at 99%
Temperature Means	2	0.119145	0.96	no
Interaction	6	0.008921	0.07	no
Subtotal	11	0.713904		
Within Groups (error)	60	0.123970		
Total	71			

TABLE 3b. A least significant difference test comparing the growth of English sole at different rations.

Ration (% dry wt)	Relative Growth Rate (% wet wt/day)	Significant Ration Comparisons	Difference	LSD Value
4%	0.0307	8% to 4%	0.5717	0.2341
8%	0.6024	12% to 4%	0.7035	"
12%	0.7342	16% to 4%	0.8577	"
16%	0.8884	16% to 8%	0.2860	"

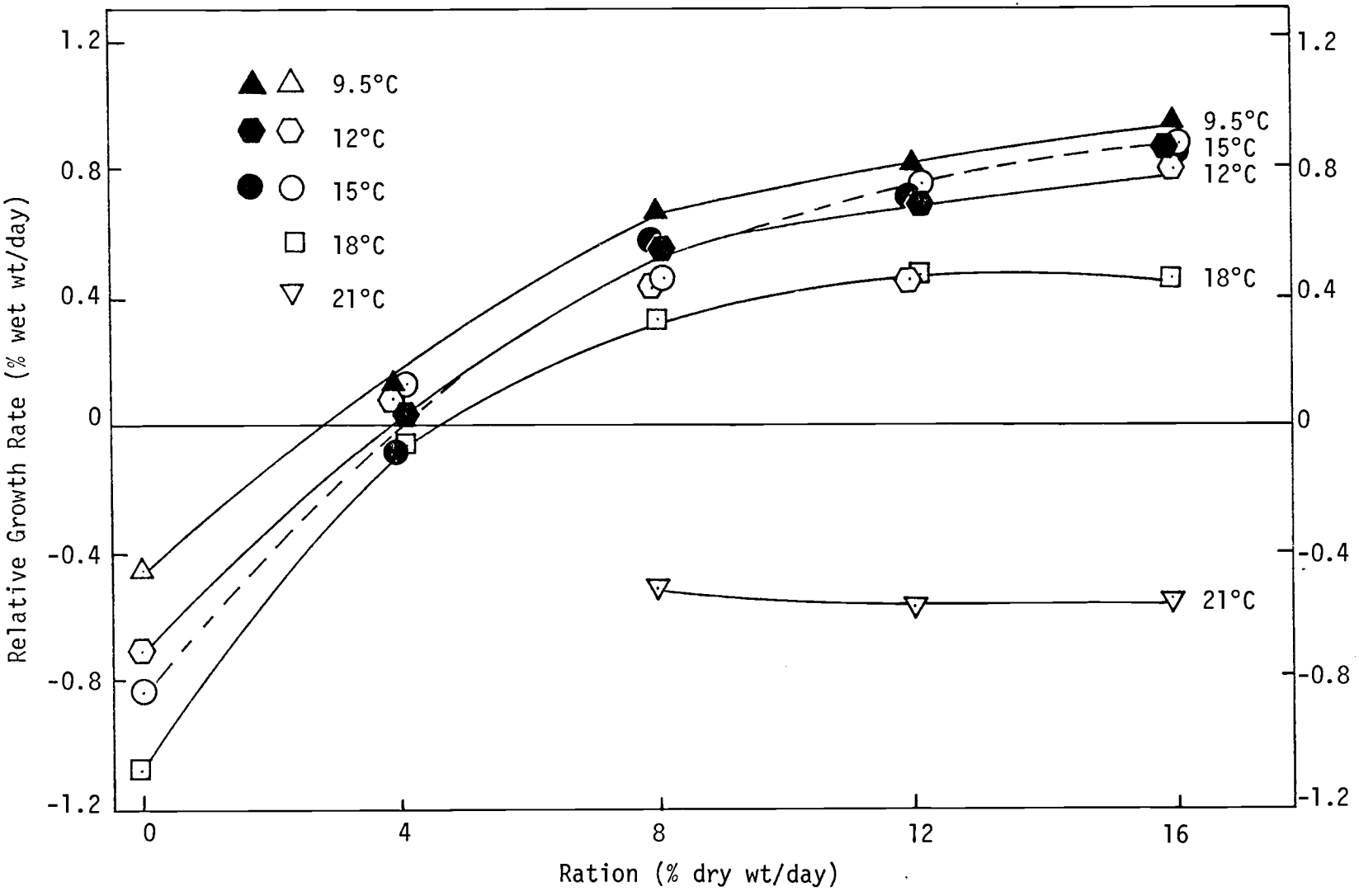

Figure 4. Relation between growth of 0-group English sole and ration at five temperatures. Experiment A: open symbols; Experiment B: closed symbols; Experiment C: starved groups. Lines fitted by eye.

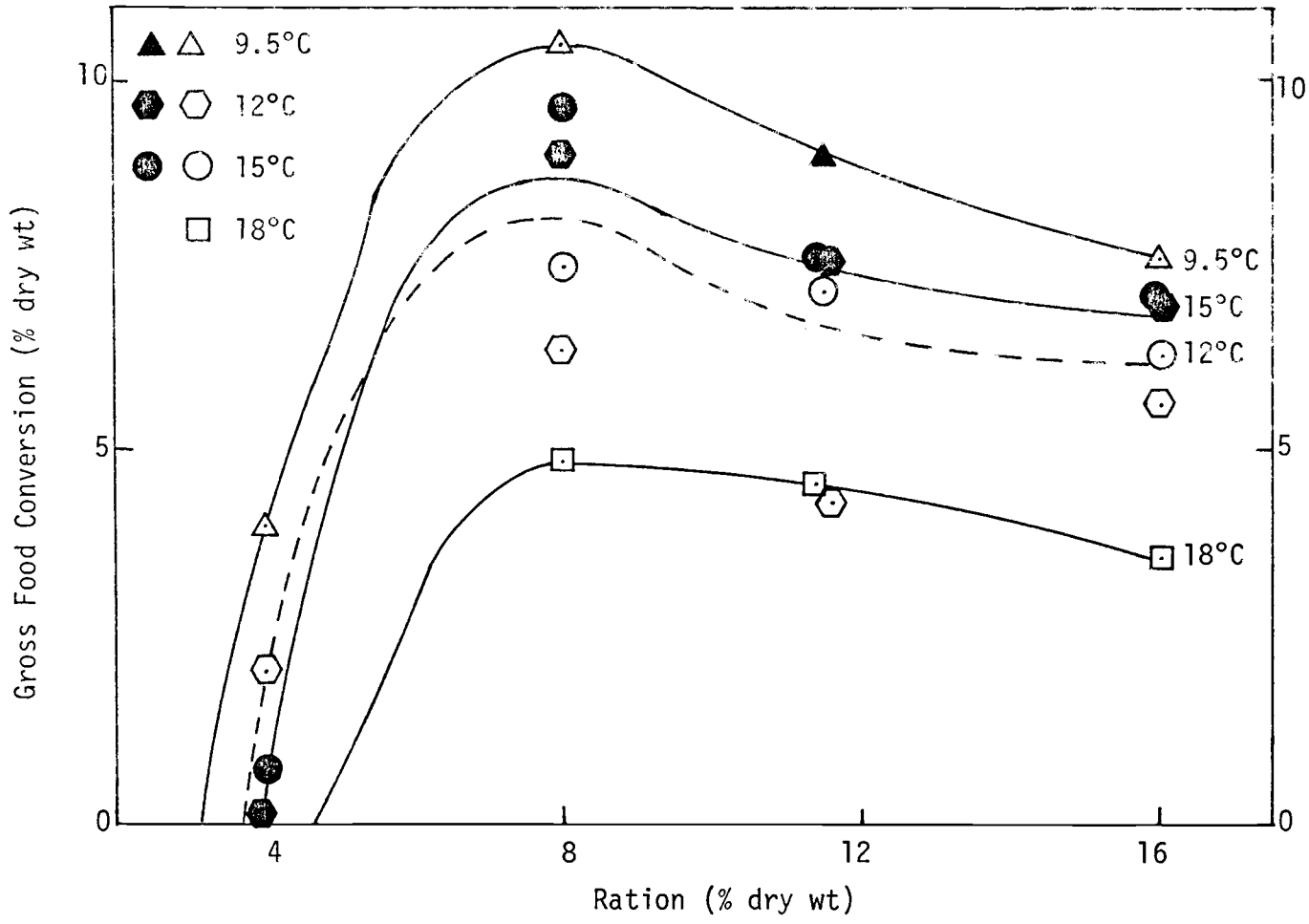

Figure 5. Relation of gross food conversion of 0-group English sole to temperature and ration. Maintenance rations (i.e., zero food conversion) were determined by interpolation in Figure 4. Experiment A: open symbols; Experiment B: closed symbols. Lines fitted by eye.

through the gut was incorporated into new tissue while the remaining 85% was used for metabolic processes.

Parameters of interest were (1) maintenance ration, i.e., the ration at which the fish sustains his weight; (2) optimum ration, i.e., the ration resulting in most efficient growth; (3) maximum ration, i.e., the smallest ration that results in the fastest, or asymptotic, growth; and (4) maximum food consumption rate, i.e., the largest amount of food consumed when feeding an excess ration. The maintenance ration was determined by interpolation as 3.1 to 4.7%, depending on temperature (Figs. 4 and 5). The optimum ration was 8%, regardless of temperature (Fig. 5). The maximum ration appeared to be somewhat above 16% of the body weight (dry weight basis) for the 0-group juveniles between 9.5 and 15° C and between 12 and 16% at 18° C (Fig. 4). It was determined in preliminary experiments that the maximum food consumption rate (% body weight) was about 20 ± 5% (range) for 5-gram fish fed in excess twice a day and kept at temperatures ranging from 11 to 15° C. The total amount consumed varied greatly from day to day within the same group of fish.

Mortality during the temperature-ration experiments was high in all groups, but the proportion of fish dying in experimental groups was higher at higher environmental temperatures and smaller rations (Table 4). Seventy-seven percent of the juveniles kept at 9.5° C survived to the end of the experiment compared to only 64% at 18° C. Survival was 76% at 16% ration over all temperatures compared to 67% at the 4% ration.

TABLE 4. Percent survival of 0-group English sole after 84 days as a function of experimental temperature and ration.

Ration (%/day)	Temperature (C)			
	9.5	12	15	18
4	72	70	58	68
8	84	64	72	72
12	72	80	72	52
16	80	82	78	64

Effect of Age

Growth rate slows as fish age (Warren 1971), so the fish culturist must determine if growth rate decreases significantly before a marketable size is reached. A single experiment was conducted with 1-group English sole for comparison with data obtained with 0-group fish in the earlier temperature-ration experiments.

Thirty-nine juveniles (average weight 33 grams), which had been used in previous growth experiments and had been kept in the laboratory for one year on an Oregon Moist Pellet diet, were evenly and randomly dispensed to three tanks, each with a different experimental temperature (viz. 12, 15, and 18° C). The fish were fed a daily 8% ration. Duration of the experiment varied between groups--12 weeks at 12° C, 6 weeks at 15° C, and 8 weeks at 18° C--because fish died from low dissolved oxygen when water lines plugged and the air supply was interrupted.

Growth did not slow by the second year of life on an 8% ration (Table 5). The differences in growth rate between 0-group and I-group English sole were not tested statistically because sample sizes differed between groups, but an examination of Table 5 shows that the two standard error ranges overlapped between treatments. These results are misleading because the maximum daily food consumption rate for I-group juveniles (33 grams) fed in excess was determined in a preliminary experiment to be about 10 ± 2% compared to 20 ± 5% for 5-gram fish and 16 ± 4% for 9-gram fish, both 0-group. Therefore, because small fish could eat more than large fish when both are fed ad libitum, the maximum growth rate of smaller fish could be expected to be higher than that of larger fish.

TABLE 5. Relative growth rate (± 2SE) of 0- and I-group juveniles fed an 8% ration at three temperatures.

age group	Temperature (C)		
	12	15	18
I-group	0.45 ± 0.26	0.45 ± 0.26	0.39 ± 0.11
0-group (exp. A)[a]	0.44 ± 0.17	0.46 ± 0.17	0.33 ± 0.16
0-group (exp. B)[a]	0.56 ± 0.31	0.57 ± 0.36	. . .

a. Data from 0-group sole are from Table 1.

Effect of Diet

Meeting basic nutritional requirements is necessary to obtain fastest growth. A suboptimal diet may allow a fish to grow, but at a reduced rate. For convenience, the Oregon Moist Pellet diet was fed exclusively in early growth experiments. This diet is a widely used hatchery diet but was developed for freshwater salmonids (Hublou 1963). Since Oregon Moist Pellet consists mainly of ground fish and plant meals (Hublou 1963), and English sole eat mainly crustaceans, polychaetes, and bivalves (Forrester 1969a), an experiment comparing growth of juveniles fed Oregon Moist Pellet and a more "natural" clam-shrimp diet was conducted at ambient temperatures, which fluctuated between 9 and 14° C. The proportion of moisture, ash, and organic materials for both diets is given in Table 6.

TABLE 6. Composition (mean ± 2SE) of Oregon Moist Pellet and clam-shrimp diets fed English sole juveniles.

Diet	Moisture (%)	Organic (%)	Ash (%)	Number of Samples
OMP	24.8 ± 1.0	65.0 ± 0.3	11.0 ± 0.05	52
Shrimp	75.2 ± 0.4	23.0 ± 0.2	2.6 ± 0.08	30
Clam	76.3 ± 0.6	22.3 ± 0.6	1.4 ± 0.03	20
OMP[a]	29.1	62.9	8.0	
OMP[b]	34.4	58.8	6.8	

a. Composition of OPR-2 formulation reported by Crawford and Law (1972).

b. Composition of Oregon Moist Pellet reported by Hublou (1963).

The juveniles had been used in previous growth experiments and had been held in the laboratory about six months prior to the start of this experiment. The fish were weighed once every two weeks for ten weeks. The clam-shrimp diet consisted of minced necks of the soft shelled clam Mya arenaria (dug in Yaquina Bay) and minced tails of the shrimp Pandalus jordani (bought in a fish market), which were fed in a 1:1 proportion on a dry weight basis. The two diets were fed at an 8% ration (dry weight/dry weight) six days a week.

English sole grew much faster on the clam-shrimp diet than on the Oregon Moist Pellet diet (Table 7). The difference between the two diets was even more striking in terms of food conversion (dry weight basis). However, in a preliminary experiment an 8% daily ration was determined to be near the maximum food consumption rate for the clam-shrimp diet whereas the maximum food consumption rate for fish this size on the Oregon Moist Pellet diet was about 16 ± 4%.

It also had been observed previous to this experiment that some of the juveniles brought in from the field would not eat Oregon Moist Pellet. These fish readily ate live food such as amphipods and polychaetes, but starved if only the artificial food was offered. The nature of this food preference was not investigated.

During the experiment, even though the fish fed the clam-shrimp diet grew much faster than fish fed Oregon Moist Pellet, the average mortality was much higher on the former diet (32%) than the latter (3%) (Table 7). The mortality of the clam-shrimp fed fish was spread evenly over the first seven weeks of the experiment with no deaths occurring during the last three weeks.

TABLE 7. The effect of diet on the weight, growth rate (± 2SE), food conversion, and survival of 0-group English sole fed an 8% ration. The initial number of fish was 25 per tank.

Treatment	No. fish surviving (n)	Initial wet wt (g)	Final wet wt (g)	Growth rate (%/day)	Gross Food Conversion (% dry wt)	Gross Food Conversion (% wet wt)
clam-shrimp						
1	15	8.9	19.8	1.13 ± 0.28	22.0	25.0
2	18	9.3	20.8	1.07 ± 0.23	22.3	25.2
3	18	9.2	19.9	1.15 ± 0.21	21.2	24.0
Oregon Pellet						
1	25	9.1	11.7	0.36 ± 0.21	6.4	22.3
2	23	8.9	11.6	0.38 ± 0.19	6.9	24.1
3	25	9.4	13.7	0.53 ± 0.27	9.4	32.9

The only external symptoms of the dying fish were a rapid expansion (over a two to six hour period) of the visceral area and a darkening of the fish. Death occurred within about six hours after the symptoms were first noticed. Upon closer examination, the peritoneal cavity and stomach were found to be full of a light yellow fluid. In some fish the stomach contained a plug of mucous or food that appeared to be wedged in the pyloric opening to the intestine.

Histopathological examinations of one healthy fish fed the Oregon Moist Pellet diet, one healthy fish fed the clam-shrimp diet, and one dying fish fed the clam-shrimp diet revealed that the only organ in the three fish in a pathological condition was the liver of the dying fish, which was simply depleted of glycogen. There was a heavy accumulation of lipids in the livers of all three fish. No bacteria were isolated from the kidneys or from the light yellow fluid of the dying fish, nor were there large numbers of bacteria on the body surface. No unusual parasites were found on or in the fish.

Effect of Population Density

The social behavior of the fish plus the quality of the water determine the density at which fish can be reared (Burrows 1972). This experiment was designed to determine if density was a factor contributing to the high mortality during the earlier temperature-ration experiments. Sole were reared at densities approximately half, equal to, and twice the initial densities in the temperature-ration experiments. Water flows were kept high and the water aerated in order to prevent accumulation of metabolic products and depletion of oxygen.

The density experiment was conducted in two plexiglass tanks (59.1 cm X 114.3 cm X 14.9 cm), which were divided into nine equal cells (59.1 cm X 12.7 cm X 10 cm), each containing 7.51 liters of water. Water flowed into the cells at 0.18 to 0.43 liters per minute. The walls of the cells were painted light brown so that fish in adjoining cells could not see each other and to provide a light background to facilitate the consumption of the Oregon Moist Pellet. Twelve cells contained one fish (averaging 3.82 g) each, three cells contained five fish (3.85 g) each, and three cells contained ten fish (4.0 g) each. The fish were weighed once every two weeks for ten weeks or until mortality terminated a group. The fish were fed a daily 16% ration.

The growth of juveniles held at the three densities did not differ significantly, as is shown by the overlapping ranges of two standard errors in Figure 6. The standard errors were smaller at the higher densities because the total numbers of fish were greater. However, the proportion of the fish starving during the experiment increased from one of 12 at one fish/cell density to two of 15 at five fish/cell density to six of 30 at ten fish/cell density. During later experiments, older juveniles were held at densities up to 13.9 kg/m^3 without fish dying or growth slowing (Table 8).

Effect of Competition

Dominance and spacing behavior limit the density at which fish can be cultured. Since tail biting had been observed in earlier experiments with English sole juveniles, the purpose of this experiment was to determine the effects of this aggressive behavior on growth.

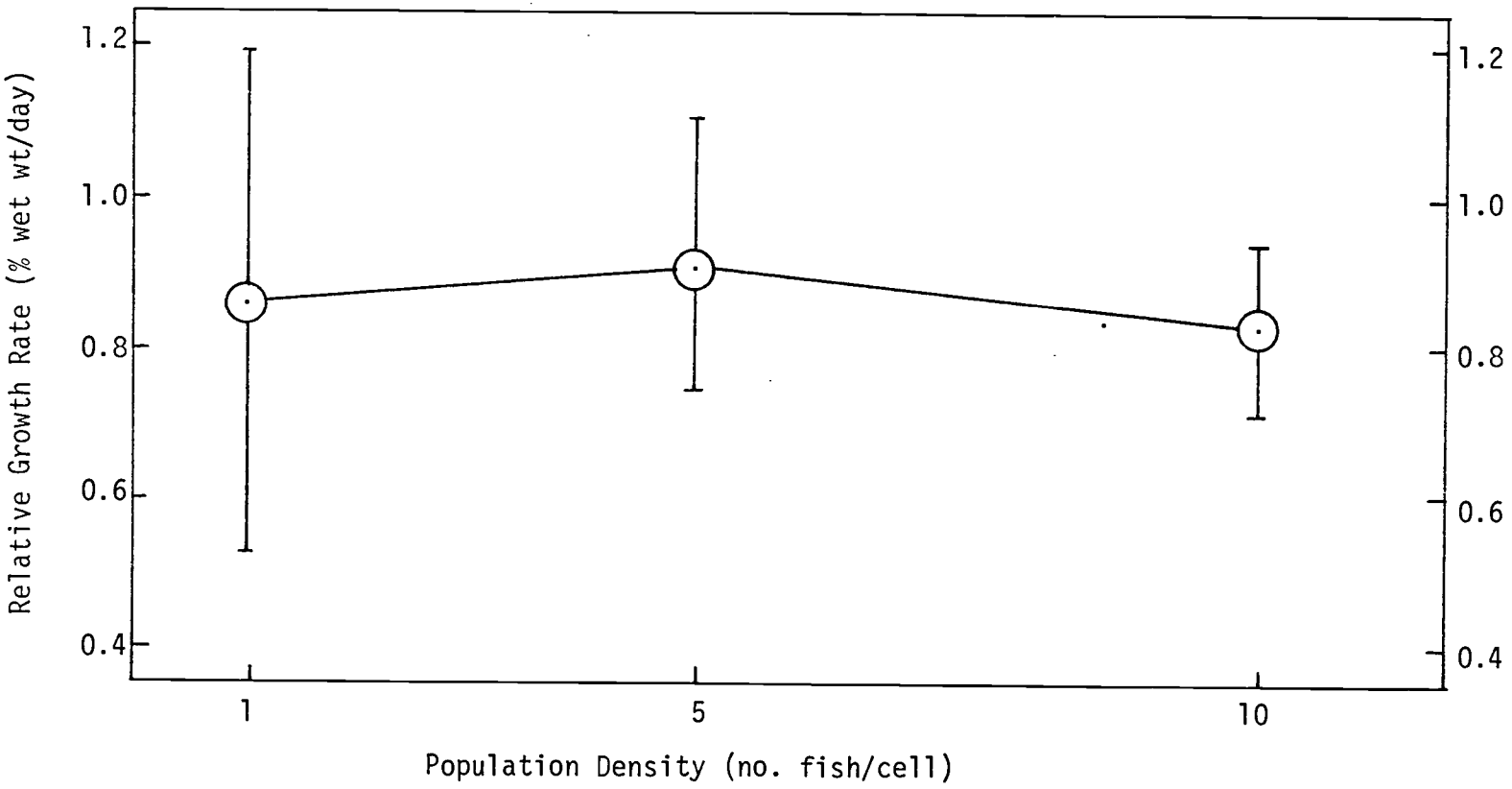

Figure 6. Growth rate (± 2SE) as a function of population density of 0-group English sole. Densities of 1 fish/cell, 5 fish/cell, and 10 fish/cell corresponded to 0.5 kg/m^3, 2.6 kg/m^3, and 5.3 kg/m^3, respectively, at the beginning of the experiment.

TABLE 8. Comparison of the average population densities of 0-group English sole before and after growth experiments.

Experiment	Population Density of Fish		
	Initial fish/m^2	Initial kg/m^3	Final kg/m^3
Density Experiment			
1 fish/cell	13	0.51	0.99
5 fish/cell	67	2.56	4.21
10 fish/cell	133	5.32	7.42
Previous Experiments			
Temperature X Ration	49	2.30	3.90[a]
Age	25	8.41	10.96
Diet	49	4.46	6.28
Competition	39	9.14	13.90

a. Includes only groups fed a 16% ration.

Forty fish were tagged individually with disc tags affixed to the animal by means of a stainless steel wire through the dorsal musculature. The fish were randomly assigned to one of two tanks and the growth rate of each fish was determined weekly for five weeks. At this point the ten fastest growing fish (above the median) from the two original tanks were placed together in one tank and the ten slowest growing fish from the two original tanks placed in another tank. The growth rate for each fish in the new groupings was then determined and compared to the growth during the first five weeks.

The fish were fed a 12% ration six days a week, feeding twice a day. The maximum food consumption rate had been determined in a preliminary experiment to be 16 ± 4% (range). The experiment was conducted at ambient temperatures, which tended to be cooler during the first five weeks (5 to 12° C) than during the second five weeks (8 to 13° C).

The average rate of growth of juveniles in the two tanks was approximately equal during the first five weeks (Table 9). At the same time the ten fastest growing fish from each tank (20 fish total) grew at a significantly faster rate ($p < 0.01$) than the ten slowest growing fish from each tank (20 fish total). The faster growing fish (22.7 grams) were significantly smaller ($p < 0.05$) than the slower growing fish (24.1 grams). When the fast growing fish were separated from the slow growing fish during the second five weeks of the experiment, both groups grew at the same rate statistically (Table 9), although the fast growers continued to grow somewhat faster than the slow growers.

TABLE 9. The effect of competition on growth (± 2SE) of 0-group English sole.

Growth Period	Group	Initial wet wt (g)	Terminal wet wt (g)	Growth rate (%/day)
1	Tank 1	23.5	28.4	0.53 ± 0.13
1	Tank 2	23.3	28.5	0.58 ± 0.07
1	Fast growers[a]	22.7	29.3	0.73 ± 0.06
1	Slow growers[b]	24.1	27.1	0.38 ± 0.07
2	Fast growers[c]	29.3	38.7	0.78 ± 0.08
2	Slow growers[c]	27.1	34.3	0.64 ± 0.14

a. These fish are the fastest growing fish above the median from Tanks 1 and 2.
b. These fish are the slowest growing fish below the median from Tanks 1 and 2.
c. The fast growers were separated from the slow growers.

The fish that grew the fastest during the first five weeks tended to be those that were more aggressive during feeding and more active throughout the day. In addition, aggressive fish were observed in this and previous experiments to nip the tails of less active fish. The less active fish were thought to be subordinate because they were less aggressive during feeding and their tails were badly bitten. However, this relationship was not established experimentally.

TOLERANCE OF JUVENILES TO LOW SALINITY

Although fishes as a group are relatively tolerant of salinity changes, Hickman and Trump (1969) in their review of the fish kidney considered English sole to be a stenohaline fish. Since the salinity of the surface water in Yaquina Bay is known to drop below 5‰ during freshets (Frolander et al. 1971), resulting in a corresponding drop in the salinity of the incoming water at the Marine Science Center during low tides, I felt it necessary to quantify the response of laboratory adapted juveniles to low salinities. Salinity tolerance was investigated by exposing groups of fish to a series of reduced salinities from 0.1 to 33.3‰ and tabulating mortality as a function of time up to four days.

Most of the test fish had been used previously in growth experiments and had been held in the laboratory for at least six months. The fish were acclimated to the test temperatures for at least four weeks but were transferred directly from full-strength sea water into the test salinities. Tolerances were investigated for 0-group sole at 9.5 and 16.5° C and for I-group sole at 16.5° C only. The temperature

range during experiments was no more than ± 1° C. The average weights (± 2SE) were 3.9 ± 0.3 g, 2.7 ± 0.2 g, and 21.9 ± 1.9 g for the three groups, respectively.

Tests were conducted in polyethylene tubs containing 30 liters of aerated sea water, which was not changed during the four-day experimental period. Water was collected at high tide from Yaquina Bay, passed through two sand filters in series to remove particulate material down to 3μ, and diluted to the desired salinity with distilled water. Fluorescent lighting (40 W) illuminated the tanks with 30 to 60 foot-candles at the water surface (Weston Illumination Meter, Model 756, Quartz Filter). Day-length was approximately nine hours.

The survival times of English sole juveniles exposed to reduced salinities are shown in Figure 7. Tolerance increased with increasing salinity, but the tests were of insufficient duration to determine the minimum salinity which could be tolerated indefinitely by 50% of the fish. However, by extrapolating from the data the lowest salinity tolerated indefinitely appears to be about 3‰ under the most favorable conditions. Tolerance tended to decrease with increasing temperature and increasing age, but the differences were not tested statistically.

DISEASES ENCOUNTERED DURING CULTURE

Because diseases of marine fishes are not well known, I have documented those encountered during my experiments.

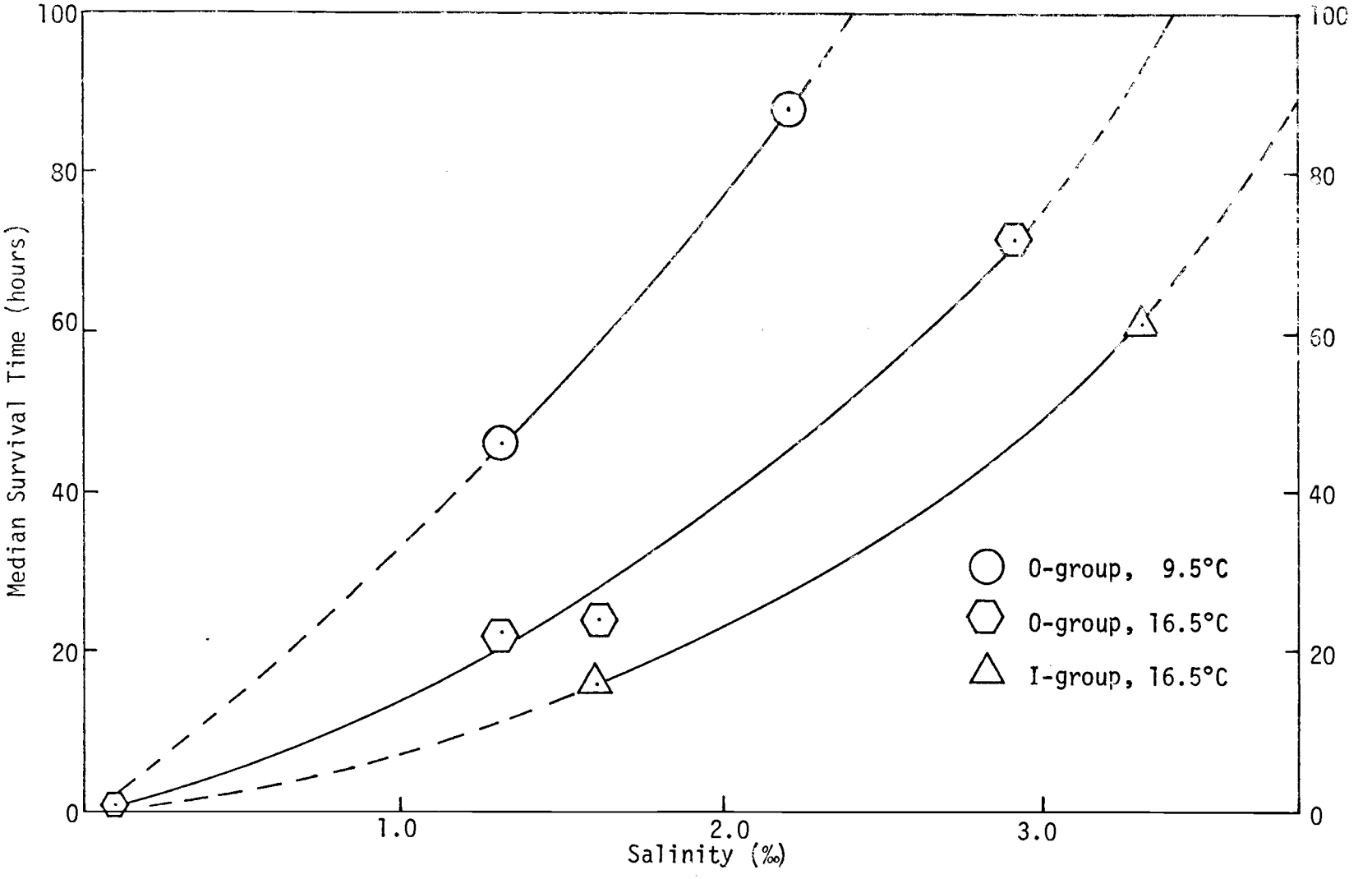

Figure 7. Median survival time of 0-group English sole as a function of salinity. Broken lines are a provisional interpretation.

Metazoan Parasites

The nematode Philometra americana was observed in English sole which had been collected during August between buoys 21 and 25 on Yaquina Bay (Fig. 1). The parasite, which encysts just under the skin, was not noticeable at the start of the experiment, but by the end of 12 weeks the characteristic "blood-red blisters" of the encapsulated nematodes were obvious. Of 201 juveniles, nine fish had one nematode and one fish had two. In all cases the worms were attached to the isthmus below the gill chambers. One English sole parasitized by P. americana was kept in the laboratory for two years during which time the fish grew from 5 grams to about 100 grams, a pace similar to that of unparasitized fish. This parasite was never observed in juveniles collected between buoys 12 and 15 during the period of May through early August.

The microsporidan protozoan, Glugea sp., was also observed only in fish which had been collected during August in the upper estuary (Fig. 1). Glugea inhabits the gut wall and in later stages causes the visceral mass to bulge markedly (Wellings et al. 1969). This symptom was first noticed in four of 201 fish three to six weeks after the start of one experiment, and within another three to four weeks the four fish died. Other fish may have harbored sublethal infections of Glugea but were not noticed.

During the summer, English sole juveniles used in growth experiments or simply held between experiments occasionally suffered from epizootics of Gyrodactylus sp., a monogenetic trematode living on the

surface of the fins and body. Symptoms of the disease included darkening of the fish and a slight fraying of the median and tail fins. Examination of the body surface revealed large numbers of gyrodactylids on the surface of the median and tail fins with fewer worms on the body surface. Gyrodactylus was effectively treated with formalin at 250 ppm for one hour. Heavy infestations required a dose of 500 ppm for two hours. When it became obvious that Gyrodactylus was to be a regular problem, fish fresh from the field were treated twice three days apart with 250 ppm formalin to remove existing parasites. The same concentration was used as a prophylaxis once a month during later experiments, but was not entirely successful in preventing epizootics. Deaths were associated with outbreaks of Gyrodactylus, but it was not determined whether the gyrodactylids killed the fish or attacked weakened fish dying from other causes. The rate of growth of several experimental groups of juveniles was observed to slow markedly after one gyrodactylid epizootic (Fig. 8). The week of slower growth may have been due to the disease or to the treatment. After prophylactic formalin treatments in 1973 experiments, growth slowed in one case and increased in another (Fig. 8).

Tail Biting and Presumed Bacterial Infections

Bacteria seemed to be involved in the deaths of many English sole juveniles held in the laboratory. However, reinfection experiments were not undertaken to demonstrate that bacteria were the primary cause of deaths. Three distinct disease conditions were identified: these were a fin rot; a cold-water, lesion-producing disease; and a

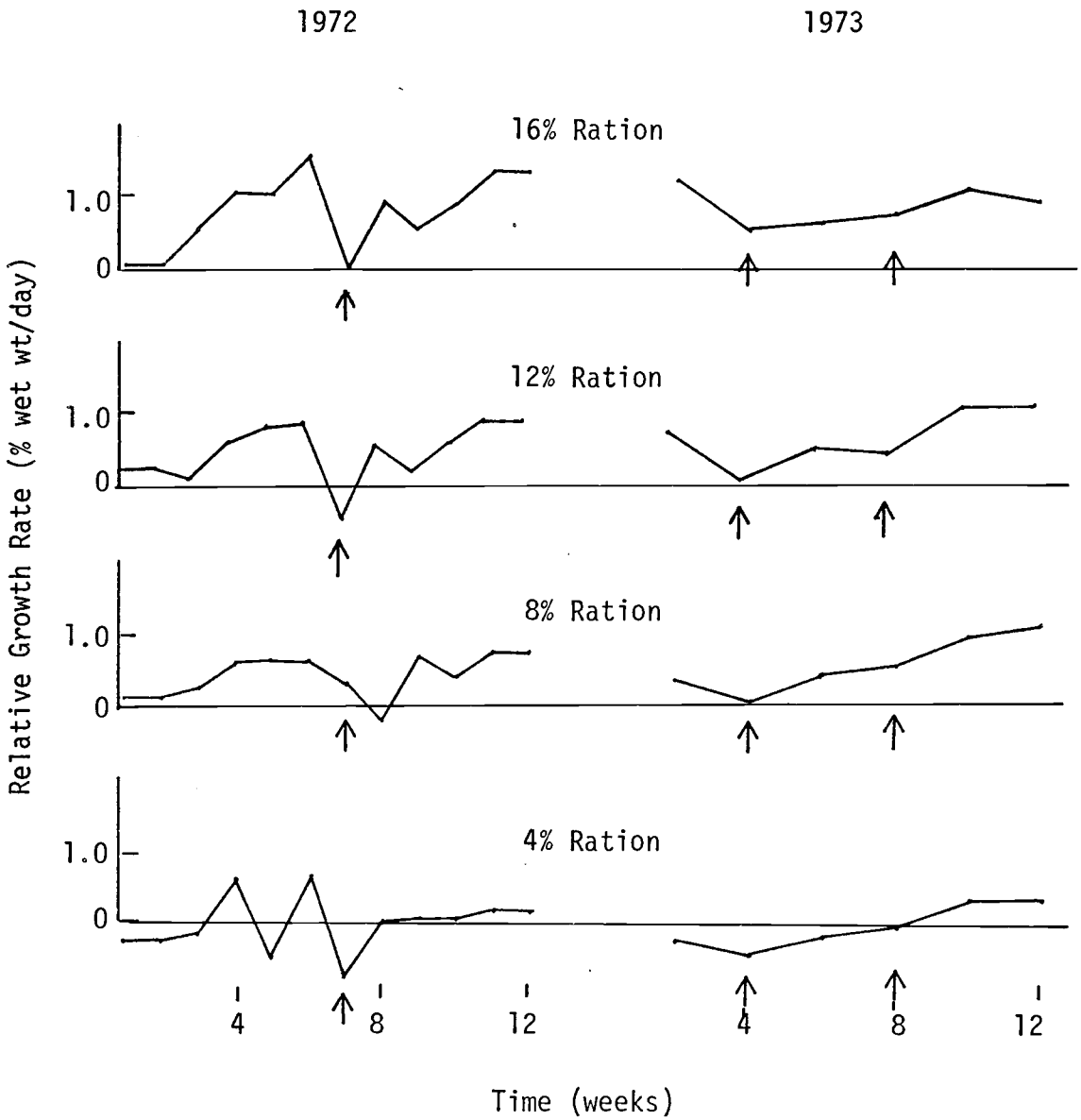

Figure 8. Effects of Gyrodactylus infection and formalin prophylaxis on relative growth rate of 0-group English sole. The data for fish at 12°C are used as an example. The arrow in the 1972 data marks a period in which a Gyrodactylus epizootic was treated with formalin. The two arrows in the 1973 data mark periods when the fish received a prophylactic treatment of formalin.

systemic disease. An unidentified species of the bacterium Vibrio was isolated from the kidneys of fish suffering from each of the three diseases. However, just as often no bacteria were found in the kidneys of fish suffering from the same diseases. In addition, an aeromonad-like bacterium was found in the kidney of one fish suffering from fin rot. The lesions often, but not always, contained motile rod-like bacteria. The following is a brief description of the gross symptoms of each disease and treatments.

Fin rot disease occurred throughout the year but was more common from mid-fall to mid-spring. The malady always began with the erosion of the tail fin (the tip was inflamed) and later spread to the dorsal and anal fins. If the disease progressed to the point where the hypural plate was exposed, the fish stopped eating and eventually died. The fins eroded slowly, over weeks or months, during which time treatment could be successfully initiated. The disease could be stopped in most cases by feeding Oregon Moist Pellet containing the antibiotic oxytetracycline as TM-50 (see Wood 1968) for two or three weeks. If the tail fin was eroded to the hypural plate, the fin rays were not replaced after recovery from the disease. It was found that the appearance of the fin rot could be prevented by feeding Oregon Moist Pellet containing TM-50 each day. Treatment was most successful when sick fish were isolated from healthy fish because the healthy fish were more aggressive in consuming the food containing the antibiotic. Fin rot disease was usually associated with tail biting, which in my experiments was a common behavioral interaction between English sole in the laboratory. Aggressive fish may have nipped the tails of other

fish to a point where any bacterium could have invaded the tissue of the fish.

Occasionally, some juveniles developed lesions in the body musculature next to the dorsal and anal fins. These lesions enlarged gradually over a period of weeks until the fish died. Most of the fish suffering from this disease also had fin rot. This disease did not respond to the antibiotic TM-50 in the food, but treatment with 1 ppm Malachite green promoted healing of the lesions in the few cases this drug was used. This disease only occurred during the winter.

During the summer months, English sole occasionally suffered from what appeared to be systemic bacterial infections. The symptoms of the dying fish were usually a reddening around the bases of the fins and on the gill cover and occasionally extensive hemorrhaging in some part of the body, usually in the body musculature, or an ulcer, also on the body. This disease struck both apparently healthy fish and fish suffering from fin rot. The occurrence of the disease was associated with warming of the incoming water. Only once was a mass mortality of juveniles associated with a presumed systemic bacterial infection, when 70% of the fish in a growth experiment died within three weeks in the middle of the experiment. Chum (O. keta) and pink salmon (O. nerka), held at the Marine Science Center at the same time experiments with English sole were conducted, frequently suffered extensive mortalities caused by the bacterium Vibrio (unpublished report, 1974, Use of heated sea water for farming oysters and salmon, Department of Fisheries and Wildlife, Oregon State University).

Skin Tumors

Skin tumors similar to epidermal papillomas of English sole reported by McArn and Wellings (1971) were observed on two juveniles during growth experiments. At the beginning of a growth experiment, one juvenile had a single small papilloma on the blind side located just ahead of the caudal peduncle and in mid-body; within eight weeks the neoplastic tissue had proliferated into a large cauliflower and spread over the surface of the caudal peduncle, at which time the fish died. The second juvenile had a single papilloma on the eyed side in the musculature just below the dorsal fin in mid-body, but the fate of the papilloma was not followed.

DISCUSSION

REARING THE LARVAE

Over the range of temperatures tested, a majority of the eggs of the English sole (Parophrys vetulus Girard) developed normally, resulting in a viable hatch of 48.5, 75, 85, and 76% at 5, 7.5, 10, and 12.5° C, respectively (Fig. 2). Alderdice and Forrester (1968) reported that for English sole eggs from Canadian fish, 90% or greater viable hatch was associated with 6.5 to 10° C and 50% or greater viable hatch associated with 4.5 to 12.5° C.

However, the primary objective of rearing the larvae to metamorphosis was not met. Even in the most successful trials, less than 20% of the original fertilized eggs became feeding larvae, and no feeding larvae survived more than two weeks longer than unfed larvae. Earlier attempts by others to culture English sole larvae to the juvenile stage also resulted in little or no success. Budd (1940) was not able to rear the larvae on a mixture of phytoplankton and the larvae of marine invertebrates. English (1966) tried unsuccessfully to rear the larvae on wild plankton, cultured diatoms, nudibranch larvae, barnacle larvae, and brine shrimp nauplii. Porter (personal conversation with P. Porter, School of Fisheries, University of Washington, August, 1972) reared small numbers of English sole larvae to metamorphosis on a mixture of the rotifer Brachionus plicatilus and the dinoflagellate Gymnodinium splendens and later brine shrimp but found the larvae of two other Eastern Pacific flatfish, Platichthys

stellatus and Psettichthys melanostictus, much easier to rear. Apparently, much remains to be learned in order to rear the larvae of English sole on a regular basis.

One may speculate that rough handling of the eggs, failure of the larvae to begin feeding, and fouling of the culture water contributed to the mortality of English sole eggs and larvae in this and previous studies. Susceptibility to mechanical shock or suboptimal physical conditions varies with the age of fish eggs and larvae, but marine fish eggs are especially sensitive until the completion of gastrulation (Blaxter 1969). In my experiments, spawning English sole by stripping, handling the eggs immediately after fertilization, and putting the newly fertilized eggs into water of a different temperature than that in which they were spawned probably contributed to the mortality during early development and stressed the survivors so the larvae were at a disadvantage during later development. British scientists avoid this handling problem with plaice (Pleuronectes platessa) by maintaining a brood stock and letting the adults spawn naturally. Eyed eggs, which are hardier, are then skimmed from the ponds (Howell 1973, Shelbourne 1964).

After the yolk is consumed, fish larvae retain the potential to initiate feeding for some days depending on species, egg size, and temperature, but there seems to be a point of no return after which larvae are alive but too weak to feed (Blaxter 1969). In laboratory experiments, larval anchovy (Engraulis mordax) had to be fed within 1.5 days after yolk absorption at 17.5° C or mass mortalities resulted (Lasker et al. 1970), while up to 10% of the larval grunion (Leuresthes

tenuis) reared at 18° C by May (1971b) retained the ability to initiate feeding at 12 days past yolk absorption. Rosenthal (1966) found the proportion of time that larval sole (Solea solea) were active was 70% at first feeding compared to 20% five to six days later, beyond which time irreversible starvation occurred. Orsi (1968) in an embryological study of the English sole, found that at hatching the eyes are unpigmented (presumably nonfunctioning) and the mouth and anus closed. By the sixth day (at 10.6° C) the eyes were pigmented and the mouth open but the anus still closed. The yolk was absorbed by the ninth or tenth day and the last larvae died by the 14th day. It appears that English sole larvae at 10° C have about six days after the eyes are pigmented in which to establish feeding.

Budd (1940) and English (1966) felt that getting the larvae to feed was the major obstacle to rearing English sole larvae. Failure of the larvae to feed was also a major problem during my studies even though the food organisms had been demonstrated adequate for English sole larvae by Porter (personal conversation with P. Porter, August, 1972). Poor illumination may have been the cause of the majority of larvae not feeding during my experiments, but the light intensity of 46 to 66 foot-candles was thought to be suitable. Blaxter (1968) reported that feeding in the younger plaice larvae (P. platessa) becomes reduced below about ten foot-candles. Shelbourne (1964) recommended 38 to 47 foot-candles at the water surface for rearing plaice larvae, while Struhsaker et al. (1973) considered 200 to 500 foot-candles optimal for the feeding of larval carangids (Caranx mate). The rearing containers in my studies were painted black to provide a

contrasting background so the larvae could see the food easier, as was recommended by Shelbourne (1964). Perhaps English sole larvae are more demanding in illumination requirements than plaice larvae.

When food organisms were added to the rearing containers during my experiments, a noticeable bacterial slime soon developed and later the pH of the water dropped. Most likely, excretory wastes and decomposing food promoted the growth of bacteria and ciliates that were also observed. A combination of accumulation of organic materials and appearance of foreign organisms probably contributed to the deaths of feeding larvae and to the stress of the first feeding larvae. Poor water quality has also been a major problem during culture of the eggs and larvae of other species (English 1966, Oppenheimer 1955, Shelbourne 1964, Struhsaker et al. 1973).

To appreciate the amount of research that has gone into rearing other flatfish larvae, it would be profitable to follow the long-term research program of the Fisheries Laboratory in Lowestoft, England, in which techniques were developed to rear larval plaice regularly on a large scale (Shelbourne 1964). Experiments in the initial stage of the program (lasting ten years) were conducted in a closed system. During this time the information needed to rear this species (e.g., feeding habits, optimum physical conditions, how to maintain water quality, etc.) was slowly accumulated. Success, measured in terms of larvae metamorphosed, was always low during the first six years but amounted to 100, 327, 1178, and 2807 larvae metamorphosed during the last four years. In 1962 an open rearing system, which made control of water quality much easier, was brought into use and up to 66% of the

original fertilized eggs in some groups metamorphosed, resulting in 18,498 juveniles. In the following 15 years, plaice larvae have been used in a variety of aquaculture and physiological experiments on a regular basis (Adron et al. 1974, Howell 1973), so a reliable technique can be said to have been developed for rearing plaice larvae. However, when the culture of another flatfish larvae, the turbot (Scophthalmus maximus), was attempted at the Lowestoft laboratory beginning in 1966, the experience with rearing plaice larvae was not as helpful as might have been expected since it was not until 1972 that small numbers of turbot larvae were reared to metamorphosis (Jones 1973). A major problem was finding an easily cultured, nutritionally adequate food organism that was small enough to be consumed by first-feeding turbot larvae, which are less than half the size of plaice larvae and cannot eat brine shrimp nauplii (Jones 1972b). The rotifer B. plicatilus was finally found to be a suitable food. In addition, the optimum conditions and procedures for culture unique to the turbot had to be defined. Although general procedures have been developed to rear fish larvae and several marine species can be cultured relatively easily (Atz 1964, May 1971a), one must expect to solve many problems before rearing many new species.

REARING THE JUVENILES

When studying the laboratory growth of an as yet uncultured species, it is desirable first to obtain as much information as possible on the general physiological and nutritional requirements of the species and later to investigate specific aspects of culture in more

detail. In this study, preliminary experiments on the effects of temperature, ration, age, diet, population density, and competition on the growth of juvenile English sole (P. vetulus) were conducted. In addition, the tolerance to low salinity was determined, and the occurrence and consequences of skin tumors and infectious diseases were documented.

Most of the growth experiments were designed to be analyzed statistically, but there was considerable variability in the data, resulting in only major effects being significant. Individual juveniles grew at different rates under presumably identical environmental conditions, reflecting the heterogeneity of the experimental fish or the experiments. During the density experiment in which 12 juveniles were held in separate containers, the average growth rates of individual fish over the ten-week period varied from -0.80 to 1.21%/day. The average growth rate of test groups in other rearing trials also differed greatly from week to week (Fig. 8).

Growth rate of English sole juveniles fed restricted rations decreased gradually with an increase in acclimation temperature over the range of 9.5 to 18° C (Fig. 3). The response on an unrestricted ration was not determined. At 21° C fish lost weight rapidly, regardless of ration, indicating that this temperature is near the upper lethal limit for the juveniles.

The increased metabolic costs of living at higher temperatures is probably reflected in (1) the increase in rate of weight loss of starved fish (Fig. 3), (2) the increase in the size of the maintenance ration (Fig. 4), and (3) the decrease in growth rate at restricted

rations (Fig. 3). The Q_{10} value for increase in rate of starvation from 9.5 to 18° C was 2.8. This is near the Q_{10} values for increase in metabolic rate with increasing temperature given in Winberg (1956): 2.9 and 2.5 over the ranges of 10 to 15° C and 15 to 20° C, respectively. However, the Q_{10} values for increase in maintenance ration and for decrease in growth rate at the 8 and 12% rations from 9.5 to 18° C were lower, being 1.8, 2.4, and 2.1, respectively. One explanation for the difference in Q_{10} values for rates between fed and starved fish is that assimilation may have been higher at higher temperatures. Anderson (1959) reported an increase in assimilation efficiency from 88 to 92% with increasing temperature over the range of 10 to 26° C for bluegill (Lepomis macrochirus). Lee (1969) determined that assimilation efficiency of largemouth bass (Micropterus salmoides) fed a 10% daily ration increased from about 77% at 20° C to 80% at 25° C; at smaller rations the increase in assimilation efficiency was smaller.

Although growth was not determined at temperatures lower than 9.5° C, it was observed that below 7° C food consumption rate decreased and near 2 to 3° C feeding ceased, so it can be assumed that growth would slow at these temperatures. Food consumption has been observed to decrease with a decrease in temperature from an optimum in the fishes Cyprinodon macularius, Salmo trutta, and Cichlasoma bimaculatum (Kinne 1960, Petelow 1939, Warren and Davis 1967).

Based on available experimental evidence, the temperature resulting in fastest growth probably lies within the range of 7 to 12° C. This is supported by the fact that older juveniles and adults on the continental shelf of Oregon live in water of about 8 to 10° C, which

varies only about 2° C during the year (Pattullo and Denner 1965, Pillsbury and Bottero 1971, unpublished manuscript, Average nearshore sections from the Newport hydrographic line during the upwelling season, Department of Oceanography, Oregon State University). The range of temperatures which English sole tolerated in the laboratory in this study for a week or more (3 to 21° C) is similar to the range of temperatures in Yaquina Bay (5 to 20° C) reported by Frolander et al. (1971).

Growth rate increased at a decreasing rate with increasing ration, tending toward a maximum above which, although more food may be consumed, no additional growth rate could be expected unless a favorable change in the environment occurred (Fig. 4). Similarly, Brett et al. (1969) found that the growth rate of sockeye salmon (Oncorhynchus nerka) increased at a decreasing rate as the ration increased until a maximum ration was reached, above which more food could be consumed but faster growth would not occur. The reason for this relationship is that the proportion of food used for non-growth functions (i.e., waste material and metabolism) increases as the ration increases, if all the food is consumed (Warren 1971). Over the range of daily rations from 0 to 15%, Averett (1969), studying coho salmon (Oncorhynchus kisutch), found that although growth rate increased with an increase in ration, feeding greater than an 11% ration resulted in a slowing of the increase in growth rate due to an increased proportion of the food lost as feces. Lee (1969), studying largemouth bass (M. salmoides), also found an increase in the proportion of waste materials with an increase in ration.

Food conversion at all temperatures between 9.5 and 18° C was highest at the intermediate 8% ration (Fig. 5). At higher rations presumably a decreasing proportion of the food is used for growth (Warren 1971). Paloheimo and Dickie (1966), in an analysis of the literature on the food consumption and growth of fish, concluded that food conversion declines with an increase in the ration above some ration near the maintenance level. Warren and Davis (1967) felt that food conversion would in some cases increase to an asymptote, if growth rate increased linearly with an increase in ration, and that if growth increased at a decreasing rate with food consumption, the maximum food conversion would occur at an intermediate ration, perhaps at two thirds of the maximum ration. Even though lacking the data to pinpoint the optimum ration and the maximum ration, for English sole fed Oregon Moist Pellet the optimum ration appears to be about one half the maximum ration (Fig. 5).

Fed the same restricted ration, I-group English sole grew as fast as 0-group juveniles at three temperatures (Table 5). However, the maximum daily food consumption rate was found to decrease with size from about 20 ± 5% (range) at 5 grams to 16 ± 4% at 9 grams to 10 ± 2% at 33 grams for juveniles fed Oregon Moist Pellet. Therefore, growth rate at an ad libitum ration could be expected to decrease with increasing size of the fish. According to Brown (1957), growth rate typically decreases with increasing size and age in fishes. The added metabolic costs of reproduction would obviously decrease growth in mature fish, but the slowing of growth rate in juvenile sockeye salmon (O. nerka) during experiments and in young hatchery brook trout

(Salvelinus fontinalis) is more difficult to understand (Brett et al. 1969, Cooper 1961). Sockeye salmon fed an excess ration (amount not specified) and held at 15° C grew almost twice as fast at 5 to 7 months as at 7 to 12 months (Brett et al. 1969). Turbot (S. maximus) grew 1.6 times faster from 1 to 20 grams in size than from 30 to 200 grams in size (Purdom et al. 1972). Since larger fish have a lower weight specific metabolic rate than smaller fish (A. Phillips 1972)--for instance, the metabolic rate of 3-gram English sole is 1.5 times that of 30-gram English sole (Hickman 1959)--the slower growth of older fish must be due in large part to decreasing food consumption. Brett (1971b) found that daily ad libitum ration decreased as sockeye salmon (O. nerka) grew larger from approximately 17 ± 4% (1SD) at 5 grams to 8 ± 1% at 50 grams to 4 ± 1% at 250 grams. By extrapolation, Brett estimated 1-gram sockeye salmon could consume a 30% daily ration. The slowing of growth may also be due in part to the environment becoming limiting in some manner as the size of the fish increases, and if the fish are given a more favorable environment the growth rate may again accelerate (Warren 1971).

Because I-group and 0-group juveniles were fed the same restricted ration and their growth rates were about the same, food conversions naturally were about equal, even though the fish were 12 months apart in age. In general, however, food conversion decreases with age because more of the food energy goes into metabolism and reproductive products (Brett 1970). Gerking (1952) found that food conversion in terms of protein fell from 33% in 10-gram longear sunfish (Lepomis megalotis) to only 5% in 105-gram fish. Food conversion in sockeye

salmon (O. nerka) dropped from 15% in 2-gram fish to 9.5% in 14-gram fish (Webb and Brett 1972). However, the food conversion in terms of wet weight of young turbot (S. maximus) only fell from 39% during the first six months of an experiment to 30% during the last six months (Purdom et al. 1972).

Oregon Moist Pellet proved an adequate diet for young English sole, but the juveniles grew twice as fast on the clam-shrimp diet as on the Oregon Moist Pellet diet at the same ration (Table 7). This may have been due to a difference in digestibility of the diets; the assimilation efficiency of English sole fed Oregon Moist Pellet was estimated to be about 66% and, while not determined for clam-shrimp fed fish, was thought to be much nearer 100% because of the almost complete lack of feces from fish fed this diet. Assimilation efficiencies reported in the literature for carnivorous fish fed natural diets are higher than I determined for English sole fed Oregon Moist Pellet. Winberg (1956) thought that assimilation efficiencies of 85% were typical of fishes. Davis and Warren (1965) feeding midge larvae at somewhat less than a maximum ration found the assimilation efficiency to be about 86% for trout (Salmo clarkii) and 82% for sculpins (Cottus perplexus). Kelso (1972) found the assimilation efficiency of walleye pike (Stizostedion vitreum) to be about 82, 84, 97, and 98% fed amphipod, crayfish, perch, and emerald shiner diets, respectively. Other published values include 91% for Megalops cyprinoides and Ophiocephalus stiatus both fed the prawn (Metapenaeus monoceras) (Pandian 1967), 89 to 98% for the red hind (Epinephelus guttatus) fed three species of fish (Menzel 1960), and 72% for pike (Esox lucius) fed minnows (Phoxinus

phoxinus) (Johnson 1966). Windell et al. (1969) have pointed out that artificial diets with a high organic content place different demands on the digestive system than the low organic content of natural foods to which fish are adapted in nature. They speculated that enzyme saturation may occur when artificial diets are fed at a high rate, resulting in a decreased assimilation efficiency. However, Brett (1971a), comparing the growth of sockeye salmon (O. nerka) when fed one of three artificial diets or one of two natural diets at "high" and "medium" rations, found that the fish grew significantly faster on the artificial diets than on the two natural diets. Growth was slowest on the frozen marine zooplankton diet, which Brett thought was unusual because the natural diet of young sockeye when they first enter the sea is marine zooplankton; he speculated that freezing diminished the quality of this diet. Comparing growth of rainbow trout (S. gairdneri) fed the diets of Oregon Moist Pellet or tubificid worms (Tubifex) at about the same rations, G. Phillips (1972) found little difference in growth rates in terms of wet weight or calories.

The higher food conversion of 22% on the clam-shrimp diet in my experiments compared to 10.5% on the Oregon Moist Pellet diet (at the most favorable temperature and ration) indicates that juveniles are more efficient at converting to flesh the food value of the clam-shrimp diet. Since most of the ingredients in the Oregon Moist Pellet come from fish and plant sources (Hublou 1963) and English sole consume a variety of invertebrates (Forrester 1969a), perhaps English sole obtain too little or too much of some factor(s) in the Oregon Moist Pellet diet to allow efficient utilization.

The important difference between the two diets may have been the ash content. I determined that the Oregon Moist Pellets used in my experiments contained about 11% ash on a wet weight basis, whereas the clam-shrimp diet contained only about 2% ash (Table 6). Therefore, a slightly greater proportion of the dry weight of the clam-shrimp diet is organic material, which is the metabolically important part of the diet. In addition, the higher ash content of the Oregon Moist Pellet diet may have imposed a higher osmoregulatory burden on fish fed this diet compared with fish fed the clam-shrimp diet. Because marine fish live in a hyperosmotic environment, it may be necessary to formulate artificial diets with a low ash content for them.

It should be emphasized that English sole were able to consume a larger amount of Oregon Moist Pellet than of the clam-shrimp diet on a dry weight basis simply because of the much higher water content of the clam-shrimp diet. There is a physical limit to the size of ration that can be consumed. Since the juveniles can eat a greater quantity of Oregon Moist Pellets, the difference between maximum attainable growth rates on the two diets is less than the difference in growth rate at a restricted ration. Presumably if a pelleted diet were developed with the nutritional requirements of English sole in mind, faster growth and lower mortality during growth experiments could be realized.

Fish fed the clam-shrimp diet suffered a higher average mortality (32%) during the experiment than fish fed Oregon Moist Pellet (3%), even though the former group grew 2.6 times faster than the latter group. The clam-shrimp fed fish which died did so rapidly after the appearance of stress symptoms, which were a darkening in color (typical of all

dying English sole), a pale yellow fluid in the gut and peritoneal cavity, and a swelling abdomen (dropsy). The latter symptom is indicative of liver malfunction due to pathogenic organisms, toxicants, or nutritional imbalances (Klontz 1973).

Infectious abdominal dropsy is a name applied to several fish diseases with similar symptoms, but which are caused by at least two separate organisms. One, bacterial hemorrhagic septicemia caused by Aeromonas liquefaciens, has been reported from many species of freshwater fishes. Common external symptoms are dropsy and ulcers. Internally, the organs undergo extreme degeneration (Amlacher 1970). Another disease, viral hemorrhagic septicemia, also results in dropsy, and internal examination reveals obvious symptoms such as hemorrhages on the organs, peritoneum, and musculature, and washed-out gills and kidney, typical of anemia (Snieszko 1972). Because no pathogenic parasites nor bacteria were found on or in dying clam-shrimp fed fish nor was there evidence of degeneration of any organ, death from this cause seems unlikely.

Some toxicants can cause dropsy and death in fishes. According to Klontz (1973), swollen abdomens are indicative of intoxication by chlorinated hydrocarbon insecticides, compounds which accumulate in fatty tissues (Amlacher 1970). The liver is a major storage depot for lipids in non-oily fish (Lagler et al. 1962), so is especially vulnerable to poisoning by such compounds. Ashley (1972) has discussed other toxicants that affect the liver in some manner; these are usually obvious because of their destruction of liver cells. It is unlikely that a toxicant was present in the water supply during the present

experiments because other English sole sharing the same water supply, but fed Oregon Moist Pellet, did not die. Furthermore, it is unlikely that toxicants were present in the clam-shrimp diet because both items in the diet are consumed by humans, the histopathological examination revealed no tissue damage to the organs examined, the mortality of the clam-shrimp fed fish occurred randomly over a seven week period, and 70% of the clam-shrimp fed fish survived to the end of the experiment without showing any abnormal effects.

Amlacher (1970) has pointed out that fishes having lived in captivity a long time always show some degree of fatty infiltration of tissues. Laboratory-reared plaice (P. platessa) fed a pelleted diet contained four times more lipid than wild fish (Cowey and Sargent 1972). Amlacher went on to say that excess food and lack of movement can lead to pathological fatty infiltration of lipid storage organs, which can cause a metabolic failure in the liver and the death of the organism. Snieszko (1972), reviewing the subject of lipoid liver degeneration (i.e., fatty infiltration of the liver), listed excessive fat, hard fat, and rancid fat in the diet as causes of this disorder. The disease develops slowly and is characterized by pale gills (anemia), an empty digestive tract containing only a pale yellow fluid, listless behavior, dropsy, exophthalmus, and a grey or yellow liver. In comparison, English sole fed the clam-shrimp diet died rapidly, and of the symptoms described for lipoid liver degeneration, exhibited only dropsy and the pale yellow fluid. In addition, although the livers of both fish fed the Oregon Moist Pellet diet and fish fed the clam-shrimp diet were heavily infiltrated with lipids, the liver cells showed no signs of the

degeneration normally associated with this disease (Ashley 1972, Snieszko 1972). One must conclude that lipoid liver degeneration was not likely the immediate cause of death.

In examining some of the dead fish fed the clam-shrimp diet, an apparent plug of food was found to be wedged in the pyloric opening to the intestine suggesting physical blockage of the digestive tract. However, blockage is unlikely because English sole have small mouths. In the final analysis, no cause was identified for the deaths of the clam-shrimp fed fish.

Over the range tested in the population density experiments (0.5 to 7.4 kg/m^3), crowding had no effect on the growth rate of English sole (Fig. 6), but as the number of fish per cell increased, the proportion of fish starving during the experiment increased from 8.3 to 20%. In other experiments juveniles were reared at densities up to 7.0 kg/m^3 for 5.3-gram fish, 4.6 kg/m^3 for 9.4-gram fish, 7.8 kg/m^3 for 30.9-gram fish, and 15.1 kg/m^3 for 48.5-gram fish (see also Table 8). In comparison, Burrows (1972) listed maximum loading densities for salmonids as 12.9 kg/m^3 for 5-gram fish, 14.1 kg/m^3 for 10-gram fish, 20.6 kg/m^3 for 30-gram fish, and 25.7 kg/m^3 for 50-gram fish. These are maximum culture densities beyond which growth rate will drop significantly; optimum culture densities in terms of maximum growth are lower. It should be noted that larger fish can be grown at higher densities than smaller fish. Purdom et al. (1972) raised turbot (S. maximus) in tanks to a density of 5.7 kg/m^3 in a recirculating system and thought higher densities could be obtained without slowing growth. Although maximum loading densities were not determined for English sole,

it appears that no experiments were conducted above those densities, thus explaining the lack of difference in growth rates between densities.

Below saturation densities there may be an optimum density at which growth is fastest. Brown (1946a) found that growth of brown trout (S. trutta) was fastest at three intermediate densities (fish/volume) than at a higher or a lower density. At the highest density the fish interacted to a degree that food consumption and food conversion were reduced. Slower growth at the lowest density was hypothesized to be caused by a lack of enough "social stimulation," which promotes growth. Hastings (1969, cited by Hastings and Dickie 1972), stocking channel catfish at different rates in ponds, found that production of the ponds increased at a slower rate than increase in initial stocking density, reflecting a decline in growth efficiency with increasing density. Willer and Schnigenberg (1927, cited by Brown 1957) found that feeding trout fry grew faster in larger rearing troughs. On the other hand, the data of Kinne (1960) and Menzel (1960) did not show a crowding effect over a wide range of density. It seems likely, therefore, that over a range of densities, growth rate remains relatively constant but that above and possibly below optimum densities growth may slow.

The final density a species will reach in a given container depends in part on its tolerance to crowding. In hatcheries, coho salmon (O. kisutch), a schooling species except as fry, can be grown more densely than rainbow trout (S. gairdneri), a territorial species (Klontz 1973).

The density of culture also depends on the rate of supplying

oxygen and removing metabolic wastes. Burrows (1972) and Klontz (1973) have discussed the interaction of these factors with stocking density to determine the carrying capacity of the water and have given methods to determine maximum fish densities given tank dimensions, water flows, and fish size.

Aggressive behavior (tail nipping) between individual English sole was observed throughout my experiments. In addition, it was determined that individually tagged juveniles, identified as slow growing and fast growing during an initial growth period, grew at statistically equal rates when the slow growing fish were separated from the fast growing fish during a second growth period (Table 9). Differential growth in groups of fish held in captivity have been reported for other species (Brown 1957). In trout hatcheries, the fry are periodically "graded" to separate large fish from small. Brown (1946a) separated a group of eight-week-old trout (S. trutta) into equal numbers of "large" and "small" fish and reared them under equal conditions, feeding an excess ration. After 28 weeks the "small" fish were almost the same size as the "large" fish, because the "small" fish grew faster once they had been separated from the "large" fish.

"Peck orders" or orders of dominance have been reported for several species of fish (Brown 1957). Stringer and Hoar (1955) observed a "peck order" among young trout (S. gairdneri) in which larger fry were more aggressive than small fry. Brown (1957) felt that the differential growth in groups of fish is probably related to this "peck order" in which larger and dominant fish grow fastest. Following the growth of individual trout (S. trutta) in groups for 20 weeks, Brown (1946a)

found that average specific growth rate declined with declining size of the fish even though small fish did not hesitate to satisfy their appetite in the presence of larger fish when excess food was fed. Brown (1957) thought that smaller fish were subject to "stress" in the presence of the larger and more aggressive fish, and this resulted in decreased growth. The apparent case of dominance found in my studies may be different in nature from those cases discussed in Brown (1957) because the faster growing English sole weighed less ($p < 0.05$) than the slower growing individuals and the faster growing fish were observed to be more active and generally consume more food, although the amount was not measured. The faster growing fish continued to be more active than the slow growing fish after the two groups were separated, although the growth rates between the two groups were nearly equal due to feeding a restricted ration.

Fenderson et al. (1968) observed that when fed an excess ration, socially dominant Atlantic salmon (Salmo salar) ate more mayfly and stonefly nymphs than subordinates under experimental conditions. No matter what the relationship is between dominance, size, and growth, the significance of observing aggressive behavior and differential growth rates in English sole is that the fish would have to be "graded" periodically to maintain an approximately equal size in the fish at marketing. Turbot (S. maximus), a flatfish grown experimentally in Great Britain, on the other hand, do not show aggressive behavior among themselves and hierarchies do not develop (Purdom et al. 1972).

A short term tolerance to low salinity was demonstrated for laboratory adapted English sole, although it is obvious that they would not

live in fresh water (Fig. 7). Neither the salinity of indefinite tolerance nor the metabolic costs of living in water of low salinity was determined. However, Hickman (1959) reported no difference in metabolic rate between English sole held in 24.3‰ versus 5.8‰ sea water.

Hickman and Trump (1969), in their review of the fish kidney, considered English sole to be a stenohaline teleost because: (1) Hickman (1959) found that newly caught English sole usually lived less than a week in 6‰ sea water and (2) Bulger and Trump (1968), after studying the morphological features of the kidney tubules, concluded that the English sole did not have the capacity for a high glomerular filtration rate needed for living in freshwater. However, Hickman and Trump (1969) pointed out that aglomerular fish may be euryhaline and that the morphology of the kidney may change in response to exposure to different salinities.

Two apparent bacterial diseases, referred to in this paper as fin rot and body erosion, were prevalent from late fall to early spring, when the water was uniformly cool. It is certain that bacteria killed some English sole, because the diseases could be prevented and cured with antibiotics, and because the bacterium Vibrio was occasionally isolated from the kidneys of sick fish. In most cases, however, the bacteria appeared to be secondary invaders of weakened fish. The fish developing body erosion tended to be the fish with fin rot; these in turn usually had nipped tails and grew slower. Fin rot is a common disease of freshwater salmonids (Bullock and Snieszko 1970), whose symptoms are similar to those I described earlier in this paper for

English sole fin rot. A variety of well-known bacterial pathogens, such as Aeromonas, and common water bacteria are found on the frayed fins but are seldom found in the kidney of fish with fin rot. Two diseases of salmonids, coldwater disease in freshwater and salt water myxobacteriosis, have symptoms similar to the body erosion disease described for English sole in this paper (Bullock and Snieszko 1970, Klontz 1973), but the causal agents, myxobacteria, were not found in the lesions of the English sole.

Mortalities also occurred occasionally from a presumed systemic bacterial disease, possibly vibriosis. Vibrio is known to be one of the most important pathogens of marine fish in captivity (Anderson and Conroy 1970, Fryer et al. 1972, Klontz 1973). Anderson and Conroy (1970) reported mortalities of flatfishes in England from vibriosis, while Purdom et al. (1972) reported a suspected Vibrio infection in the turbot (S. maximus) during cultivation trials. It is fortunate that English sole appear to have a degree of resistance to this pathogen. While Vibrio epizootics caused large mortalities in two species of salmonids held at the Marine Science Center, mortalities in English sole during the same period were light.

Gyrodactyliasis, which reached epizootic proportions during growth experiments and while holding fish between experiments during the summer, was associated with slowed growth (Fig. 8) and deaths. This disease has also been implicated in the deaths of flatfishes in aquaculture experiments in Great Britain (MacKenzie 1970, Pearse 1972). Fortunately, gyrodactyliasis is easily cured by treating with formalin and could probably be prevented by providing sand in the tanks, which

would enable the juveniles to scour off external parasites.

The nematode (Philometra americana) and microsporidan (Glugea) parasites and the skin tumors reported in this paper do not appear to be serious problems in the culture of English sole because their modes of transmission probably preclude a rapid increase in numbers. However, large mortalities caused by Glugea have been reported from trout hatcheries (Putz 1969) and in young flatfish kept in aquaria (Buckmann 1952, cited in Amlacher 1970), and heavy infections of Philometra make sole unmarketable (Olson 1972).

Most communicable diseases of fishes held in captivity are thought to occur only when the fish are stressed (Klontz 1973) or at least the effects of the disease are magnified under artificial culture conditions when the environment is suboptimal (Herman 1970). Wedemeyer (1970), reviewing the literature dealing with the relationship between stress and fish disease, listed high population density, excessive handling, low or high temperature, and low oxygen as factors associated with epizootics in fishes and listed anesthesia, fright, forced exertion, injury, etc., as stressing agents which could lower the disease resistance of fish. Mortalities may be delayed, obscuring the connection between the stress and the deaths.

Based on the preceding information, several stresses can be identified that might have promoted the deaths of English sole juveniles. These are use of the anesthetic MS-222, handling during weighing, low oxygen levels while water lines were plugged, and high temperature. That mortality was higher in groups fed low rations and/or held at high temperatures (Table 4) was probably indicative of the stress of those

two factors. In addition, formalin treatments, which I used to control gyrodactyliasis, are known to cause epithelial separation, hypertrophy, and necrosis in the gills, requiring at least 24 hours recovery time (Wedemeyer and Yasutake 1974). These treatments also cause substantial mortalities in hatchery salmonids when used in excess or at inappropriate times (Wood 1968). If these stresses could have been avoided, survival of English sole in the laboratory may have been expected to be higher than reported in Table 4. Laboratory adapted English sole kept between experiments at ambient temperatures and fed an adequate ration and an occasional dose of antibiotic suffered little or no mortality for months at a time.

The highest values for growth rate (% wet weight/day), dry food conversion (% dry weight growth/dry weight food), and wet food conversion (% wet weight growth/wet weight food) during my English sole studies under most favorable conditions were 0.95%/day, 10.5%, and 36%, respectively, for fish fed an artificial diet and 1.11%/day, 22%, and 25%, respectively, for fish fed a "natural" diet (Tables 1 and 7). To put these results of this study into perspective, the growth rates and food conversions in published studies with other species of flatfish have been listed in Table 10. There is a trend toward faster growth on "natural" diets than on artificial diets, indicating that the nutritional requirements of flatfishes are not well known. Salmonid species in hatcheries generally grow at rates faster than those reported for flatfish in Table 10, probably reflecting a greater knowledge of the culture requirements of the species. Sockeye salmon (O. nerka) have been reared at relative growth rates of 6.64%/day (0.9 to 4.2-gram fish)

TABLE 10. Growth and food conversion of flatfishes in the laboratory.

Fish Species	Approximate Size (g)	Food	Relative Growth Rate (% wet wt/day)	Food Conversion (% wet wt)	Food Conversion (% dry wt)	Author
Parophrys vetulus	4-15	artificial	0.95	36	10.5	Williams (this study)
	15-50	artificial	0.45	20	6.6	"
	7-20	clam-shrimp	1.11	25	22	"
Limanda yokohamae	49-120	annelid worms	0.90	18	--	Hatanaka et al. 1956a
Kareius bicoloratus	87-234	clams	1.42	19	--	Hatanaka et al. 1956b
Pseudopleuronectes americanus	37-105	bivalve siphons	0.11	9	--	Frame 1973
Scophthalmus maximus	1-20	trash fish	1.03	29	--	Purdom et al. 1972
	30-200	trash fish	0.65	32	--	"
Pleuronectes platessa	2.7-11	artificial	0.54	--	4.2	Kirk and Howell 1972
	3.5-25	enchytraeid worms	1.86	50	38	"
	2-22	artificial	0.90	--	13.1	Cowey et al. 1970
	1.2-20	artificial	1.07	--	--	Cowey et al. 1971
	4-20	artificial	0.92	--	13.5	Cowey et al. 1973
	0.3-2.6	mussels	0.66	25	--	Colman 1970
	10-80	mussels	--	17	--	Buchmann 1952
	25-149	mussels	--	14	--	Dawes 1930a, 1930b
Platichthys flesus	0.01-0.06	Oligochaet worms	--	27	--	Bregnballe 1961

and 1.44%/day (7.7 to 25.5-gram fish) on artificial diets, obtaining food conversions up to 27% (dry weight basis); the progress in defining the optimum environment for growth of sockeye salmon, which ultimately resulted in these high rates of growth, has been discussed in Brett et al. (1969), Brett and Sutherland (1970), and Shelbourn et al. (1973).

If one started with English sole that weighed five grams each on June 1 and if it were possible to grow them at a rate of 0.95% wet weight/day, the fish would reach the minimum commercial size of 30.5 cm (Forrester 1969a) and about 230 grams (Holland 1969) 237 days later, or during January of the following year. A more reasonable growth rate of 0.50%/day over the total period would result in marketable fish in about 450 days, or during August of the following year. Allowing four months for larval development and growth to five grams in size, English sole could theoretically be marketed at the minimum size within 12 to 17 months after fertilization of the eggs. However, much more basic biological information and knowledge of the economics involved is prerequisite to a serious evaluation of English sole for aquaculture.

RECOMMENDATIONS

Areas needing additional study to further the culture of the larvae include (1) factors affecting the initiation of feeding, (2) techniques to maintain the water quality while the larvae are weak swimmers and especially sensitive to a suboptimal environment, and (3) methods to prevent the occurrence of pathogenic organisms.

To further the culture of the juveniles, it is necessary to (1) develop a nutritional, artificial diet for English sole, (2) determine the important communicable diseases during artificial culture and find cures and prevention techniques for those diseases, (3) further define the effects of temperature and ration at low temperatures and on excess rations, (4) study the effects of salinity and light on growth and survival, and (5) determine maximum and optimum population densities for growth in order to permit estimation of land and water needs for large scale culture.

SUMMARY

Larval rearing experiments were conducted with the artificially spawned offspring of wild English sole, Parophrys vetulus Girard. The purpose was to develop a basic technique to rear the larvae through metamorphosis to the benthic juvenile stage. In addition, the effect of temperature on the rate of development and survival was studied.

Stripping the reproductive products from adults and artificially fertilizing the eggs proved to be a successful technique for spawning English sole, but delaying spawning more than 48 hours after capture of the adults resulted in a lower percent fertilization. At optimum temperatures, survival of the eggs and yolk-sac larvae was relatively high, but the older larvae could not be reared to metamorphosis under the culture conditions provided. Rate of development increased with increasing temperature.

Experiments were conducted with wild juvenile English sole from Yaquina Bay, Oregon. Different environments were offered and growth, food conversion, and survival studied. The purpose was to develop a basic technique to rear the juveniles to a marketable size and to begin defining optimum conditions for culture.

Growth rate depended on temperature. Growth rate on restricted rations was highest at 9.5° C and decreased with increasing temperature. No growth occurred at 21° C. Although not determined, growth was thought to decline below 7° C because of a drop in food consumption rate with decreasing temperature.

Growth rate also depended on daily ration (dry weight food/dry

weight fish). Growth was maximal on the highest restricted ration of the Oregon Moist Pellet diet and decreased with decreasing ration. The ration producing fastest growth was estimated to occur at somewhat above a 16% daily ration at temperatures between 9.5 and 15° C but was estimated to be nearer 12% at 18° C. The maintenance ration was estimated to increase from 3.1% at 9.5° C to 4.7% at 18° C. The optimum ration in terms of highest food conversion occurred at the 8% ration, regardless of temperature.

Food conversion (increase in dry weight of fish divided by dry weight of the food eaten, multiplied by 100) was highest at an intermediate ration. It decreased in the following order: 8%, 12%, 16%, and 4%. Food conversion was also influenced by temperature. It was greatest at 9.5° C and decreased with increasing temperature.

At three temperatures and on the same restricted ration, growth rate and food conversion did not differ between juveniles four to seven months old and juveniles 16 to 19 months old, but maximum food consumption rate decreased with increasing size and age.

English sole tolerated low salinities for short periods of time. Juveniles survived without mortality for 96 hours at 3.3‰ and 36 hours at 1.3‰. The time to 50% mortality at 1.3 to 1.6‰ was two times greater at 9.5° C than at 16.5° C and one and one half times greater for 0-group than I-group juveniles.

English sole grew well on the artificial diet Oregon Moist Pellet, but grew two and one half times faster on a more "natural" clam-shrimp diet on the same restricted ration. Juveniles consumed a larger total ration of the artificial diet than the "natural" diet.

Over the range of initial densities tested (0.5 to 5.3 kg/m^3), no decrease in growth rate was observed. Starvation mortality increased slightly with an increase in density. In later experiments juveniles were reared at densities up to 15.1 kg/m^3 without mortality or a slowing of growth.

Aggressive behavior between fishes in the form of tail biting was observed during the growth experiments. In an experiment in which all the fish were marked, it was determined that aggressive fish grew faster than less aggressive fish. Aggressive fish were characterized by swimming actively throughout the day, feeding readily, and biting the tails of other fish. Less aggressive fish swam infrequently, fed hesitantly, and had badly nipped tails. At the start of the experiment, the aggressive fish, as a group, were smaller than less aggressive fish.

Occasionally, juveniles suffered from epizootics of the monogenetic trematode Gyrodactylus and from three diseases with which bacteria were associated (a fin rot, a coldwater lesion-producing disease, and a systemic bacterial infection). Also observed during experiments were the parasites Philometra americana (Nematoda) and Glugea (Microsporida) and skin tumors.

Mortality of juveniles during experiments increased with increasing experimental temperatures and decreasing rations.

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