AGE AND GROWTH OF THE NEHU, *STOLEPHORUS PURPUREUS* (PISCES: ENGRAULIDAE), FROM THE HAWAIIAN ISLANDS AS INDICATED BY DAILY GROWTH INCREMENTS OF SAGITTAE

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ABSTRACT

Direct evidence is presented that the sagittae of nehu, *Stolephorus purpureus*, grow by discernible daily increments. Aging by daily growth increments provides the means to establish a general growth curve for the first 6 mo of life for this species. Adult nehu exhibit nearly linear growth between 30 and 60 mm standard length. Preliminary evidence is presented that the nehu population of Pearl Harbor may grow more rapidly than that of Kaneohe Bay.

Attempts to age tropical fishes by conventional methods have generally been thwarted by the absence of well-defined annuli in calcarious structures and protracted spawning periods which make length-frequency mode progression analyses difficult. Recognizing that exceptions to the above statement exist, Pannella's work (1971) providing indirect evidence of the presence of daily growth layers and periodical deposition patterns in the sagittae (otoliths) of three species of boreal fishes from the western North Atlantic suggested a means for conducting age and growth studies of tropical species. He concluded in that report: "Preliminary observation of growth patterns in sagittae of other species, living at various depths and different climates, appears to support the idea that daily growth may be a universal feature of fish otoliths." Pannella's (1974) later work in Puerto Rico provided circumstantial evidence of daily growth layers in sagittae of several species of tropical fishes.

To gain direct evidence that daily growth increments exist in tropical fishes we studied the nehu, *Stolephorus purpureus* Fowler, a small engraulid endemic to the Hawaiian Islands. The nehu is the basis of a live-bait fishery producing about 4,000 metric tons annually of skipjack tuna, *Katsuwonus pelamis* (Linnaeus), from the vicinity of the Hawaiian Islands. *Stolephorus purpureus* is a short-lived species (less than 1 yr) and has been the subject of relatively numerous studies: Nakamura (1970) has summarized the biological knowledge of this species available through 1965. Our work provides evidence of the presence of daily growth increments in the sagittae of nehu and permits the assembly of a growth curve for the first 6 mo of life for this species.

Brothers et al. (1976) have recently demonstrated the presence of daily growth increments in larval *Engraulis mordax* Girard and *Leuresthes tenuis* (Ayres) and presented evidence that the phenomenon occurs in several other species of California fishes.

METHODS AND MATERIALS

The nehu samples were taken with three types of gear in Pearl Harbor and the southeastern end of Kaneohe Bay, Oahu, Hawaiian Islands. Adults and juveniles (> about 30 mm standard length (SL)) were sampled with commercial bait seines (square mesh measuring 3.2 mm to a bar) in Pearl Harbor. Postlarvae (about ≥ 20 mm SL), juveniles, and adults were obtained in Kaneohe Bay by a similar seine having a bar mesh measurement of 1.6 mm. Larvae (< 20 mm SL) were obtained near Coconut Island by personnel of the Hawaii Institute of Marine Biology with 0.5-m ring nets with mesh sizes of 550 μm.

Three separate holding experiments were conducted to test the hypothesis that the sagittae of nehu grow by discernible daily increments. All animals for these experiments were collected in Pearl Harbor and held in tanks of 38-kl capacity at the National Marine Fisheries Service (NMFS) Kewalo Basin Facility. The tanks were supplied with well seawater of 23°-24°C and 33-35‰ salinity at a rate of about 300 liters/min. The nehu

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were fed with frozen and live brine shrimp, *Artemia* sp., under variable regimes as described below. Each experimental population of nehu was sampled during placement in holding tanks, and then subsampled at various time intervals as described for each experiment. Otoliths were extracted from most specimens within a few hours of sampling. The remaining samples were frozen in seawater or preserved in 75% solution of isopropanol until extraction of otoliths (removal of tissue from otoliths of alcohol preserved specimens is difficult).

The first holding experiment was begun 5 April 1972. A 16-day sample (21 April) and a 34-day sample (9 May) were obtained from this population. The animals were fed once a day with frozen and/or live brine shrimp. The second holding experiment was begun 15 December 1972. This population was initially fed once a day. A high mortality was observed during the first 2 wk, after which food was provided twice daily. Samples were collected weekly after 1 mo of captivity. We examined sagittae from animals collected on 19 January and 26 January 1973. The third holding experiment was begun 4 May 1973. This population was fed two or three times daily with frozen brine shrimp. Samples were obtained weekly between 4 May and 6 July. We examined sagittae from animals collected 25 May and 8 June 1973.

Wild populations of larval, juvenile, and adult nehu were sampled 13 times in Kaneohe Bay between 19 March 1972 and 13 July 1973 to obtain estimates of growth rates at various seasons. Although a second species of *Stolephorus* (*S. buccaneeri* Strasburg) occurs in Hawaii, larvae of this species have not yet been collected in the southeastern end of Kaneohe Bay (Watson and Leis 1974; W. Watson pers. commun.).

After extraction, the sagittae were cleaned and etched for up to 3 min in a 1% solution of HCl, then washed and mounted whole on glass slides with the mounting medium Euparal[2] and covered with glass cover slips. Short lengths of monofilament line were used to prevent the contact of the specimen by the cover slide. Although the smallest growth increments are microscopically discernible immediately after extraction their detection was enhanced after about 30 days of clearing in the mounting medium. Sagittae used in the first holding experiment and those collected from Kaneohe Bay and Pearl Harbor during spring 1972 were placed in glycerine on slides and covered. Some erosion of the sagittae edges was noted after about 5 mo, and this practice was discontinued after the first experiment. Slides were either labeled with date of collection and length of fish or assigned a five digit random number for identification.

Our initial counts were taken from thin sections of sagittae taken on the frontal plane. After mounting the sagittae in epoxy resin, the initial plane of polishing was made with rough sandpaper. As the surface approached the desired section, fine wet silicon carbide sandpaper (400 grit) was used. Final polishing of the surface was done with suspensions of aluminum oxide particles having diameters of 15, 5, and 0.3 μm. The section was thinned on the opposite side to a practical thickness and etched in a 1% solution of HCl for variable periods up to 3 min. A few attempts to make acetate peels of the small nehu sagittae sections as described by Pannella (1971) and Pannella and MacClintock (1968) were unsuccessful. We eventually abandoned the sectioning of sagittae because of the time required and the difficulty in obtaining a precise section from the nucleus to the posterior edge of the sagitta.

Sagittae were obtained from larvae less than about 20 mm SL by placing the specimen on a slide and gently teasing the otoliths from the head region. The sagittae were then mounted in Euparal and read immediately. These otoliths tended to clear completely within a few hours, and photographs are the only permanent record of these specimens.

The smallest growth increments of the mounted sagittae were counted with a compound microscope at magnifications of 400-800×. The smallest growth increment in all fish otoliths consists of both an organic and an inorganic layer (Degens et al. 1969). These two layers in the nehu otolith together measure about 1-4 μm thick. A zoom feature of the microscope was found to be extremely useful. Counts were maintained on a hand tally.

Enumeration of the smallest growth increment layers in whole sagittae is tedious, and reliable counts can be obtained only after a moderate amount of experience has been acquired. Enumeration is, obviously, much easier in sagittae from smaller fishes (Figure 1). Usually, readings cannot be made in a direct line from the nucleus to the selected point on the edge of the sagitta;

[2]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

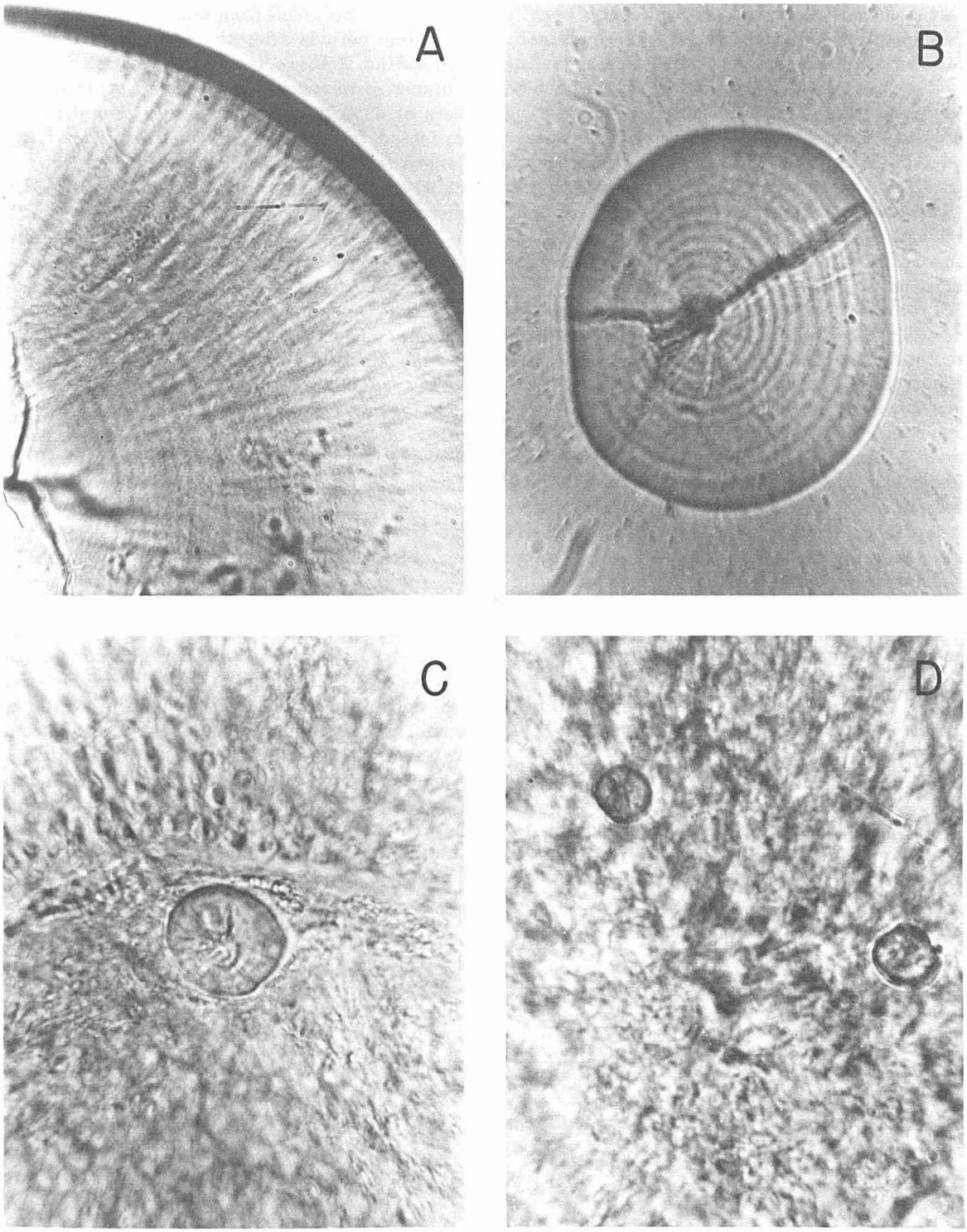

FIGURE 1.—Sagittae of larval *Stolephorus purpureus*. A: Portion of sagitta from a 28.8-mm SL individual with about 65 growth increments. B: 12.6 mm SL, 14 increments. C: 7.3 mm SL, 7 increments. D: 3.9 mm SL, 1 increment.

rather, a somewhat circuitous route must usually be taken from one area of the sagitta to another by following a prominent growth increment.

Each sagitta was counted several times in succession, the number of counts (up to 10) being proportional to the size of the sagitta. Counts were made from the nucleus to the antirostrum, rostrum, and postrostrum (terminology of Messieh 1972). A consistent count for the number of lamellae was then obtained. Verification counts were then made by the same reader at a later time. Verification counts were made by a second reader on 167 otoliths from the second and third holding experiments, as well as randomly selected sagittae representing the wild populations: 26.3% of these counts agreed with the original count; 48.5% differed by less than 1%; 72.5% differed by less than 2%; 86.9% differed by less than 3%; 92.9% differed by less than 4%; and 95.9% differed by less than 5%. Errors of less than 5% were considered acceptable, and the median values of the two readers were then utilized in the analyses. In cases where the results differed by more than 5%, the sagittae were reexamined and either a consensus of opinion reached or the data discarded.

Standard lengths were taken to the nearest 0.01 mm with dial calipers. Sagittae were measured with a micrometer eyepiece.

RESULTS

Holding Experiments

The holding experiments were undertaken as one means to determine if the smallest growth increments observable in the sagittae of nehu represent daily growth increments. We examined sagittae of specimens from samples taken at various time periods after the initial collection to determine if there was an increase in mean number of increments approximating the numbers of days between sampling. (Length data collected from all samples indicate that the length-frequency distributions of most of the captive populations studied were normally distributed.)

The data obtained for each holding experiment were subjected to analysis of covariance and the results are summarized in Table 1 and Figures 2-4. There was homogeneous variance within the samples for each of the three experiments as indicated by Bartlett's test of homogeneity (chi-square values = 0.56, 3.59, and 0.59, respectively).

In the first experiment there were no significant differences between the means of the independent variable (standard length) for each of the three samples at the $P<0.05$ level. There were significant differences between the regression coefficients and the elevation of the regression curves for each sample at the $P<0.01$ level (Table 1, Figure 2).

The significant differences between regression coefficients seems best explained by the effects of captivity. Hypothetically, the regression coefficient of the initial sample of 5 April represents the relationship between number of growth increments and standard length in the wild population. The smaller regression coefficient value of the 21 April sample indicates a slower growth rate of the captive population during the 16-day interval between sampling. This is probably due to less than optimal food supply and/or other effects of captivity. The intermediate regression coefficient value of the 9 May sample indicates that the

TABLE 1.—Summary of analysis of covariance for three holding experiments.

Sampling date	Dependent variable (increments) Unadjusted $\bar{y}$	Adjusted $\bar{y}$	r^2	n	s^2	*F* ratios Independent variable (standard length)	Regression coefficient	Elevation
5 Apr. 1972	84.9	86.7	0.77	30	38.2			
21 Apr. 1972	101.1	100.6	0.76	24	10.8			
9 May 1972	118.1	116.4	0.74	24	20.6			
First experiment						1.2	5.4**	206***
19 Jan. 1973	114.8	114.0	0.95	25	14.7			
26 Jan. 1973	120.8	121.6	0.85	24	31.1			
Second experiment						0.1	1.3	31***
25 May 1973	124.9	132.1	0.97	23	13.8			
8 June 1973	140.0	133.4	0.95	24	6.1			
Third experiment						34***	1.1	1.1

**$P \leq 0.01$.
***$P \leq 0.001$.

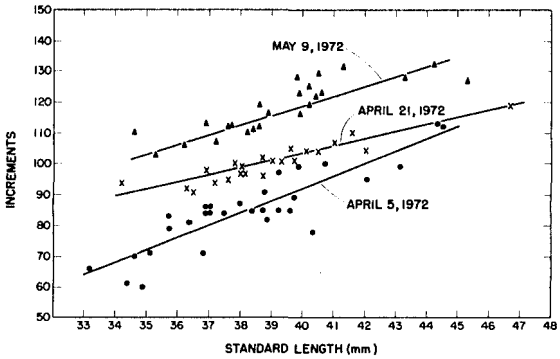

FIGURE 2.—*Stolephorus purpureus*: First holding experiment.

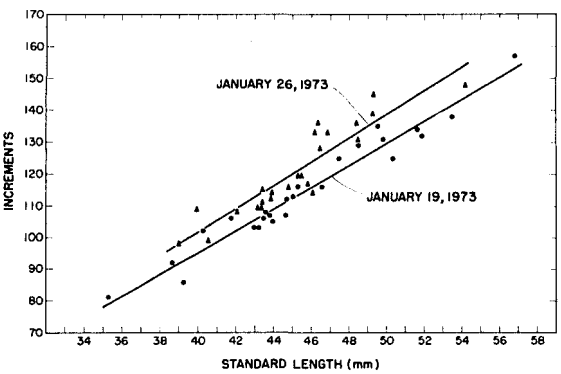

FIGURE 3.—*Stolephorus purpureus*: Second holding experiment.

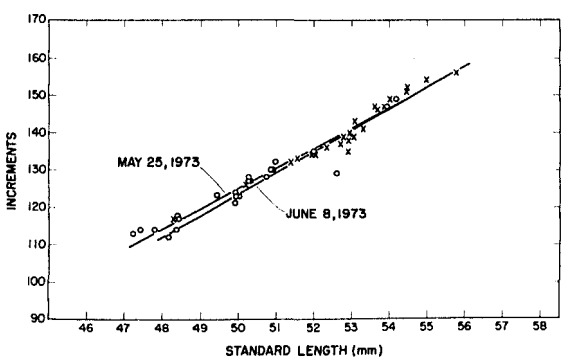

FIGURE 4.—*Stolephorus purpureus*: Third holding experiment.

growth rate has increased in the captive population after 34 days in captivity, but has not reached the value of the wild population from which it was taken.

In the first holding experiment, the second and third samples were collected 16 and 34 days, respectively, after the initial sample. For unadjusted $\bar{y}$ values, these samples differed from the initial sample by 16.2 and 33.2 increments, whereas for the adjusted $\bar{y}$ values, they differed from the initial sample by 13.9 and 29.7 increments (Table 1).

The results of the two samples (collected after more than 30 days in captivity) collected 19 and 26 January 1973, and compared in the second holding experiment, are summarized in Table 1 and Figure 3. There were no significant differences between the means of the independent variables or the regression coefficients at the $P \leq 0.05$ level. The elevations of the two regression curves are significantly different at the $P \leq 0.001$ level. The differences in number of increments between unadjusted $\bar{y}$ values (6.0) and adjusted $\bar{y}$ values (7.6) again closely approximate the expected difference of 7 days between samples.

The results of the samples of 25 May and 8 June 1973 compared from the third holding experiment are given in Table 1 and Figure 4. In this experiment there was a significant difference between the means of the independent variable ($P < 0.001$), but no differences between the regression coefficients and elevations of the two regression curves at the $P \leq 0.05$ level. The significant difference in mean length between the two samples is probably attributable to the increased amount of food provided to the captive population and the resulting high growth rate exhibited throughout the duration of the experiment. Because the treatment significantly affected the independent variable, further examination of the regression statistics is unwarranted. However, if the two samples are subjected to a two-group comparison, there is a significant difference between the mean number of increments for each sample ($P < 0.05$). The difference between the means for each sample (25 May, $\bar{y} = 124.9$; 8 June, $\bar{y} = 140.0$) closely approximates the expected difference of 14 days between samples.

We conclude from the relatively good agreement between the increase in mean number of growth increments and the number of days between collection of samples, that these data from the holding experiments provide direct evidence of the presence of daily growth increments in the sagittae of nehu.

Growth of Sagittae

The total lengths of sagittae from the 5 April and 9 May 1972 nehu samples (the initial sample from the wild population and the 34-day sample)

of the first holding experiment were taken in order to examine the effects of captivity on sagittal growth. Four measurements for the 5 April sample were arbitrarily deleted because their values were well below the distribution of the majority of the sample. All 24 measurements from the 9 May sample were utilized. There are significant relationships between sagitta length and fish length for the two samples ($P < 0.001$, r^2 values: 5 April, 0.82; 9 May, 0.70) (Figure 5). The first experiment demonstrated that there was a significant increase in the mean number of increments between the two samples. Analysis of covariance of sagittae lengths indicated that there were no significant differences between the means of the independent variables, regression coefficients, or elevations of the regression curves for the two samples (respective F ratios: 2.5, 1.0, 1.2) presumably because of intrinsic variation, limited precision of measurements, and the relatively short time period between samples. Although there were no statistically significant differences found in the comparison of the two curves, the two regression coefficients exhibit perhaps expectable trends. The lesser regression coefficient and r^2 value for the 9 May sample may be indicative of a decreased growth rate and more variable responses of individuals in the population to the highly variable, and probably less than optimal, conditions of the holding facility. In addition, the differences between the unadjusted and adjusted means of sagittal lengths between the 5 April (1.094 mm; 1.070 mm, respectively) and 9 May (1.176 mm; 1.201 mm, respectively) samples of 0.082 mm and 0.131 mm are to be expected with daily growth increments of about 3-4 μm.

We have noted one apparent example of provisioning rates affecting the growth rates of sagittae of captive nehu. Sagittae from the 19 January sample of the second holding experiment usually exhibited 23-24 distinctive, more widely spaced increments on the edge of the otolith. The numbers of distinctive increments approximately correspond to the number of days during which the daily amount of food provided the sample population was double the initial ration. As might be expected otoliths collected 7 days later in the 26 January sample exhibited 30-31 distinctive increments. Indeed, the wider increments observed after provisioning rates were doubled were much more effective in "labeling" the sagitta than our attempts to accomplish the same objective with Tetracyclene. Possibly, controlled experiments with rapidly growing fish species incorporating this treatment would be a much more expeditious test of the daily growth increment hypothesis.

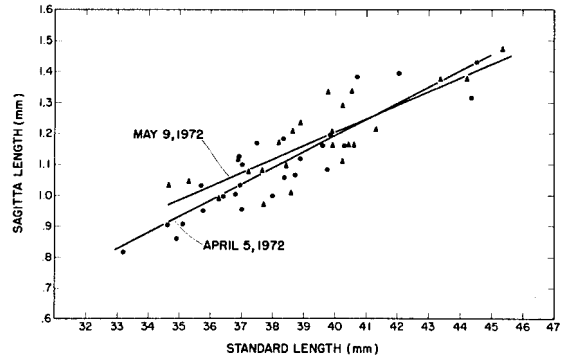

FIGURE 5.—*Stolephorus purpureus*: Growth of sagittae during first holding experiment.

Age and Growth in Wild Populations

We examined larval, juvenile, and adult nehu collected in Kaneohe Bay to obtain an estimate of age and growth of a wild population based on the assumption that the smallest observable growth layers in the sagittae represent daily growth increments. We examined 213 specimens from 13 collections made during most seasons between spring 1972 and summer 1973 (no collections were made in the months November through January). The growth curves obtained from the individual collections are given in Figure 6. Because all individuals in a sample have been exposed to the vagaries of the environment during their observed lifespan, a composite growth curve for all collections is presented in Figure 6F. Although some variation between samples is apparent, the composite scattergram serves as a first estimate of the growth pattern of nehu in Kaneohe Bay.

There are two well-defined segments to the composite growth curve (Figure 6F). Young larvae exhibit exponential growth to a length of about 15-17 mm. At about 20 mm the population enters an almost linear growth phase to about 60 mm. The composite scattergram obscures another, lesser inflection at about 20-30 mm exhibited by the spring 1972 collections (Figure 6A). Yamashita (1951) has demonstrated that nehu have completed larval metamorphosis at about 30 mm. The major inflection at a length of about 17 mm appears to reflect the fact that nehu begin to exhibit exponential growth in body depth at this

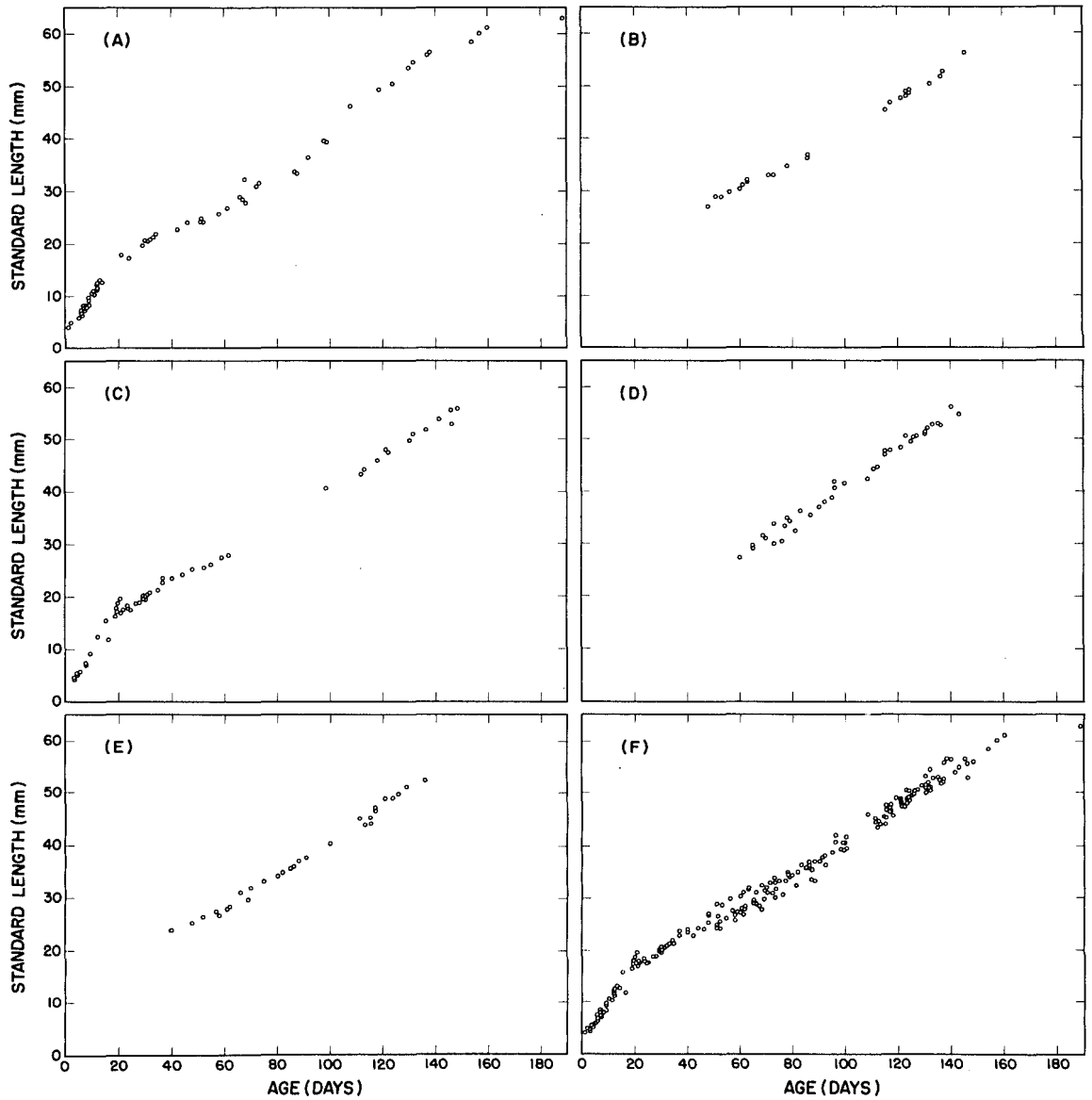

FIGURE 6.—*Stolephorus purpureus:* Age-length relations of 213 individuals from 13 collections in Kaneohe Bay. A: 19 March 1972 (21 individuals, 66-189 days); April 1972 (13, 1-24); 1 May 1972 (11, 6-12); 26 May 1972 (16, 21-68). B: 26 August 1972 (all specimens). C: 7 October 1972 (8, 16-23); 14 October 1972 (23, 19-62); 19 October 1972 (13, 99-148); 25 October 1972 (9,3-9). D: 12 February 1973 (12, 60-87), (11, 115-140); 19 March 1973 (15, 78-125). E: 5 May 1973 (8, 40-69); 13 July 1973 (27, 66-136). F: Composite scattergram of all observations.

size (cf., Nakamura 1970, fig. 4). Thus, much growth of individual nehu is directed to allometric growth of body depth, rather than body length. The growth rate of young nehu (<17 mm) indicated by the composite scattergram is consistent with the estimates of larval growth rates presented by Tester (1951) and Yamashita (1951).

The possibility that the inflection at 15-17 mm is related to a change in diet was examined. Burdick (1969) investigated the feeding habits of larval nehu from hatching to a length of 25 mm in Kaneohe Bay. He found that young nehu less than 5 mm long fed almost exclusively on copepod nauplii. At lengths of 5-7 mm, the diet shifted to a

preponderance of small, adult copepods representing two genera. Larvae less than 20 mm fed exclusively during day, at 20 mm they began occasional feeding at night, and when they attained a length of 25 mm they fed regularly at night. None of these changes in feeding habits seem related to the 15-17 mm inflection.

Only one fish, estimated to be 189 days old at a length of about 63 mm, indicated that the Kaneohe Bay population of nehu may enter an asymptotic growth phase at about 60 mm. Obviously, additional collections of older fishes are required to elucidate this portion of the growth curve.

The absence of large adults might be explained by the heavy exploitation of this stock by commercial fishermen. Another possible explanation relates to the observations of Muller[3] on *Stolephorus heterolobus* Rüppell in the Palau Islands of the western Pacific. He found that large spawning adults occur in open lagoon waters 2-4 km offshore over depths of 30-40 m during night. The daytime distribution of these individuals is unknown, but it is thought that they occur near bottom in the open lagoon. In the case of nehu, however, the explanation of an absence of adults in the asymptotic growth phase by invoking an offshore spawning movement is argued against by a recent study demonstrating that this species is capable of spawning at a length of 35-40 mm (Leary et al. in press).

These readings of whole-mounted sagittae from Kaneohe Bay nehu did not reveal any periodic deposition patterns of increments or spawning checks as reported by Pannella (1971).

Geographical Comparison of Growth Rates

One of the more exciting aspects of being able to accurately determine growth rates of young fishes is the tool that it provides to examine the effects of various environmental conditions. As an exercise, we compared the linear segments of the growth curves of two samples (n = 15) of nehu collected during March and April 1972 in Pearl Harbor and Kaneohe Bay (Figure 7). Unfortunately, the differences in size ranges of the two samples and the small sample sizes resulted in significant heterogeneity of variance ($P < 0.05$). The analysis of covariance did indicate, however, that there may be

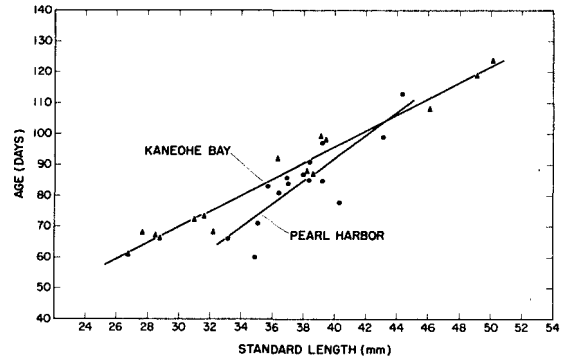

FIGURE 7.—Comparison of *Stolephorus purpureus* growth rates in Pearl Harbor and Kaneohe Bay, spring 1972.

significant differences between the regression coefficients ($P < 0.05$) and elevations ($P < 0.01$) of the two population curves, the Pearl Harbor sample exhibiting a faster growth rate to a length of about 44 mm. Similar, but more intensive, studies should provide a wealth of insight into a variety of aquatic situations.

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