

Mortality of Starry Flounders (*Platichthys stellatus*) with Skin Tumors

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The incidence of skin tumors among starry flounders (*Platichthys stellatus*) in Bellingham Bay, Washington, varied both spatially and temporally. Incidence was 37% in the young-of-the-year inshore fish, declining to near-zero values by age II. My results indicate that tumor regression and spatial segregation of tumorous fish were not responsible for the decline, but that tumorous individuals had a high mortality rate relative to normal conspecifics. Selection against tumorous individuals was indicated by a sharp decline in tumor incidence in 1-yr-old fish at the same time as mean tumor number per fish declined and tumorous fish became significantly smaller than their normal counterparts. There were no differences in susceptibility to stress between normal and tumorous fish until age I; age I tumorous fish had a higher mortality rate under stressful conditions. The flounder skin tumors are lethal to a large proportion of each year-class, and therefore represent one of the largest known sources of repetitive, disease-induced mortality of fishes.

Key words: starry flounder, skin tumor, X cell, mortality

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La fréquence de tumeurs cutanées chez le flet étoilé (*Platichthys stellatus*) de la baie Bellingham (Washington) varie dans le temps et dans l'espace. Chez les poissons côtiers de moins de 1 an, l'incidence s'élève à 37 % et diminue aux environs de zéro chez les poissons de l'âge II. Les résultats portent à croire que la régression des tumeurs et la ségrégation spatiale des poissons atteints ne sont pas responsables du déclin, mais que les individus malades ont un taux de mortalité élevé par rapport aux individus sains. La sélection s'effectuant au détriment des poissons atteints est révélée par un déclin marqué de la fréquence de tumeur chez les poissons de 1 an; en même temps, le nombre moyen de tumeur par poisson baisse, et la taille des poissons malades diminue de façon significative par rapport aux poissons en santé. Aucune prédisposition au stress n'a été notée chez les deux types de poissons avant l'âge I mais, à ce stade, les poissons malades ont un taux de mortalité plus élevé en présence de conditions difficiles. Les tumeurs sont létales pour une grande partie de chaque classe d'âge et représentent donc une des sources les plus importantes de mortalité entraînée par la maladie.

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SKIN tumors in flounders are among the most intensively studied of the fish tumor systems, due to epidemic proportions in certain areas. At least 17 species of pleuronectids are regularly affected in the north Pacific, although tumor incidence varies widely among populations. Tumor presence has been recorded in California (Cooper and Keller 1969; Mearns and Sherwood 1976), Puget Sound/Strait of Georgia (Wellings et al. 1976; Stich et al. 1977), the Bering Sea (McCain et al. 1978), and Japan (Oishi et al. 1976). With many commercially important species being susceptible, and up to 58% of a population year-class being affected (Stich et al. 1977), the problem has economic as well as biological significance.

Skin tumors first appear on the body and fin surface several months after the fish has metamorphosed from the larval stage

(corresponding to an approximate age of 6–10 mo in most species). Although tumor appearance may be delayed by several months in individual fish, no new tumors appear once this period has passed. Therefore, tumor appearance is restricted to young-of-the-year flounders. Tumor development is similar in all of the flounder species studied (Miller 1969; McArn and Wellings 1971; Wellings et al. 1976).

Whether the skin tumors or epidermal papillomas are virally induced neoplasms (Peters et al. 1978) or simply parasitic infestations (Dawe et al. 1979) is controversial. Acknowledging both possibilities, I use the term "tumor" throughout this report in a loose sense. If truly neoplastic, the tumors are benign, malignancy having been reported only in isolated instances (Peters et al. 1978).

Because skin tumors appear early in the life history, the greatest number of affected individuals exists in the youngest

age-classes (Stich et al. 1977). However, the proportion of tumorous individuals in the population appears to decrease with age; few, if any, tumorous individuals reach sexual maturity on the coasts of British Columbia or California. Therefore, infected fish are probably subject to indirect selective mortality, because benign tumors are not generally directly lethal (Wellings et al. 1964; McArn and Wellings 1971).

Three possible explanations for the loss of tumorous fish are: (1) tumor regression, (2) emigration of tumorous individuals from the study site, and (3) natural selection against tumorous fish relative to normal fish. The object of this study was to distinguish between these alternatives to ascertain the importance of skin tumors in the life history of starry flounders, *Platichthys stellatus*.

Materials and Methods

Collections for this study were made in Bellingham Bay, Washington. Site selection was determined by the high incidence of epidermal papillomas on starry flounders, the epizootiology of which is representative of the other flounder species affected (McArn and Wellings 1971). A number of pleuronectids have been observed with skin tumors in this area; however, starry flounders are more easily monitored in situ because of their inshore habits, both as juveniles and adults.

Inshore, age 0–I flounders were collected with a 9-m seine with wings of 1.3-cm stretch mesh and a bag of 0.6-cm stretch mesh. Collections were made monthly (or more frequently) between June 1979 and August 1980, and in the summer and fall of 1980 and 1981. Seine samples were taken within 1 h of low tide at various locations on the sand flats of the Nooksak River estuary. Collection depth was 0.1–1.5 m. On several occasions I made collections at widely different sites on the estuary on the same tide, to check for intersite differences in the monitored fish characters.

Trawl collections were made monthly between July 1979 and July 1980, with the exception of January 1980. A shrimp "try net" with a 2.5-m lead line, 3.5-cm stretch mesh wings, and a cod end liner of 1.1-cm stretch mesh was towed at a velocity of 1–2 m/s behind a herring skiff powered with a 40 hp engine. Three collection sites off the mouth of the Nooksak River, at depths of 2–15 m, were sampled on each trawling date.

In November 1980, the effects of gear selection on age 0 fish were examined. In water depths ranging from 0.2 to 1.5 m, paired parallel hauls of both seine and trawl were made. Three 10-min seine hauls were alternated with three 100-m hand-pulled trawl hauls. The trawl was pulled with a 100-m tow line (to avoid fish disturbance before the trawl mouth) at a constant velocity of 1.5 m/s. Visual observations of the action and shape of the trawl in this experiment matched those made by SCUBA divers during a regular, boat-pulled trawl collection. Seine and trawl collections were immediately placed in buffered formalin and analyzed later for differences in standard length, weight, and tumor incidence.

Most monthly collections were placed immediately in buffered formalin and processed later. To test the possibility of tumor loss, however, we collected normal and tumorous young-of-the-year and age I fish at various times during the year and reared them in the laboratory for periods of up to

4 mo. They were fed tubificid worms daily. Normal and tumorous fish were not separated. During these periods, fish and tumor development were monitored.

Both preserved and fresh fish were processed 24 h after collection. Because formalin preservation alters fish length and weight through time, the same length of time was left between collection and examination for all samples. Some fresh samples were measured and weighed before and after 24-h of formalin preservation to determine the extent of any shrinkage. Adjustment factors for length (0.985) and weight (1.12) were applied to fresh-specimen data before any comparison between fresh and preserved fish.

Fish were processed by recording standard length, weight, age via otoliths, sex (of all but young-of-the-year), presence or absence of skin tumors, and data on the skin tumors — number, stage of development, location, diameter, height, and weight. The validity of age determination by otolith annuli was confirmed for age 0–I fish by length–frequency analysis. The "birth date" was arbitrarily set at May 1, because this is when the new recruits were first collected. Samples of skin tumors were confirmed as being those described by McArn and Wellings (1971) by staining tumor thin sections with hematoxylin and eosin; the X cells unique to this type of papilloma (Brooks et al. 1969) were observed in all sections.

Stress tolerance experiments were performed on both field-collected and laboratory-maintained fish of ages 0 and I. Field tests were designed to compare tolerance of normal and tumorous fish to transportation stress. Throughout the year, fish were transported to the laboratory under varying degrees of temperature and/or density-induced stress. Several experiments involved paired normal and tumorous fish of comparable size in each transport container. On arrival at the laboratory, I noted the number of dead individuals and their status as normal or tumorous. The proportion of tumorous individuals in the initial collection was then compared with the proportion present among the survivors.

Laboratory stress experiments were performed throughout the year by placing pairs of normal and tumorous individuals of comparable size in aquaria and subjecting them to heat (initial = natural temperature; final = up to 28°C) and/or salinity (initial = 3‰; final = up to 35‰) shifts. These experiments were continued from 1 to 5 d, with the stress level increased each day, until mortality equalled or surpassed 50%. The order of individual mortality (with respect to presence or absence of tumors) was recorded.

Results

Over 3600 starry flounders, most aged 0–II years, were sampled from Bellingham Bay during this study (Table 1). The general pattern of tumor epizootiology as described by Wellings et al. (1976) was observed, although specific details differed. The differences were probably due to a previous lack of emphasis on the shallow-water (and generally smallest) individuals.

From 1979 to 1981, angioepithelial nodules (AEN) first appeared on young-of-the-year flounders in July, although postmetamorphic juveniles were collected as early as May. First appearances of AEN continued until September, after

TABLE 1. Collections of age 0–I starry flounder made between 1979 and 1981 by seine and trawl. Fish older than age I are not listed.^a

Date of collection	Seine				Trawl			
	No. of age 0	Proportion of tumorous fish	No. of age I	Proportion of tumorous fish	No. of age 0	Proportion of tumorous fish	No. of age I	Proportion of tumorous fish
June, 1979	35	X	23	0.26	—	—	—	—
July	56	0.41	7	0.14	36	0.11	12	0.08
Aug.	63	0.27	3	0	7	0.43	21	0.05
Sept.	38	0.42	—	—	42	0.26	107	0.07
Oct.	5	0.20	7	0.14	27	0.22	66	0
Nov.	—	—	—	—	104	0.35	23	0
Dec.	42	0.40	—	—	21	0.19	20	0
Jan., 1980	15	0.47	6	0	—	—	—	—
Feb.	38	0.32	20	0.15	13	0.23	—	—
Mar.	40	0.23	2	0	9	0.11	7	0.14
Apr.	35	0.34	9	0	34	0.12	87	0
May ^b	100	X	178	0.30	9	X	52	0.16
June	413	X	38	0.26	—	—	42	0.07
July	458	0.07	—	—	—	—	63	0.03
Aug.	241	0.40	—	—	—	—	—	—
Nov.	181	0.42	—	—	—	—	—	—
March, 1981	64	0.42	—	—	—	—	—	—
May ^b	—	—	30	0.40	—	—	—	—
June	31	X	—	—	—	—	—	—
Sept.	251	0.26	—	—	—	—	—	—

^aX = tumors have not yet appeared, — = data not available.

^bTime of "birthday."

which time, no new AEN were noted. This stage of the tumor was relatively short-lived. By September most fish had replaced the AEN by a distinctive transitional stage characterized by a low, plaque-like appearance and rapidly spreading edges. Although its cytological makeup was similar to that of an epidermal papilloma (EP), the low spreading tumor was sufficiently long-lived and morphologically distinct to be classified separately as a low epidermal papilloma (LEP). LEP were observed on young-of-the-year flounders between August and April, although they were most prevalent during the fall and early winter. EP were observed as early as September, although most developed from LEP during the winter. Once developed, they grew both laterally and vertically, with no apparent size threshold. EP and angioepithelial polyps (AEP) are morphologically similar and I considered them as synonymous. The progression of AEN to LEP to EP was confirmed in laboratory-kept fish.

Given that new tumors stop appearing in young-of-the-year fish in September, and assuming that regression does not occur and that emigration is minimal, the proportion of tumorous fish within a population should remain constant with time. This constancy was not observed. Tumor incidence decreased radically with increasing age of the flounder, and changed significantly with the time of year and sampling gear used. Year-to-year variations in this pattern were minimal. Inshore young-of-the-year fish had a mean tumor incidence of 37.4% ($n = 1119$) whereas only 12.7% ($n = 741$) of age I possessed skin tumors. Six out of 343 (= 1.7%) of age II fish were tumorous, and no older afflicted individual was collected.

Diseased flounders possessed up to nine tumors each. Tumor incidence was neither sex-related (χ^2 test, $P > 0.1$) nor

related to the type of eye rotation (χ^2 test, $P > 0.1$) (starry flounders are unusual among flatfish in that the ratio of left-handed to right-handed individuals may vary by region). Although the spatial distribution of the skin tumors was random, there was a slight tendency for the growths to occur on the pigmented side of the fish during the 1st year of life (χ^2 test, $P < 0.05$). This tendency was not significant for age I fish ($n = 75$) or for the young-of-the-year during the first 3 mo of tumor appearance ($n = 305$).

Because the young-of-the-year had the highest tumor incidence, the proportion of affected fish was monitored both inshore (by seine) and offshore (by trawl). Concurrent, widely spaced seine collections indicated that no intersite differences in size or tumor incidence of the young-of-the-year existed. Intersite differences in abundance were common; therefore, all fish collected on a sampling date were pooled. A similar pattern was observed in trawl collections; relative abundance varied between collection stations, but at a given depth all monitored fish characters were similar. Trawl samples on a given day were therefore pooled.

TUMOR INCIDENCE

Tumor incidence of the 1979 young-of-the-year inshore fish increased during the months of July–September as new tumors appeared. After this point it stabilized, remaining at roughly 37% until the following June (Table 1). By July 1980, despite continued sampling, the catch per unit of effort (CPUE) of the year-class decreased sharply. This rapid decline did not appear to be due to size selectivity of the seine, as greater quantities of larger fish were caught in the seine at

other times of the year. Moreover, the same pattern of tumor incidence and young-of-the-year abundance/decline was recorded both for the 1978 year-class in the summer of 1979 and for the 1st year of the 1980 year-class. Therefore, the decline in CPUE was interpreted as representing a decline in abundance.

Offshore monitoring of the 1979 year-class revealed a different seasonality in tumor incidence. The proportion of affected fish increased during the tumor formative months, although not as rapidly as in the seine catches. By October, the percentage of tumorous fish had leveled out at about 27%, remaining there until April, at which time the percentage quickly decreased to near zero by July (Table 1). Qualitative measures of CPUE remained high during the spring and summer. The 1978 year-class, which was monitored between the summers of 1979–80, showed similar patterns in the timing and magnitude of the decrease in tumor incidence. The age I fish from the 1978 year-class never rose above a 0–2% proportion of tumorous fish.

A comparison of tumor incidence between two different gear types is only valid if there is no significant difference in gear selection. The gear selection experiment demonstrated that both gear types captured tumorous young-of-the-year flounders in a proportion not significantly different from one another (one-tailed Fisher exact test, $P > 0.05$). In addition, the seine and trawl sampled fish of the same standard length (1-way ANOVA, $P > 0.1$), although trawl-caught fish were significantly heavier (1-way ANOVA, $P < 0.05$). This difference in weight could affect the comparison of gear samples if normal and tumorous fish had different weights at this time of year. However, diseased and normal fish were not significantly different with respect to either length or weight at the time of this experiment (1-way ANOVA, $P > 0.1$). Trawl and seine young-of-the-year catches were therefore deemed comparable from November onwards (because continued fish growth would reduce the loss of the youngest fish through the trawl mesh), and non-comparable before November as a result of selection of larger fish by the trawl. Capture of large numbers of age II fish with the seine suggested that the seine was a representative sampler from the youngest stages to at least age I+.

Because the size selectivity of the two gear types was minimal from November onwards, a comparison of the age 0 fish caught inshore (by seine) and offshore (by trawl) is justified. Such a comparison demonstrates that tumor incidence varies with depth (Table 1). However, inshore and offshore young-of-the-year flounders also differ in mean size (Fig. 1), suggesting that tumors are less common at depth and/or in larger fish of the same age. The cause/effect relationship between tumor incidence and depth of habitat cannot be deduced from Fig. 1.

TUMOR DEVELOPMENT

The conspicuous decline in the proportion of tumorous flounders in the wild suggests that affected individuals either lose their EP (through tissue sloughing or regression) or are selected against. Newly metamorphosed flounders, collected in May and reared in the laboratory, developed the same tumor incidence and grew at about the same rate as their

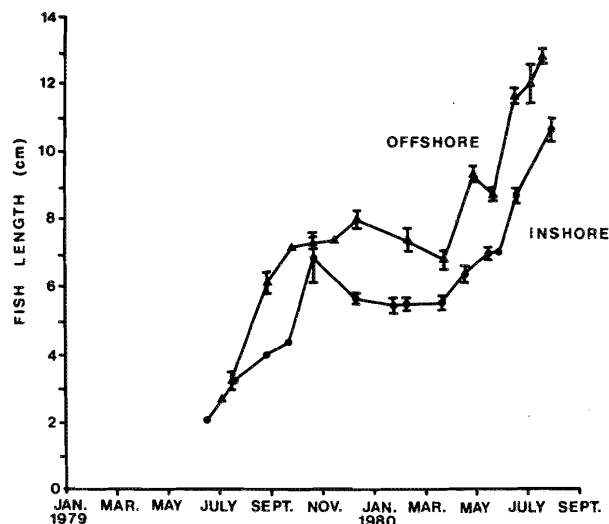


FIG. 1. Growth of the 1979 year-class of starry flounder both inshore (●) and offshore (▲). Size selection of the trawl may be present prior to November. Vertical bars represent the mean \pm 1 SE (except where omitted when SE < diameter of data point).

counterparts in situ ($n = 164$). No new tumors appeared after September. In addition, tumor development (stage and growth) paralleled that of the wild fish.

Once tumors had appeared, tumor growth was monitored. In virtually all cases, tumors increased substantially in surface area (and volume, once the flat LEP stage was passed). No instances of tumor regression were recorded ($n = 63$).

Because tumors in wild fish could regress, and considering I could not distinguish between a shrinking tumor leaving healing epidermis behind it and a growing EP with a flat spreading edge, I looked for other evidence of tumor growth or regression. Values for mean tumor diameter divided by mean fish length for each year-class within a sample were plotted against time. The division by fish length was included to allow for isometric growth of the tumor with the fish. Seine and trawl results were similar, so samples taken within 2 wk of each other were pooled. Tumor diameter increased relatively constantly throughout the year, suggesting that regression did not occur (Fig. 2). The previously discussed decline in the proportion of tumorous fish in late spring was not reflected by a change in tumor diameter.

SELECTION AGAINST TUMOROUS FLOUNDERS

In addition to the decline in the proportion of tumorous fish in the face of continuous tumor growth, additional evidence suggests that tumorous individuals are selected against relative to normal individuals of the same age. Prior to the end of their 1st year of life, young-of-the-year flounders show no significant differences in length or weight between normal and tumorous fish. Beginning in late May in the trawl collections, normal 1-yr-old fish are significantly longer and heavier than their tumorous counterparts (1-way ANOVA, $P < 0.05$). This trend continues and becomes more significant as the fish get older.

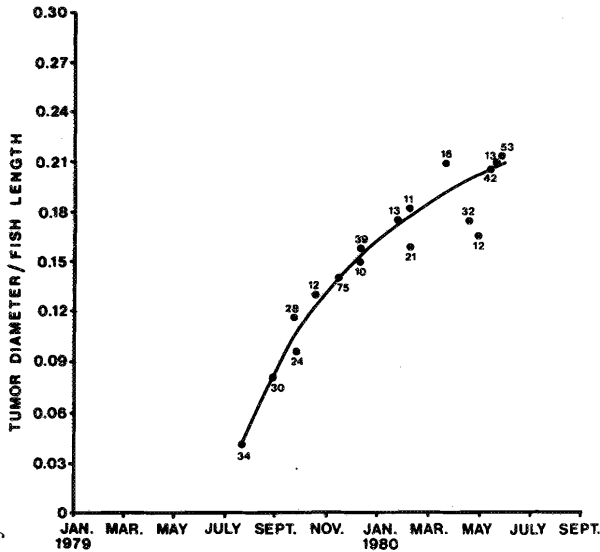


FIG. 2. Progression through time of mean tumor diameter relative to mean fish length. Numbers represent sample sizes.

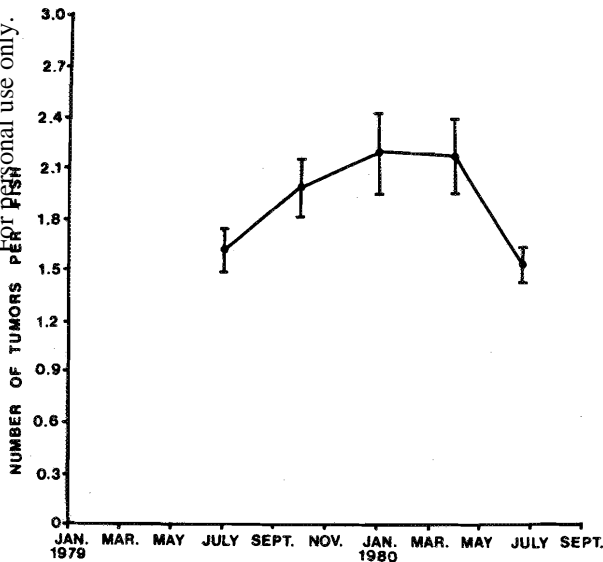


FIG. 3. Change in mean number of tumors per tumorous fish through time for the 1979 year-class of starry flounders. Vertical bars represent the mean \pm 1 SE.

If tumorous fish were being selected against at the end of their 1st year of life, one might expect that those individuals with the most tumors would be the first to die. A plot of mean number of tumors per fish against the date indicates that tumor number per fish remains relatively constant until late May, at which time a sudden decline occurs (Fig. 3). The rise in the curve between July–November is most likely due to the appearance of new tumors. Seine and trawl samples were pooled for this graph, because of the similarity of their re-

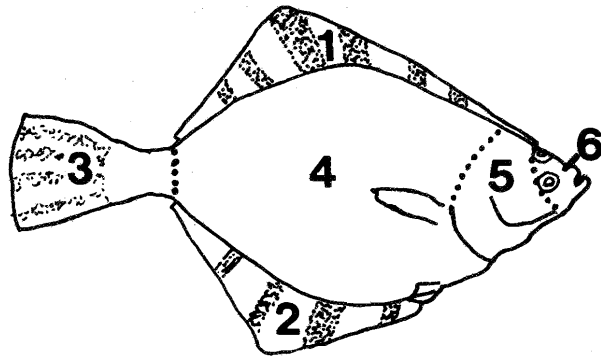


FIG. 4. Sectioned starry flounder for the purpose of recording tumor location.

spective curves.

A test comparing the mean number of tumors per fish before and after May (the period of decline) indicates that the difference is significant (1-way ANOVA, $P < 0.05$). However, during spring and summer, tumors grow rapidly, and in some cases merge with an adjoining tumor. This process of fusion would result in a lower value for the tumor count on an individual. Therefore, using empirical values for mean tumor area and the total surface area of a fish in June, I developed a simple mathematical model to determine the probability of one or more tumors merging on an individual possessing one to seven tumors. Using the observed frequency distribution of tumors on affected fish, I calculated the expected mean number of tumors per fish, allowing for those tumors that do merge with others. The result is significantly higher than the observed value (1-way ANOVA, $P < 0.05$), indicating that tumor fusions alone cannot account for the reduced number of tumors per young-of-the-year fish in late spring and summer.

It appears, therefore, that fish with the most tumors are selected against the most heavily. However, it may be that it is not tumor number per se that selects against its owner, but the location of the tumors. To test this possibility, tumor location was recorded and allocated to one of six sections on a standardized starry flounder (Fig. 4). The location of the sections was determined on the basis of biological utility to the fish. Tumors located on a fin may interfere with swimming ability, on opercula with respiration, on eyes with sight, etc. I hypothesized that newly formed tumors are randomly scattered over each side of the flounder; therefore, I determined the surface area of each section and calculated the expected frequency distribution of tumors in each section. Observed frequencies from 3-mo periods, starting in July and ending 1 yr later, were compared to the expected frequencies for young-of-the-year fish. The observed frequency distribution for all age I fish was also determined. Comparisons (by χ^2 goodness of fit tests) were then made between the various age-groups to see if certain sections became underrepresented through time, indicating selection against fish with tumors in those sections. The results were negative, with all sections fitting the expected frequency distribution model at all ages ($n = 796$).

TABLE 2. Results of transport stress experiments on age 0–1 starry flounder in which total mortality exceeded 5%. (**Significant at 95% confidence level.)

Experiment date	Sample size	Proportion of tumorous fish in collection	Proportion of tumorous fish among survivors	Significance
Aug. 23, 1979	63	0.27	0.28	n.s.
Sept. 21	38	0.42	0.45	n.s.
Oct. 20	27	0.29	0.20	n.s.
Feb. 8, 1981	38	0.32	0.15	n.s.
March 21	15	0.40	0.33	n.s.
April 18	35	0.34	0.23	n.s.
May 26	23	0.39	0.33	n.s.
May 29	73	0.33	0.13	**
June 17	39	0.26	0.00	**

STRESS EXPERIMENTS

Mortality records of laboratory-reared flounders indicated that the survival of tumorous individuals was similar to that of normal fish between September and April ($n = 281$). Paired stress experiments were not suggestive of increased mortality of tumorous fish ($n = 36$). Transport stress experiments performed during this period reflected the same pattern (Table 2).

Beginning in late May, significantly fewer of the tumorous fish were able to survive the stress of transport compared to normal fish (Table 2). Similarly, in a sample of age I flounders maintained in the laboratory in late May for a period of a month, the mortality rate of tumorous fish was significantly higher than that of normal fish (χ^2 test, $P < 0.05$). Virtually all of the tumorous fish that died in the May and June stress experiments possessed one or more EP or a transitional stage between LEP and EP. Transport mortalities did not differ significantly in length from that of the survivors (t -test, $P > 0.1$).

Discussion

Tumor incidence in Bellingham Bay decreased dramatically from 37% in age 0 fish to virtually zero in age II individuals. Because no new tumors appeared after the first 6–9 mo of life, some process must have occurred to reduce the apparent proportion of tumorous individuals in the population. Otherwise, the proportion should have remained constant throughout the life of the cohort.

Tumor regression was not demonstrated, or even suggested, in laboratory-maintained fish. Most tumors grew rapidly in size at all times of the year, and in no instances did they regress. Despite the artificial environment, this evidence is rendered more credible by the close parallel in tumor development and fish growth between laboratory and wild fish.

Evidence for continuous tumor development in wild flounders was less direct but still persuasive. Mean tumor size in both seine and trawl collections increased steadily with age. Tumor growth occurred even relative to fish growth, and at all times of the year. Continual tumor growth would not be expected if tumor regression were occurring, especially because such a large proportion of fish would have to lose their tumors between late age 0 and early age I (the period of

major decline in tumor incidence). Tumor regression has not been observed in other studies where fish were maintained in flow-through, saltwater aquaria (Wellings et al. 1964; Miller 1969), although its existence has been implicated (Nigrelli et al. 1965; Cooper and Keller 1969).

The potential for behavioral differences between normal and tumorous individuals suggests that their respective distributions could differ. This possibility was tested by sampling both inshore and offshore throughout the year. Stich et al. (1976) have observed that normal English sole, *Parophrys vetulus*, will move offshore during the winter before their tumorous counterparts, biasing any inshore incidence sample. Such a pattern was not observed in Bellingham Bay starry flounder, where the proportion of normal and tumorous individuals inshore remained relatively constant throughout the year. CPUE decreased for all age-classes of flounder during the winter months, both inshore and offshore; however, the emigration reversed itself by April, returning the same proportion of tumorous fish to the bay. Emigration of fish with EP at any other time of the year was unlikely, because Bellingham Bay is semienclosed with the opening well away from the estuary where the flounders lived. No age 0 or I flounders were ever captured near the bay mouth.

From at least November onwards, young-of-the-year flounders caught offshore were significantly larger than their conspecifics inshore. Such size-depth aggregations are common among many species of flounders, where the largest individuals move offshore into deeper water (Gibson 1973). In Bellingham Bay, the offshore samples held a consistently lower percentage of tumorous young-of-the-year than did the inshore samples. If a variable growth rate between normal and tumorous fish were responsible for this distribution, one would expect that the inshore tumor incidence would increase with time, as the faster growing normal fish moved into deeper water. This did not occur; the observed depth-related variation in tumor incidence is so far unexplained.

There was no evidence supporting tumor regression or segregation through time of tumorous and normal flounders. However, my study strongly suggests that tumorous fish near the end of their 1st year of life undergo a high mortality rate relative to the normal individuals of their cohort. Offshore tumor incidence declined rapidly in the late spring with no corresponding increase in the inshore samples. Inshore

incidence did not vary, but CPUE dropped sharply in June. If the inshore fish simply moved offshore, one would expect an increase in the tumor incidence there, not the decrease that actually occurred. Therefore, it appears that most tumorous fish died during this period. It is impossible to tell if the normal inshore fish died off as well, or if they simply emigrated offshore.

Several other measures demonstrated a reduced fitness of tumorous fish. Although normal and tumorous fish had similar lengths and weights throughout their 1st year, significant differences occurred by late May, and increased in magnitude from that point on, suggesting that the now-large EP were reducing the growth rate of the affected fish. Miller (1969) observed the same pattern in flathead sole (*Hippoglossoides elassodon*), with significant differences occurring only in age I fish.

The observed decline in the mean number of tumors per fish also occurred in late spring, after a year of relative constancy. Such a decrease would be expected if tumor possession incurred an increased mortality rate — fish with the most tumors should be at the most risk. A correction factor for tumor fusion through growth demonstrated that after May, tumor number per fish was still lower than expected, implying that those fish with the most tumors were being selected against first.

The strongest piece of evidence for differential mortality was obtained from the stress experiments. Susceptibility to stress did not differ between normal and tumorous fish from the time of first tumor appearance to the spring. Then, in late spring, at the same time that all of the other indicators pointed towards high mortality of tumorous fish, the latter became significantly more susceptible to various forms of stress. Mortality of stressed tumorous fish has been noted previously, although it was not quantified (McArn and Wellings 1971). The experiments performed here demonstrated that stressful situations occurring *in situ* could account for the mortality that is actually observed.

Mortality of tumorous individuals in late spring is consistent with other fish tumor studies. Stich et al. (1977) observed a sudden decline in tumor incidence among age I English sole near the end of spring. Similarly, late spring is a pivotal time for esocids with lymphosarcomas; affected individuals either die or their tumors regress (Sonstegard 1976).

The idea that tumorous flounders may suffer a relatively high mortality rate is not a new one (Wellings et al. 1964; McArn and Wellings 1971). If the tumors are truly neoplastic, they are generally considered to be biologically benign, and therefore nonlethal. However, it has been suggested that mortality of tumorous fish occurs by indirect means, such as interference with swimming, feeding, escape from predators, etc. (Wellings et al. 1964; Peters et al. 1978). Two results from my study do not support this hypothesis. The first involves the distribution of tumors over the body of the fish, irrespective of the fish's side. Soon after tumor appearance, the AEN are small enough that it is improbable that they could interfere with any of the fish's activities, no matter where the tumors are located. A random distribution of tumors would therefore be expected, and indeed was observed. By late spring, tumor size had increased enough that they might be considered a liability to the fish if positioned in certain crucial

areas. Yet the tumor distribution of all age 0 and I fishes remained random, with large EP observed not uncommonly on opercula, over eyes, and on jaws. These observations militate against the hypothesis of selection against tumor location. Secondly, tumorous age I flounders reared in the laboratory in late spring under apparently ideal conditions (abundance of food, oxygen, and natural temperature) died off over a period of a month, whereas the resident normal flounders survived and grew. Under such conditions of absence of predators and apparent lack of competition, it is difficult to conceive of any location-specific tumor liability.

Although stress experiments indicate a reduced fitness of age I tumorous flounders, they do not show the means by which stress mortality may occur in the wild. Experimental temperature/salinity shifts and crowding were found to induce mortality, as they may do naturally, but were not actually observed to do so in nature. Certainly a large increase in water temperature is one of the characteristics of late spring, suggesting its involvement in tumorous fish mortality. To this end, the observation of tumorous age X rock sole (*Lepidopsetta bilineata*) in the cold waters of the Bering Sea (McCain et al. 1978) alludes to a temperature effect on survival, as very few age III tumors have ever been observed in warmer areas (Wellings et al. 1976; Stich et al. 1977).

Flounder skin tumors may cause general stress, perhaps through nutrient requirements of the tissues involved. Although speculative, this idea is supported by the slower growth of tumorous fish that survive the spring die-off. Continuous stress can weaken an organism, rendering it more susceptible to death (Selye 1976). Increased temperature could be such a source of stress, especially as higher temperatures hasten growth, and tumor growth rate exceeds that of the fish. Virtually any proposed etiology of the skin tumor is consistent with this hypothesis.

This study provides evidence that tumorous starry flounders in Bellingham Bay undergo almost total mortality near the end of their 1st year. It is improbable that this process is unique to this species or location. All of the flounder species studied to date (except for rock sole in the Bering Sea described earlier) display a similar pattern of tumor incidence, which decreases to near zero after 1–2 yr (Wellings et al. 1976). It is likely that this is due to mortality of the affected individuals. Nigrelli et al. (1965) suggested that tumor regression occurred in sand sole, *Psettichthys melanostictus*, although their conclusion was based only on a presumed similarity between skin tumors and other forms of skin disease. It is now known that flounder tumors are histologically quite different (Peters et al. 1978).

In a study done near San Francisco, Cooper and Keller (1969) concluded that the yearly decline in tumor incidence was also due to tumor regression. They produced no evidence that regression actually occurred, but did not find a significant difference in size between normal and tumorous English sole. The study presented here and that of Miller (1969) demonstrated that no such difference occurs until age I is reached. Ages were not determined in the California study (Cooper and Keller 1969), but most fish sampled were most likely age 0.

Flounders produce large numbers of offspring, most of which die in their 1st year. By age I, the survival rate has increased to at least 5% (Campana unpublished data). There-

fore, the deaths of a large number of tumorous age I fish, 37% in Bellingham Bay, in addition to the "normal" mortality sources, must be of some import to the affected population, especially as sexual maturity is not reached until ages II-III. The effect may only be temporary if other ecological processes compensate for the decrease in population density. Nevertheless, the flounder skin tumor system must be considered to be one of the largest-scale, repetitive sources of disease mortality known for fishes.

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