Development of Sediment Bioassays using Newly Settled California Halibut (*Paralichthys californicus*)

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The California halibut (*Paralichthys californicus*) is an important flatfish to recreational and commercial fisheries in Southern California (CDFG 1989). Adult halibut inhabit sandy bottoms along the coast and spawn from February to September (Plummer *et al*. 1983). Larvae spend approximately one month in the plankton before settling and migrating to semiprotected bays, harbors, and estuaries (Allen and Herbinson 1990). Newly settled halibut (Figure 1) live directly on the sediment and have high surface-to-volume ratios. They are, therefore, likely to suffer toxic effects from contact with contaminated sediments. Since the nursery areas are being impacted by dredging and urban runoff, it is important to determine if juvenile halibut are being affected by sediment contamination. The objective of this study was to develop a long term (28 d) flow-through sediment toxicity test for newly settled California halibut. This test will measure and evaluate the effects of sediments on halibut survival and development.

MATERIALS AND METHODS

Prior to developing the toxicity test, preliminary experiments were conducted to determine a suitable sediment and test container, and to examine the effect of sediment renewal on growth and survival. The toxicity test was then used to determine the effect of sediment collected from five sites in Southern California on growth and survival of juvenile halibut. These sites included a reference station in Mission Bay, three industrialized harbors (Los Angeles Harbor East Turning Basin, Outer Los Angeles Harbor, and the San Diego shipyard) and an area near a large municipal wastewater outfall (Palos Verdes shelf) (Figures 2a and 2b). Sediment was collected with a $0.1 m^2$ Van Veen grab. Only the upper 2-cm layer of each grab sample was removed for toxicity testing and grain-size analysis. This layer was then thoroughly homogenized before being separated into subsamples. The toxicity test sediments were stored in 1-L polyethylene jars at 0 to 5º C for three days before being used in the test. The remaining subsamples were placed in 4-oz plastic cups and stored at 0 to 5º C until analyzed for grain size by the methods of Plumb (1981).

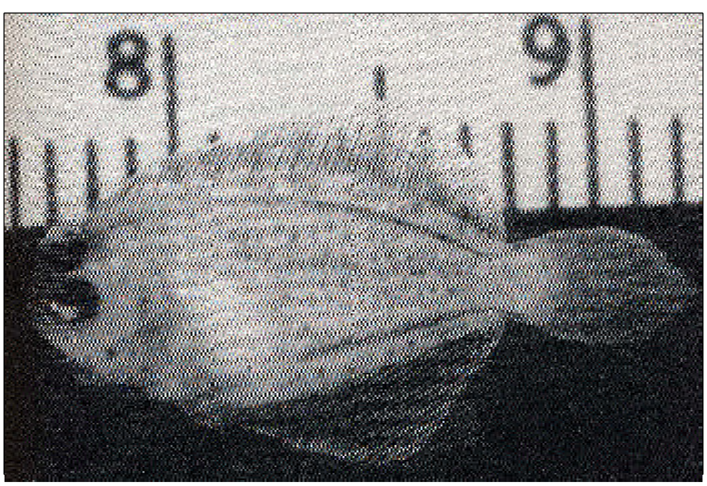

FIGURE 1. Newly settled California halibut (*Paralichthys californicus*), 12.5 mm standard length.

California halibut were provided by the Los Angeles County Natural History Museum Halibut Hatchery Project, Redondo Beach, California. Four hundred larval halibut were siphoned into separate plastic bags (100 fish/bag) filled with seawater. The bags were then placed into ice chests to keep the fish at 15 ± 1º C. Upon arrival at the laboratory the fish were transferred into 33 L holding tanks with seawater. Seventy-five larval California halibut were placed in each tank. Halibut cultures were maintained at 15 ± 1º C with mild aeration and fed newly hatched brine shrimp, *Artemia* sp., nauplii seven days a week until they had completely settled (approximately three weeks). Settled fish are defined as having fully migrated eyes, shortened dorsal rays, and lying on the substrate except when swimming up to feed (Gadomski *et al*. 1990).

An artificial sediment was created to simulate a field-collected sediment from Alamitos Bay, Long Beach, California, (a representative juvenile halibut site) both chemically and physically. Grain size analysis for Alamitos Bay determined a coastal sand type sediment composed of fine- and medium-grained sand (> 0.125 mm diameter) (Table 1). Two different artificial sediments, one representing 93% sand and the other 50% silt/clay (< 0.063 mm grain diameter), were then made following the formulated sediment procedures of the United States

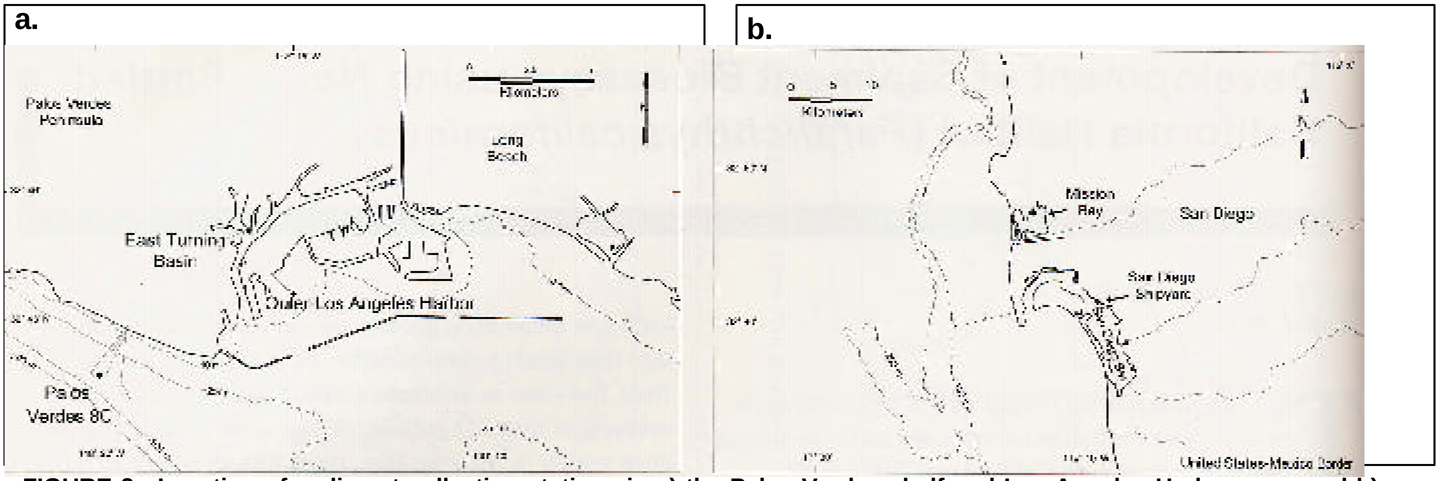

FIGURE 2. Location of sediment collection stations in a) the Palos Verdes shelf and Los Angeles Harbor area, and b) Mission Bay and San Diego Bay, California.

Environmental Protection Agency (USEPA 1994). Silica sand was washed and placed in a drying oven overnight at 60º C. The sand was then sieved to represent coarse (0.5-2.0 mm), medium (0.25-0.5 mm) and fine (0.05-0.25 mm) sand particles. ASP 400®, an aluminum silicate, was used to represent the silt fraction. ASP 600, ASP 900 (also aluminum silicates), and montmorillonite clay were used to represent the clay fraction. Since the silts and clays averaged a pH of 3.5, $CaCO_3$ was added as a pH buffer. The $CaCO_3$ was sieved to <0.05 mm. The silt, clays, and $CaCO_3$ constituents were ashed at 550º C for 1 h in a muffle furnace to remove organic matter. Peat moss was then used for the organic carbon source. The peat moss was rinsed and then soaked in deionized water for 5 d with daily water renewal. Moist peat moss was then sieved to provide an average particle size of 0.84 mm. All constituents were then mixed dry in 5 L plastic tubs before adding filtered seawater. After preparation, a conditioning period of at least 7 d was required for pH stabilization. Conditioning involved static renewal of the overlying seawater.

All experiments were 28 d exposures conducted with a 12:12 h light:12 h dark photoperiod in temperature (15 ± 1º C) controlled water baths. A 28 d exposure period was chosen to facilitate comparisons to other long term sediment bioassays conducted at the Southern California Coastal Water Research Project (i.e. *Lytechinus*, *Amphiodia* and *Grandidierella*). Only robust fish, defined as settled, well-pigmented, and with full guts, were used in the experiments. Artificial and field-collected sediments were placed in either small 1.8-L polyethylene plastic tubs (ST) or tall 4-L glass jars (TJ). One day prior to the start of the experiments the sediments were added to the test containers and the seawater flow was initiated. Each tub or jar contained a 2 cm layer of sediment and 1 L of overlying filtered seawater (3 L for the glass jars). Each replicate received mild aeration and a seawater flow rate of 4 mL/min. Experiments were initiated by adding 10 (five in the initial test) California halibut which had been digitally imaged to each of five replicate containers per treatment. Since California halibut are visual feeders (Haaker 1975), brine shrimp nauplii, *Artemia* sp. were added to the test containers early in the day to maximize feeding potential. Each day, old brine shrimp were removed from the test containers using a 60 µm net and 15 mL of new *Artemia* (20 *Artemia*/mL) were added using a 25 mL

TABLE 1. Sediment grain size and organic content of sediments used in bioassays with newly settled California halibut (*Paralichthys californicus*).

Station	Station Code	% Gravel	% Sand	% Silt	% Clay	% TOC
Alamitos Bay	AB	-	93.0	3.8	3.0	0.50
Agua Hedionda	AH	-	33.2	62.4	4.3	NA
93% sand	93% AS	-	92.7	3.9	3.2	0.50
50% silt/clay	50% AS	-	50.0	28.0	22.0	0.50
Play Sand	PS	0.1	99.5	0.2	0.2	NA
Los Angeles Harbor						
East Turning Basin	LAHETB	4.5	22.8	51.8	21.0	0.59
Mission Bay (Reference)	MB	-	13.8	46.1	40.1	1.63
Outer Los Angeles Harbor	OLAH	-	48.3	36.8	14.9	0.69
Palos Verdes 8C	PV 8C	-	38.9	49.4	11.6	3.10
San Diego Shipyard	SDS		29.1	35.9	35.0	2.83

AS=artificial sediment.
NA=not analyzed.
TOC = total organic carbon.

pipette. Water quality measurements (dissolved oxygen, pH, salinity and ammonia) were taken three times a week. Flow rates and mortality were checked daily. Dead fish were preserved for histological analyses in 70% ethanol and then fixed using Davidson's fixative. California halibut still alive at the end of the experiment were again digitally imaged, preserved, and fixed for histological analyses.

Standard length measurements were made at the beginning and end of each experiment on the digital images using Optimus™ software. Halibut growth was measured by subtracting the mean standard length (SL) of all the fish in each replicate at the beginning of the experiment from the mean SL of all the surviving fish in each replicate at the end of the experiment. The test end points were mortality, defined as no visible signs of fish movement after gentle prodding, and growth, defined as the increase in SL during the bioassay.

The final sediment bioassay on field-collected sediments varied slightly from the preliminary experiments. The sediment was not changed after two weeks for this experiment and, due to a shortage of robust California halibut only nine fish were added to each test container. In addition, each field-collected sediment sample was press sieved through a 1.0 mm mesh screen to remove potential infaunal predators before being added to the test containers. Since the preliminary experiments indicated there was no significant difference in container type, plastic tubs were selected for use in this experiment.

Sediments used in our exposures have not yet been analyzed for total organic carbon content (TOC), trace metals, and synthetic organics. Historical chemistry data from the collection sites can be found in the Bay Protection and Toxic Cleanup Program database (CDFG 1994) and from SCCWRP data on the Palos Verdes shelf (SCCWRP 1994).

The proportions of California halibut surviving in all experiments were evaluated using a One-Way Analysis of Variance (ANOVA) with the following modified arcsine square root transformation:

$$\theta = \arcsin \sqrt{\frac{Y + 3/8}{n + 3/4}}$$

Dunnett's test was used to locate differences between the treatment means. Effects of the sediments on California halibut growth were tested using One-Way ANOVA (Sokal and Rohlf 1995).

RESULTS

Preliminary Experiments

The initial experiment was conducted to determine a suitable reference sediment. California halibut were exposed to a field-collected sediment (Agua Hedionda), to commercial play sand, and to artificial sediments. Exposure to these different sediment types had variable effects on juvenile halibut survival. Percent survival of California halibut was highest (88%) for the artificial 93% sand sediment (Figure 3). On the formulated 50% silt/clay, the play sand, and the Agua Hedionda sediment types halibut survival varied from 60 - 76%. There was no significant difference among the sediment types (F = 1.47; P = 0.249). However, because survival was highest on the artificial 93% sand sediment, it was chosen as the reference sediment for the toxicity test. Since the power for the initial experiment was so low (1-β = 0.135), the sample size was increased to 10 fish for the next experiment (small vs. tall container and not changing vs. changing the sediment).

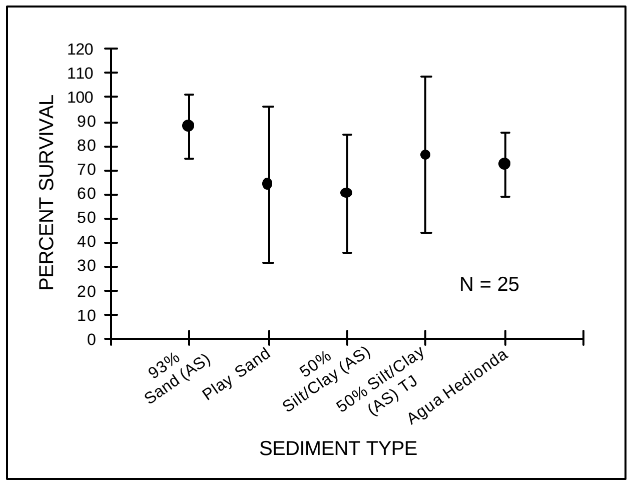

FIGURE 3. Percent survival of newly settled California halibut (*Paralichthys californicus*) on artificial and natural sediments. The error bars indicate the 95% confidence interval. AS = artificial sediment; TJ = tall jars.

The next experiment was conducted to determine a suitable test container and to test for effects of sediment renewal on California halibut survival using the artificial 93% sand as the substrate. After 14 d, sediment was changed on two of the treatments (one small tub treatment and one tall jar treatment). Juvenile halibut survival was fairly low for all treatments (Figure 4). Percent survival was similar between small tubs and tall jars (18-34%) without sediment renewal. Likewise, percent survival was also similar between small tubs and tall jars (38-50%) with sediment renewal. In addition, when the sediment was renewed, a similar increase in halibut survival occurred between the small tubs and the tall jars. Results indicated that there was no significant difference in survival among container types or sediment renewal status (F = 2.1; P = 0.119). However, in both cases where sediment was renewed, the results suggested a trend toward increased halibut survival.

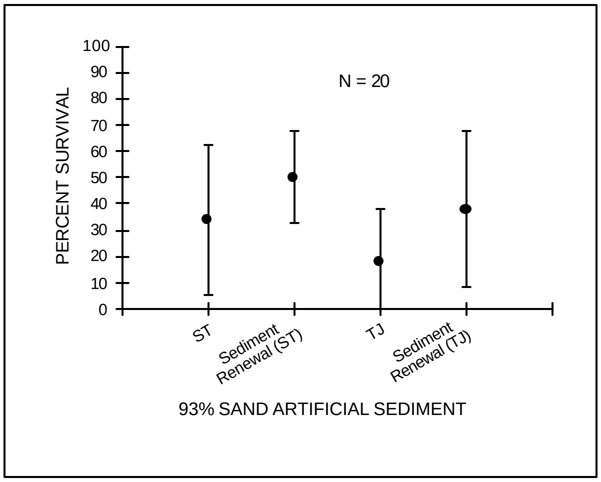

FIGURE 4. Percent survival of newly settled California halibut (*Paralichthys californicus*) in different test containers and on original and renewed sediment. The error bars indicate the 95% confidence interval. ST = small tubs; TJ = tall jars.

Test of Sediment Toxicity

The final sediment bioassay was conducted to evaluate the effects of contaminated sediments on California halibut growth and survival. Percent survival varied from 4 to 31% on the East Turning Basin, Palos Verdes 8C, San Diego Shipyard, and Outer Los Angeles Harbor sediments (Figure 5). Percent survival was higher on the artificial sand and Mission Bay reference sediments and ranged from 44 to 47%. Even though control survival was poor for this experiment an ANOVA showed that there was a statistically significant difference in halibut survival among the treatment groups (F = 11.2; P < 0.001). A Dunnett's test showed that the reference sediments (Mission Bay and the artificial sand) had significantly higher survival than the San Diego shipyard, Palos Verdes 8C, and the Los Angeles Harbor East Turning Basin sediments.

Mean halibut growth did not differ significantly (F = 0.423; P = 0.826) by sediment type. There was, however, a trend toward less growth for treatments that had the lowest survival (Table 2). Newly settled California halibut at the beginning of the experiment had a size range of 6 to 9 mm SL within each replicate. The difference between the means of all the fish at the start of the experiment from those fish surviving at the end indicates an average of only 4 mm of growth.

DISCUSSION

The initial experiments demonstrated that artificial sediment can provide suitable substrate for California halibut through 28 d of exposure. The results of our final exposure on the field-collected sediments indicate that juvenile California halibut are tolerant of a wide range of sediment particle sizes (<0.004 mm - 2.00 mm). The reference site, Mission Bay, which had the highest halibut survival, has a fairly small grain size with sediment composed of silt and clay (Table 1). Artificial 93% sand, which had the next highest survival had a larger grain size containing a mixture of coarse, medium, and fine sand grain sizes. The Outer Los Angeles Harbor, San Diego shipyard, and the Los Angeles Harbor East Turning Basin sediments were all composed of smaller grain size (<0.06 mm) silty clay. Palos Verdes 8C sediment was silt. Since the highest California halibut survival occurred on contrasting grain sizes of Mission Bay and artificial 93% sand grain size is not a major factor influencing survival. While our results showed that the halibut can survive on a wide range of sediment grain sizes, Drawbridge (1990) and MBC (1991, 1992) found that recently settled fish preferred clay/silt sediment over coastal sand.

The reason the survival results for the preliminary experiment (small vs. tall container and not changing vs. changing the sediment) and field-collected exposures were sharply lower than the initial experiment may have been due to an unhealthy halibut brood stock. During these experi-

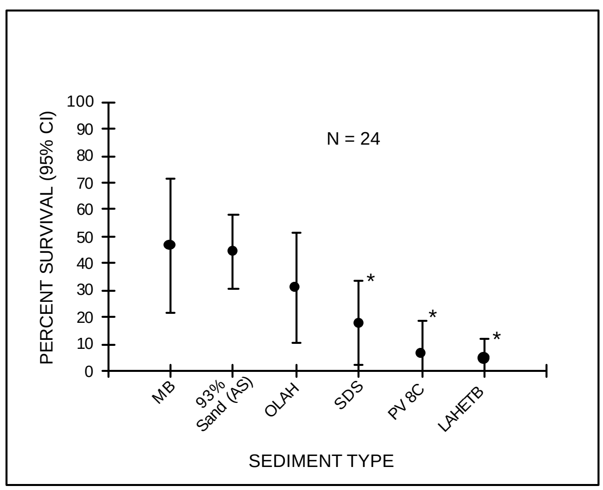

FIGURE 5. Percent survival of newly settled California halibut (*Paralichthys californicus*) on contaminated sediment. The error bars indicate the 95% confidence interval. See Table 1 for station abbreviations. * = Treatment groups that are significantly different from Mission Bay and the 93% sand artificial sediments.

TABLE 2. Mean and standard deviation of newly settled California halibut (*Paralichthys californicus*) growth on artificial, contaminated, and natural sediments from coastal, bay, and harbor areas off Los Angeles and San Diego, California.

	Mean	SD	N
MB	4.87	1.34	5
AS	4.40	0.51	5
OLAH	4.41	0.48	5
SDS	4.33	2.06	5
PV 8C	4.12	1.18	2
LAHETB	3.40	0.34	2

See Table 1 for meaning of station abbreviations.

mental periods the halibut hatchery had been experiencing an unusually high mortality rate (>80%) of larval fish possibly causing the low percent survival seen in these two experiments. Laboratory halibut mortality is normally less than 10% (Caddell *et al*. 1990). Alternatively, the limited diet fed to the halibut may also have effected their survival. In the future, a variety of prey such as copepods, amphipods, mysids, and cumaceans should be provided to the halibut to enhance their nutrition and improve their survival.

The PV 8C, Los Angeles Harbor East Turning Basin (LAHETB) and the San Diego shipyard (SDS) sediments have had elevated concentrations of metals and/or organics in previous studies (Anderson et al 1988). The LAHETB and SDS stations have previously been found to be toxic to the amphipod, *Grandidierella japonica* (Anderson et al 1988). However, until the sediments from our exposures are analyzed, no definitive source of the decreased survival of the juvenile halibut on these sediments can be determined.

Since mortality was so high in the final field-collected sediment experiment, it is possible that the data could be biased if more small or large fish were dying. This issue may be addressed by ranking each fish by size at the beginning and the end of the experiment. Those fish that died during the experiment and those corresponding to the same rank at the start as those that died would be removed from the analysis. Growth would then be measured on the remaining fish. The growth endpoint may be a more valuable tool to assess effects when survival is not affected.

While sediment tests using juvenile California halibut show some promise as a research tool, there is much further study that needs to be done. Testing using spiked sediments and reference toxicants should be done to discover the range of response. Feeding tests should be performed to optimize growth and survival. The most important factor for future tests is to insure the experiments are started using healthy animals. At this time, there is only one source of juvenile halibut and their availability is limited to late spring through summer.

CONCLUSIONS

Sediment testing using newly settled California halibut juveniles is technically feasible. The halibut are tolerant of a wide range of sediment particle sizes (<0.004 - 2.00 mm). Artificial sediment is a suitable substrate for maintaining California halibut to 28 d of exposure. We were able to detect a significant difference between sediments thought to be clean and those assumed to be contaminated. Much further study is needed to improve the methodology.

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