Mark retention and growth of jet-injected juvenile marine fish

John F. Thedinga*
Adam Moles
Jeffrey T. Fujioka
Auke Bay Laboratory, Alaska Fisheries Science Center
National Marine Fisheries Service, NOAA
11305 Glacier Highway, Juneau, Alaska 99801-8626
*E-mail address: John.Thedinga@noaa.gov

The type of mark or tag used for a particular study depends on the objectives of the study. Retention and recognition of a mark are critical to the success and reliability of a study. External tags have been the most common fish tags used (McFarlane et al., 1990), but they may affect survival, behavior, and growth of fish (Andersen and Bagge, 1963; McFarlane and Beamish, 1990). Each tag type has limitations and capabilities, but ideally external tags should be easily and rapidly applied to many fish, be inexpensive, easily identified, not easily lost or entangled, and not be stressful enough to alter fish survival, behavior, or growth. Studies that require such characteristics, therefore, are restricted in the type of tag that can be used and thus must rely on internal marks or dyes to identify fish. Identification of internal marks, however, generally requires that fish be killed, thus eliminating any possibility of repeated measurements of individual fish. Choice of mark is further restricted when marking juveniles that are of small size and that exhibit rapid growth.

Jet injection is a method of applying external marks to fish (Hart and Pitcher, 1969) that is relatively fast and can apply a variety of colors for either batch or individual marking. Jet injection does not affect survival or growth of juvenile salmonids in the laboratory (Cane, 1981; Herbinger et al., 1990; Thedinga and Johnson, 1995) but may contribute to increased mortality in field situations (Thedinga et al., 1994). Injection by Panjet has been used primarily on freshwater fish species (Hart and Pitcher, 1969) in addition to salmonids (Cane, 1981; Pauley and Troutt, 1988; Laufle et al., 1990). Juvenile flatfish have been marked with needle-injected latex (Riley, 1966) as well as by freeze branding (Dando and Ling, 1980), but not by jet injection. To our knowledge, juvenile sablefish have been marked only with Floy anchor tags (Rutecki and Meyers, 1992). Our objectives were to evaluate retention of jet-injected marks and their effect on growth of four marine fish species, as well as their effect on the tissue structure of three marine species held in the laboratory.

Methods

We tested the retention of jet-injected marks and effects of marks on growth of four species of marine fish and histological effects on three species of marine flatfish. We injected two substances into juvenile sablefish, *Anoplopoma fimbria*, and one substance into juvenile yellowfin sole, *Pleuronectes asper*, rock sole, *Pleuronectes bilineatus*, and Pacific halibut, *Hippoglossus stenolepis*. Sablefish were captured by hand-jigging in St. John Baptist Bay near Sitka, Alaska, September 1993 (Rutecki and Meyers, 1992). Sole were captured by beach seining in Auke Bay, Alaska, May to July 1994, and halibut were collected by trawling in Sitkinak Strait and Ugak Bay near Kodiak Island, Alaska, August 1994.

A total of 28 sablefish and 30 flatfish were injected with a Panjet in 1993–94. Sablefish were marked with alcian blue dye (65 mg/mL aqueous solution) and fluorescent orange acrylic paint (Liquitex, 50% aqueous solution); 10 of each flatfish species were marked with alcian blue dye. All sablefish were marked on the abdomen between the pelvic fins (Fig. 1). Flatfish, however, were marked with individual identifying marks on the ventral surface at one to four locations along the lateral margin and on the caudal peduncle (Fig. 2); 12 sablefish and 10 of each flatfish species were left unmarked as controls.

The Panjet was held about 25 mm from the fish's skin during marking. Sablefish were anesthetized with tricaine methanesulfonate (MS-222) before marking but flatfish were not. After marking, excess dye or paint was rinsed off with water to check mark quality. If a mark was good, the fish was put in a recovery tank; if poor, the fish was remarked.

Sablefish and flatfish were held in different environments. After being marked, sablefish were held in 600-L flow-through tanks for 238 days. Because of space restrictions, blue-marked and control fish were held in one tank, but each group was kept in separate compartments. Orange-marked fish were held in another tank but died prematurely in a laboratory accident

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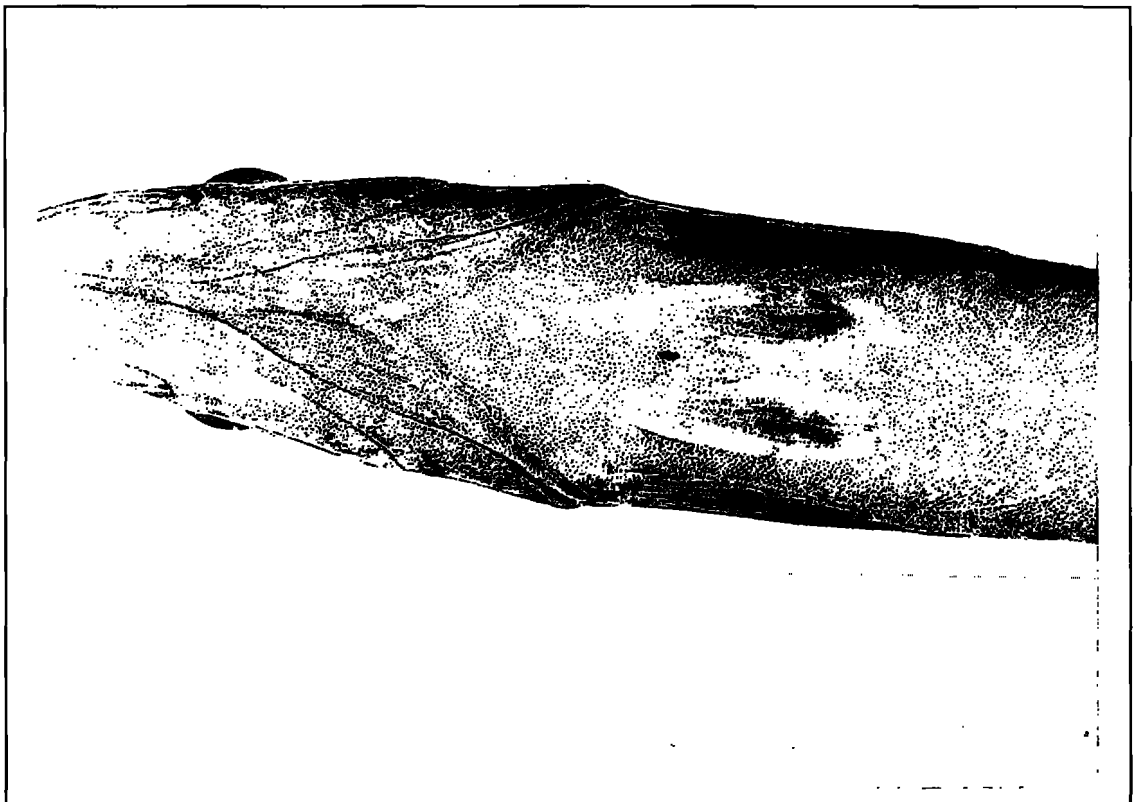

Figure 1
Panjet-injected alcian blue dye mark between the pelvic fins of a juvenile sablefish.

after 189 days and were frozen prior to analysis. Flatfish were held for 90 d in six 70-L flow-through tanks on their preferred bottom type (mud substrate for soles [Moles and Norcross, 1995], sand substrate for halibut). For each flatfish species, control and marked fish were held in separate tanks. Sablefish were held indoors and were provided about 8 h of fluorescent light daily, whereas flatfish were held outdoors under an awning with 12 h of fluorescent light daily. Sablefish were fed ad libitum, and flatfish were fed 10% of their initial body weight per day throughout the study. The substrates in the flatfish tanks were initially frozen three days to kill meiofauna and macrofauna. Mark recognition for sablefish was checked about every 3 weeks, flatfish about every 4 weeks. Blue marks were viewed under fluorescent light, orange marks under fluorescent and ultraviolet (UV) light. Mark retention was rated subjectively as acceptable (retained) or unacceptable (not retained) by the same person each time the fish were checked. Fish lengths were recorded at the beginning and end of the study: fork length (FL) for sablefish, total length (TL) for flatfish. Because we were obtaining additional data (histological) from flatfish, we also recorded flatfish weights when we recorded their lengths. Differences in acceptable mark retention were tested with a chi-square test, and differences in fish size and growth rate were tested with a t-test.

Absolute growth rate in length of flatfish was calculated as

$$L = TL_2 - (TL_1/90)(10),$$

where L = absolute growth in length;
TL_2 = total length at 90 days; and
TL_1 = total length at day 1 (beginning of the study).

Instantaneous growth rate in weight of flatfish was calculated as

$$W = (\log_e W_2 - \log_e W_1)/90,$$

where W = instantaneous rate of increase in weight;
W_2 = weight at 90 days; and
W_1 = weight at day 1 (beginning of the study).

All flatfish were examined for histological changes. Fish were examined for gross pathology at 50× with a dissecting microscope. Gill and liver tissues were

Figure 2
Panjet-injected alcian blue dye mark on the ventral lateral margin of a yellowfin sole.

excised and fixed in 10% buffered seawater. Tissues were then placed in 70% ethanol for two days, dehydrated in a graded ethanol series, cleared in xylene, embedded in paraffin, and sectioned at 6 μ. Sections were stained with hematoxylin and eosin and examined for lesions and evidence of wound healing.

Results

For sablefish, mark retention varied with mark color and method of detection. Retention of marks was significantly higher ($P<0.001$) for alcian blue dye (100%) than for fluorescent orange acrylic paint when orange marks were viewed under fluorescent light but was similar ($P=0.99$) when orange marks were viewed under UV light (Fig. 3). Retention of orange marks viewed under fluorescent light was 100% after 21 days but decreased to less than 20% after 84 days. Retention of orange marks viewed under UV light, however, decreased only to 92% after 84 days and remained at that level throughout the remainder of the study.

Mean length at the end of the study was similar ($P=0.24$) between blue-marked sablefish (280 mm) and controls (288 mm) (Table 1). Despite the potential for tank-linked effect due to holding the marked fish in a separate tank, mean length of the orange-marked sablefish (270 mm) was not significantly different from that of the controls (288 mm) ($P=0.06$) (Table 1). Mortality was zero.

Table 1
Mean fork length of jet-injected and control juvenile sablefish at time of injection and after 33 weeks. Fish were injected with alcian blue dye and fluorescent orange acrylic paint. Standard error is in parentheses.

	Mean fork length (mm)	
	Initial	After 33 weeks
Blue	212 (0.80)	280 (1.07)
Orange	212 (0.96)	270 (1.18)
Control	218 (1.16)	288 (1.48)

For all flatfish, mark retention was 100% throughout the study, and growth was similar between marked and control fish (Table 2). The instantaneous rate of increase in length and weight at the end of

the study was similar ($P \geq 0.10$) for marked and control fish, indicating that marking did not affect growth. Again, mortality was zero.

Histological examination of flatfish indicated that the marks were nonirritating and nontoxic. Necropsy of the flatfish revealed no evidence of damage to skin or musculature and no alteration in the structure of dyed tissue. All liver hepatocytes were normal, indicating no toxic exposure. There was no evidence of increased macrophage aggregations in the liver or hyperplasia in the gills to suggest cellular responses to dyes.

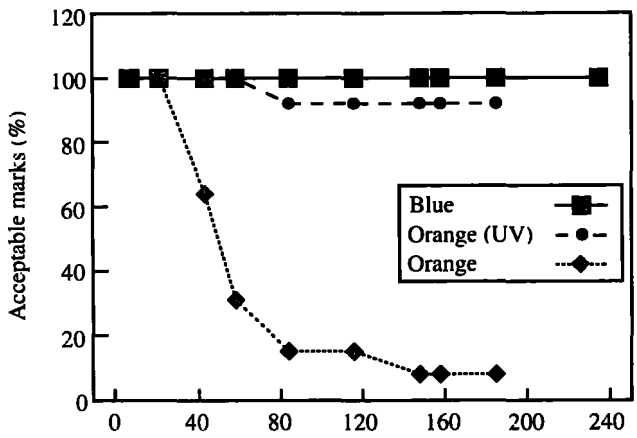

Figure 3

Percentage of acceptable jet-injected marks on juvenile sablefish by color: alcian blue dye and fluorescent orange acrylic paint. Dashed line is orange marks viewed under ultraviolet light (UV), and dotted line is orange marks viewed under fluorescent light.

Discussion

Marine fish can be jet injected rapidly with many individual marks, often without anesthesia. For example, in this study we marked several nonanesthetized flatfish per minute. Jet-injected alcian blue dye produced a highly visible intradermal mark that was retained for at least 8 months by juvenile sablefish, 3 months by juvenile flatfish. Detection of alcian blue dye marks on flatfish is easy and requires minimal handling. Usually marks can be detected without anesthesia and without turning fish over. Increased visibility of jet-injected marks, however, could make fish more conspicuous to predators. Fluorescent orange acrylic paint marks, however, faded rapidly, making marks less visible to predators but necessitating the use of UV light for detection.

Mark retention was similar to that reported for other species. Thedinga and Johnson (1995) reported 96% retention of alcian blue dye and fluorescent orange acrylic paint marks on the caudal fin of juvenile coho salmon, *Oncorhynchus kisutch*, and sockeye salmon, *O. nerka*, after nearly 4 months, and Herbinger et al. (1990) reported 96% retention of alcian blue dye-marked Atlantic salmon, *Salmo salar*, after 6 months. Few studies have been published that used marked juvenile sablefish or flatfish, and only one used a dye mark. Kelly (1967) injected Fast Blue 8GXM and hydrated chromium oxide by needle into the heads of juvenile winter flounder, *Pleuronectes americanus*, and had 100% retention after 4 months.

Jet-injected marks did not affect growth or mortality. Unlike Petersen disc and roll tags, which depressed growth rates (Andersen and

Table 2

Mean initial total length (mm) and weight (g) and mean absolute growth rate in length and instantaneous growth rate in weight of marked (jet-injected with alcian blue dye) and control juvenile yellowfin sole, rock sole, and halibut 90 d after marking. Standard error is in parentheses.

	Initial size		Growth rate after 90 d	
	Marked	Control	Marked	Control
Length				
Yellowfin sole	67.9 (1.04)	74.2 (1.45)	0.198 (0.067)	0.196 (0.054)
Rock sole	59.7 (0.92)	71.6 (1.56)	0.161 (0.068)	0.137 (0.073)
Halibut	72.3 (0.81)	71.8 (0.88)	0.228 (0.059)	0.241 (0.050)
Weight				
Yellowfin sole	3.5 (0.49)	5.2 (0.64)	0.823 (0.144)	0.841 (0.120)
Rock sole	2.3 (0.32)	5.2 (0.68)	0.610 (0.171)	0.817 (0.153)
Halibut	4.1 (0.36)	4.1 (0.36)	1.193 (0.109)	1.175 (0.111)

Bagge, 1963) and resulted in increased mortality in sablefish (McFarlane and Beamish, 1990), jet-injected marks did not affect flatfish and sablefish growth or survival. Marks, however, may not be retained as long under natural conditions where growth is faster: sablefish in their natural habitat average 31–33 cm FL in spring (McFarlane and Beamish, 1983) in contrast with 28 cm FL recorded at the end of our laboratory study in spring.

Jet-injected marks did not affect fish histology. There was no evidence of lesions in skin or musculature and no alterations in either the cells or the structure of dyed tissues. Changes in liver hepatocytes occur when fish have been exposed to toxicants (Hinton et al., 1992) but test hepatocytes in our preparations were normal.

Jet-injection of either alcian blue dye or fluorescent orange acrylic paint is a good method for mass marking or individually marking juvenile marine fish and meets most criteria for an effective external marker (Kelly, 1967). The marks are effectively retained and nontoxic and nonirritating; they do not affect mortality, can be used rapidly, and are inexpensive, readily visible, and permit numerous different mark combinations (Thedinga et al., 1994). Their application requires minimal training and equipment. Most importantly, jet injection does not alter the growth or tissue structure of fish. A limitation of jet-injection marking is the nonpermanent nature of the mark (Thedinga and Johnson, 1995). As a moderate-lasting marking method of juvenile marine fish, it is superior to most available external marking methods.

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