

## Factors affecting the post-release survival of cultured juvenile *Pseudopleuronectes americanus*

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Laboratory experiments were performed with cultured and wild juvenile winter flounder *Pseudopleuronectes americanus* to evaluate differences in behaviour and adaptation affecting post-release vulnerability to predation. Studies revealed that the cryptic abilities of cultured winter flounder increased over time. Sediment-naïve, cultured fish required a minimum of 2 days to improve their burying skills and at least 90 days for colour adaptation to match the sediment. Cultured winter flounder selected sediments consisting of small grains and colours matching their own pigment. Cultured winter flounder, regardless of their colour, were significantly more vulnerable to predation by birds. Additionally, cultured fish reacted differently than wild winter flounder when exposed to cues from a potential predator. © 2004 The Fisheries Society of the British Isles

Key words: crypsis; predation vulnerability; *Pseudopleuronectes americanus*; stock enhancement.

### INTRODUCTION

For any stock enhancement programme, the extent to which the released fishes contribute to the natural population is the ultimate measure of success. Therefore, a crucial first step towards this ultimate goal is maximizing stocking survival. In many stock enhancement programmes, however, initial survival rates of newly released cultured fishes are low (Svåsand & Kristiansen, 1990; Pitman & Gutreuter, 1993; Iglesias & Rodríguez-Ojea, 1994; Leber & Arce, 1996; Tsukamoto *et al.*, 1997; Tanaka *et al.*, 1998), and therefore, techniques to minimize this early mortality are critical.

Many factors can contribute to a high mortality at stocking. Rapid changes in temperature and salinity (Wallin & Van Den Avyle, 1995), increased stress due to excessive handling (Pitman & Gutreuter, 1993; Wallin & Van Den Avyle, 1995), stress induced impairment in avoiding predators (Olla *et al.*, 1992; Masuda & Ziemann, 2003) and intensified intraspecific competition due to artificially high densities (Fenderson & Carpenter, 1971; Kellison *et al.*, 2002) are known to lower survival of hatchery-reared fishes upon release. Behavioural anomalies in the cultured fishes may also decrease survival. Inability to switch from a formulated diet to live prey and the ability to effectively capture the

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prey can diminish chances of survival (Johnsen & Ugedal, 1989; Nordeide & Salvanes, 1991; Hossain *et al.*, 2002). It also has been shown that cultured fishes experience sensory deprivation, which can lead to the fish's inability to recognize, react to and avoid predators upon release. Deficiencies in predator avoidance behaviour have been shown for a number of species, including zebra danio *Brachydanio rerio* (Hamilton) (Dill, 1974), coho salmon *Oncorhynchus kisutch* (Walbaum) (Patten, 1977), minnows *Phoxinus phoxinus* (L.) (Magurran, 1990) and red drum *Sciaenops ocellatus* (L.), and these deficiencies can contribute to reduced survival.

In flounder (pleuronectid) enhancement programmes, low post-release survival has been attributed to a lack of cryptic abilities. Flatfishes have the ability to conceal themselves by changing both skin colour and pattern to match sediment, and by burying into the sediment. These abilities help obscure flatfishes from the view of visually hunting predators. Abnormal pigmentation in both Japanese flounder *Paralichthys olivaceus* (Temminck & Schlegel) (Tanaka *et al.*, 1998) and plaice *Pleuronectes platessa* L. (Anon, 1966), along with unnatural off-bottom behaviour in Japanese flounder (Tsukamoto *et al.*, 1997), have increased the visibility of the released, cultured flounders to predators. In order to maximize their cryptic abilities, studies have suggested that flatfishes reared in tanks devoid of any sediment require an acclimation period to 'learn' to bury effectively in sediment and to adjust their colour to match that of the background prior to release (Blaxter, 1970; Howell, 1994).

This difference in colouration between wild and cultured flounders is due to many factors, some of which are poorly understood. These factors include effects of rearing tank colour, hatchery lighting and pseudoalbinism caused by inadequate nutrition in the larval stage (Bolker & Hill, 2000).

In flounder enhancement programmes, lighter, cultured fishes released onto dark sediments inhabited by dark, wild fishes may be more conspicuous and suffer greater predation than the cryptically coloured, wild fishes. Increasing the cryptic abilities of cultured fishes may be an effective way to increase their survival and offset the typical high initial mortality rates upon release. Colour alone, however, may not be sufficient to explain the initial high mortality of cultured flatfishes. In both laboratory (Kellison *et al.*, 2000) and field studies (Furuta, 1996; Hossain *et al.*, 2002; Iglesias *et al.*, 2003), cultured flatfishes were more vulnerable than wild flatfishes to predation. This high predation is probably caused by the unnatural behaviour of cultured flounders, attributed to artificial hatchery conditions and lack of predator experience.

The purpose of this study was to assess the factors affecting the possible post-release vulnerability of cultured, juvenile winter flounder *Pseudopleuronectes americanus* (Walbaum) to predation. To examine the cryptic abilities of cultured fish, the amount of time to bury in sediment and to change colouration to match the substratum was evaluated. Additionally, an experiment was conducted to look at grain size and colour preference of light and dark coloured cultured fish. To determine the effect of winter flounder colouration on vulnerability to predation, the rate of predation on light coloured and normal coloured cultured winter flounder on dark sediment to avian predators was investigated. Further, to discern whether inherent behavioural differences exist between cultured and wild winter flounder, vulnerability to avian predation was

tested between similarly coloured fish. Finally, the reactions of cultured and wild winter flounder exposed to cues from a potential predator were studied.

## MATERIALS AND METHODS

### SOURCES OF FISH

Cultured winter flounder were reared at the University of New Hampshire's Coastal Marine Laboratory (CML) from a wild caught broodstock (Fairchild, 1998). Wild winter flounder were collected from Beverly Harbor, MA (42°32' N; 70°53' W) using a 17 m beach seine. Wild fish were brought back to the CML where they were allowed to acclimate for at least 1 week prior to use in any experiment. These fish were maintained on a daily diet of chopped blue mussel *Mytilus edulis* (L.) while in the laboratory.

### CRYPISIS OF CULTURED FISH

#### *Experiment 1: burying ability*

Fifteen cultured fish ( $54.7 \pm 5.7$  mm total length,  $L_T$ ) were placed individually into rectangular, plastic aquaria (15 × 12 × 10 cm) and monitored during 48 h from 11 to 13 June 1998 at the CML. Temperature ranged from 10 to 12° C. The sediment used in the aquaria was collected locally from an area where winter flounder are known to occur. The sediment was sieved to a diameter of  $\leq 1$  mm so that even the smallest fish would be able to bury (Tanda, 1990; Gibson & Robb, 1992; Moles & Norcross, 1995) and added to a depth of 1–2 cm in each tank (Ellis *et al.*, 1997). All aquaria were placed in a shallow tank and supplied with individual lines connecting to a flow-through seawater system. Light from three, 60 W overhead lights illuminated all aquaria for a photoperiod of 12L:12D.

Data were collected every 6 h, including 1 h before and after light changes, for 48 h. At these predetermined time intervals, each fish was inspected from above and the per cent of the fish's dorsal surface covered by sediment was subjectively estimated and recorded to the nearest 5%. When the sampling interval occurred during a dark photoperiod, a flashlight was used to determine how much of the fish was buried in the sediment. Since measuring fish burial took <30 s, the fish were not disturbed enough to move. Because winter flounder are most cryptic when completely buried in sediment, the data were analysed by comparing the proportion of individual fish buried 100% in the sediment to the total number of fish in the trial at each sampling interval over time.

#### *Experiment 2: colour adaptation ability*

Using the same system as in experiment 1, two trials were conducted from January to May 1998. In the first trial, 15 fish were used for control and experimental groups, and 13 fish were used in the second trial. For both trials, fish (mean  $\pm$  s.d.  $51.5 \pm 5.8$  mm  $L_T$ ) were divided randomly and equally into the experimental and control treatments. Experimental fish were maintained in aquaria with sediment, while control fish were kept in aquaria without sediment. Water temperature increased over time from 3.3 to 6° C, and from 3 to 11° C in trials 1 and 2, respectively.

All tanks were lit by artificial light from three, 60 W overhead bulbs and natural light from a nearby window. The artificial light, on a photoperiod of 10L:14D, attempted to mimic natural light intensity for a 10 cm water depth, but yielded only a maximum of 18 microeinsteins  $s^{-1} m^2$  at the tank surface. Natural sunlight at a similar water depth yielded as much as 2000 microeinsteins  $s^{-1} m^2$ . It was assumed that attenuation in the tanks was negligible.

The control fish were maintained in separate tanks and treated similarly to all other hatchery-reared, non-experimental flounder in the CML. They remained in 0.9 m diameter round, light blue, fibreglass rearing tanks devoid of any sediment. Although no overhead lights were mounted above the tanks, light emanated from other experimental

set ups, overhead fluorescent lights and some natural light from nearby windows. These tanks also were connected to the flow-through system that supplied each tank with individual water lines.

Throughout the experiment, all fish were fed a formulated, dry diet once daily. Tanks were siphoned clean of excess food as necessary to prevent background colour contrast in the control tanks and to prevent anoxia in the treatment aquaria. The sediment in the experimental aquaria was changed twice during the course of each trial in an effort to prevent anoxic sediment discolouration.

Ellis *et al.*'s (1997) methodology, that employed the use of the Munsell notation system (Munsell, 1971) to quantify colour change, was used. In this system, colour consists of three variables: hue, value and chroma. Hue is the principal component of colour that distinguishes one colour group from another (*i.e.* yellow from green, red from blue), value is a measure of the shade of a colour quantifying the varying degree of lightness or darkness and chroma is a measure of the intensity of a colour quantifying the amount of grey in the colour.

Colour charts for describing soil colour with hues of 2.5Y and 5Y (yellow) were applicable to this experiment (Munsell Colour, 1994). Both value (darkness) and chroma (greyiness) were measured on a scale of 0–8. A score of 0 indicated a dark value or dull chroma; a score of 8 indicated a light value or bright chroma. The colours of each experimental fish and control fish in each tank were measured at intervals over the course of 89 days. Measurements for the fish were determined by transferring each individual from its aquarium into a glass dish that was then moved over the polyethylene-protected Munsell colour charts (Davenport & Bradshaw, 1995; Ellis *et al.*, 1997). This took <1 min per fish and no noticeable colour change occurred during these measurements. In addition, a sediment sample from each aquarium was measured, since it was assumed that the colour of the sediment was the target colour for the experimental fish. Sediment colour was measured by randomly scooping a small amount of sediment into a glass dish and comparing it with the colour charts.

The time taken for the experimental fish to colour adapt to match the sediment determined the length of the experiment (Davenport & Bradshaw, 1995). Colour data were reciprocally transformed in order to achieve normality (Zar, 1996). Each colour variable (hue, value and chroma) was analysed independently at each sampling interval using a randomized complete block  $2 \times 3$  factorial analysis followed by Tukey's posterior test.

### *Experiment 3: sediment preference*

Light coloured fish (mean  $\pm$  s.d.  $31.9 \pm 6.7$  mm  $L_T$ ;  $0.5 \pm 0.3$  g) reared under normal grow-out conditions in light blue, fibreglass tanks devoid of sediment were tested from 20 June to 18 July 2000. Dark coloured fish (mean  $\pm$  s.d.  $19.7 \pm 4.4$  mm  $L_T$ ;  $0.1 \pm 0.1$  g) reared under normal grow-out conditions in black, fibreglass tanks devoid of sediment were tested from 26 June to 12 July 2001. Both experiments employed the same methods.

Eight glass tanks, each with a bottom surface area of  $248 \text{ cm}^2$ , were maintained in a flow-through water table with natural illumination. Each tank bottom was partitioned into four sediment types, each covering *c.*  $62 \text{ cm}^2$  (filled to a depth of 2 cm). The four sediment types tested consisted of two colours (natural and black) and two grain sizes (small =  $250\text{--}1000 \mu\text{m}$  and large =  $1000\text{--}2000 \mu\text{m}$ ).

At the start of each 24 h trial, each tank was filled with 1 l of sea water and temperature was recorded. A 5 cm wide plastic tube was placed in the centre of the tank where all sediment types converged. A single winter flounder was introduced into the tank *via* the tube. Once the fish settled to the bottom, the plastic tube was removed, and the trial began. Observations were made five times over the course of a 24 h period: observations were made hourly for the first 4 h and a final observation was made at 24 h. During these observation intervals, the sediment occupied by the greatest portion of each individual fish was recorded.

Before a new trial was initiated, the tank was flushed, new water was added, and the tank was rotated  $90^\circ$  to negate any light effects. A new winter flounder was used for each trial.

To determine if the winter flounder exhibited a sediment preference, a  $\chi^2$ -test of independence was used at each of the sampling intervals. In the case that there was no observed difference between two or more sediment types, these treatments were grouped and the  $\chi^2$ -test was rerun (Zar, 1996).

## PREDATION OF CULTURED FISH BY BIRDS

Two field studies investigated the vulnerability of cultured fish to predation by birds. For both experiments, two floating, open-topped wooden pens ( $106 \times 74 \times 31$  cm) filled with 2 cm of sediment were used. These pens were attached to a floating dock away from any human and boat disturbances in the waters near the CML. A stationary motion detector camera photographed all bird activity in the pens. Live fish were stocked into each pen for a 5 h period of predation.

While other flatfish enhancement studies have shown that cultured fishes are more vulnerable to predation than wild fishes (Furuta, 1996; Kellison *et al.*, 2000; Hossain *et al.*, 2002; Iglesias *et al.*, 2003), this trend had not been evaluated in winter flounder. This research was necessary to determine if the overall success of the restocking programme would be affected and if the cultured flounder would lack behavioural survival skills upon release. If it was discovered that cultured winter flounder were more at risk compared to their wild counterparts, rearing and release strategies could be altered to compensate for any morphological or behavioural deficiencies prior to a large scale release of winter flounder. Exposing live fish to live predators was the only method which enabled the vulnerability of winter flounder to these avian predators to be tested.

### *Experiment 4: predation of light-coloured and normal-coloured cultured fish*

The first avian predation experiment was tested in September 1999. Light-coloured winter flounder were reared under normal conditions in light blue tanks devoid of sediment. Normal-coloured (dark) winter flounder were cultured fish that had been acclimated for several weeks in tanks containing sand.

For each trial, a total of 10 winter flounder ( $53.1 \pm 14.2$  mm  $L_T$ ; five light and five normal) were stocked into each pen and made available to birds for a 5 h period. At the end of the trial, all remaining fish were removed and the survivors identified by colour. Survival data were analysed using  $\chi^2$  goodness of fit test.

### *Experiment 5: predation of normal-coloured cultured and wild fish*

A second avian predation experiment was tested from October to November 2001. Cultured winter flounder ( $65.0 \pm 13.1$  mm  $L_T$ ) used in this experiment were fish that had been acclimated for several weeks in tanks containing sand. Wild winter flounder ( $64.7 \pm 16.1$  mm  $L_T$ ) used in this experiment were fish collected by beach seining from Beverly Harbor. To distinguish between the cultured and wild fish, all fish were tagged with VIE tags (Northwest Marine Technology, Inc., WA, U.S.A.) injected subcutaneously on the ventral side of the fish. In each trial, a total of 10 winter flounder (five cultured and five wild) were stocked into each pen and made available to birds for a 5 h period. At the end of the trial, all remaining fish were removed and the survivors identified by tags. Survival data were analysed using a  $\chi^2$  goodness of fit test (Zar, 1996).

## BEHAVIOUR OF CULTURED FISH

### *Experiment 6: comparison of cultured and wild winter flounder reactions to cues from sand shrimp*

To examine behavioural responses of juvenile winter flounder ( $55.4 \pm 15.5$  mm  $L_T$ ) when exposed to cues from a potential predator, a  $2 \times 3$  factorial design experiment was conducted at the CML during August to October 2002. During this period, temperature ranged from 13 to 18° C.

The experiment was conducted using two aquaria: a 401 tank ( $0.5 \times 0.25 \times 0.3$  m) containing the predators and an 821 shallow raceway ( $2.0 \times 0.6 \times 0.08$  m) containing the winter flounder. Water from a flow-through seawater system passed from the predator tank into and down the length of the raceway where it drained at the far end. The raceway was slightly angled to create this water gradient from the predator tank to the outflow pipe. In addition, the predator tank was covered with black plastic so that the winter flounder only received chemical and not visual cues. The raceway contained 2 cm of sand evenly distributed along its length. Marks were scored on the side of the raceway every 10 cm to gauge distance. Black plastic curtains were hung around the system to exclude interference from the laboratory. Overhead fluorescent light illuminated the experiment.

Two types of winter flounder (wild and cultured) were exposed individually to three types of olfactory cues (predator, predator-prey and control). For the predator cue, sevenspine sand shrimp *Crangon septemspinosa* (Say), collected locally by beach seine were stocked into the predator tank. For the predator-prey cue, both sand shrimp and winter flounder were stocked into the predator tank. A control treatment was run in which the predator tank was empty.

Multiple trials were run for each of the treatment levels (winter flounder type and predator cue) using a new winter flounder and shrimp for each replicate. Prior to the start of these 15 min trials, the predator tank and raceway were flushed with new sea water to rinse out any residual chemical cues and the sand in the raceway was graded to smooth out any burying spots or fish tracks from the previous trial. For a predator trial, *c.* 25 shrimp were stocked into the predator tank. If the trial tested the predator-prey cue, then in addition to the shrimp, two winter flounders were added to the predator tank 1 min after the shrimp were stocked. Lastly, a single winter flounder was introduced *via* a 5 cm wide plastic tube into the raceway at the midpoint between the predator tank and the outflow pipe. The trial began when the winter flounder settled to the bottom and the plastic tube was removed. An observer recorded how many seconds it took the flounder to bury (bury time), how much it buried (%bury) and the maximum distance (cm) it travelled from the starting point during the 15 min interval (distance). If the fish moved towards the predator tank, the distance was marked as negative. If the fish moved away from the predator tank towards the drainpipe, the distance was scored as positive. In addition, distance data were converted to absolute values (absdistance) so that direction was negated.

Bury time and %bury data were square root and arcsine transformed, respectively, to normalize the distribution. All variables were tested by one-way ANOVA's followed by Tukey's posterior tests.

## RESULTS

### CRYPISIS OF CULTURED FISH

#### *Experiment 1: burying ability*

The percentage of fish completely buried in the sediment generally increased over time, so that by the end of 48 h, 87% of the winter flounder were completely concealed beneath the sediment (Fig. 1).

#### *Experiment 2: colour adaptation ability*

During the 89 day experiment, hue took the longest to change of the three colour variables. Both experimental and control fish started with a hue of 5Y while the sediment of the experimental aquaria was maintained at a hue of 2.5Y [Fig. 2(a)]. Neither the sediment nor the control fish changed colour over the duration of the experiment.

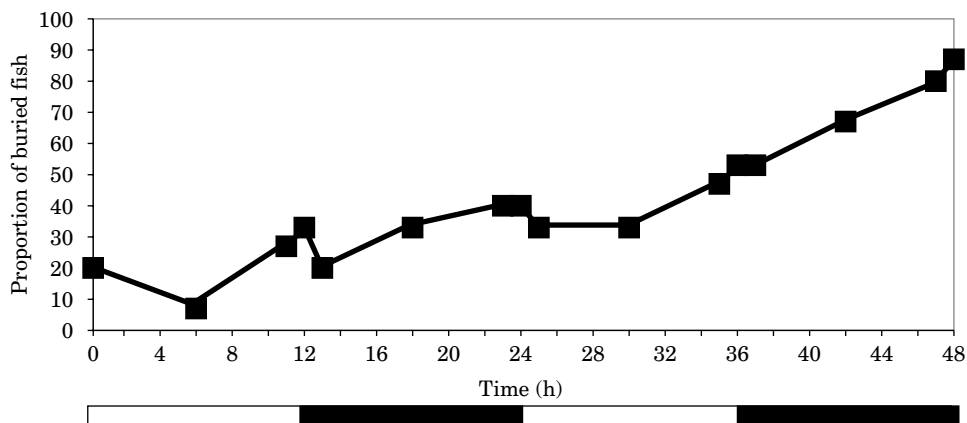


FIG. 1. The proportion of winter flounder ( $n=15$ ) buried completely in sediment over 48 h. The photoperiod was 12L:12D.

For the first 19 days, no change occurred in the hue of the experimental fish. From days 19 to 49, hue changed gradually such that by day 38, the experimental fish had a significantly different colour from that of the control fish. After this period of gradual colour change, a sharp drop occurred in experimental fish hue from 4.2Y to 3.3Y over 3 days. From days 55 to 76, hue did not change overall. Then hue decreased steadily to approximate that of the sediment. With a hue of 2.6Y at the termination of the experiment on day 89, hue of the experimental fish still had not reached that of the sediment. The difference, however, was relatively small (ANOVA, d.f. = 2 and 58,  $P < 0.001$ ; Tukey, d.f. = 58,  $P = 0.035$ ).

Value (darkness) changed more rapidly than hue. Although there were slight changes in the values of both the control fish and the sediment, the change in value was more pronounced in the experimental fish [Fig. 2(b)]. The control group started with a median value of 7.0, but after 10 days the value decreased and remained at 6.36 for the duration of the experiment. Sediment value was maintained at *c.* 4.0 throughout the experiment.

The value of the experimental fish dropped sharply from 7.0 to 4.7 over the first 13 days. By day 7, the value of the experimental treatment was significantly different from that of the control treatment (ANOVA, d.f. = 2 and 80,  $P < 0.001$ ; Tukey, d.f. = 80,  $P < 0.001$ ). The mean value of the experimental fish continued to darken until it matched that of the sediment on day 46 (ANOVA, d.f. = 2 and 62,  $P < 0.001$ ; Tukey, d.f. = 62,  $P = 0.082$ ). There was no further change during the remaining 43 days of the experiment.

Chroma took the least time to change of the three colour variables. Both experimental and control fish started with a chroma of 1.0 [Fig. 2(c)]. The initial chroma of the sediment in the experimental aquaria was 3.0 but decreased to 2.3 from days 13 to 35 with the onset of anoxia. Once the sediment was replaced, chroma increased sharply to 3.0. The experimental fish changed chroma almost immediately.

The chroma of the experimental fish quickly changed from 1.0 to 3.0 in 31 days. By day 4, the chroma of the experimental fish had deviated from that of the control group (ANOVA, d.f. = 2 and 82,  $P < 0.001$ ; Tukey, d.f. = 82,  $P < 0.001$ ),

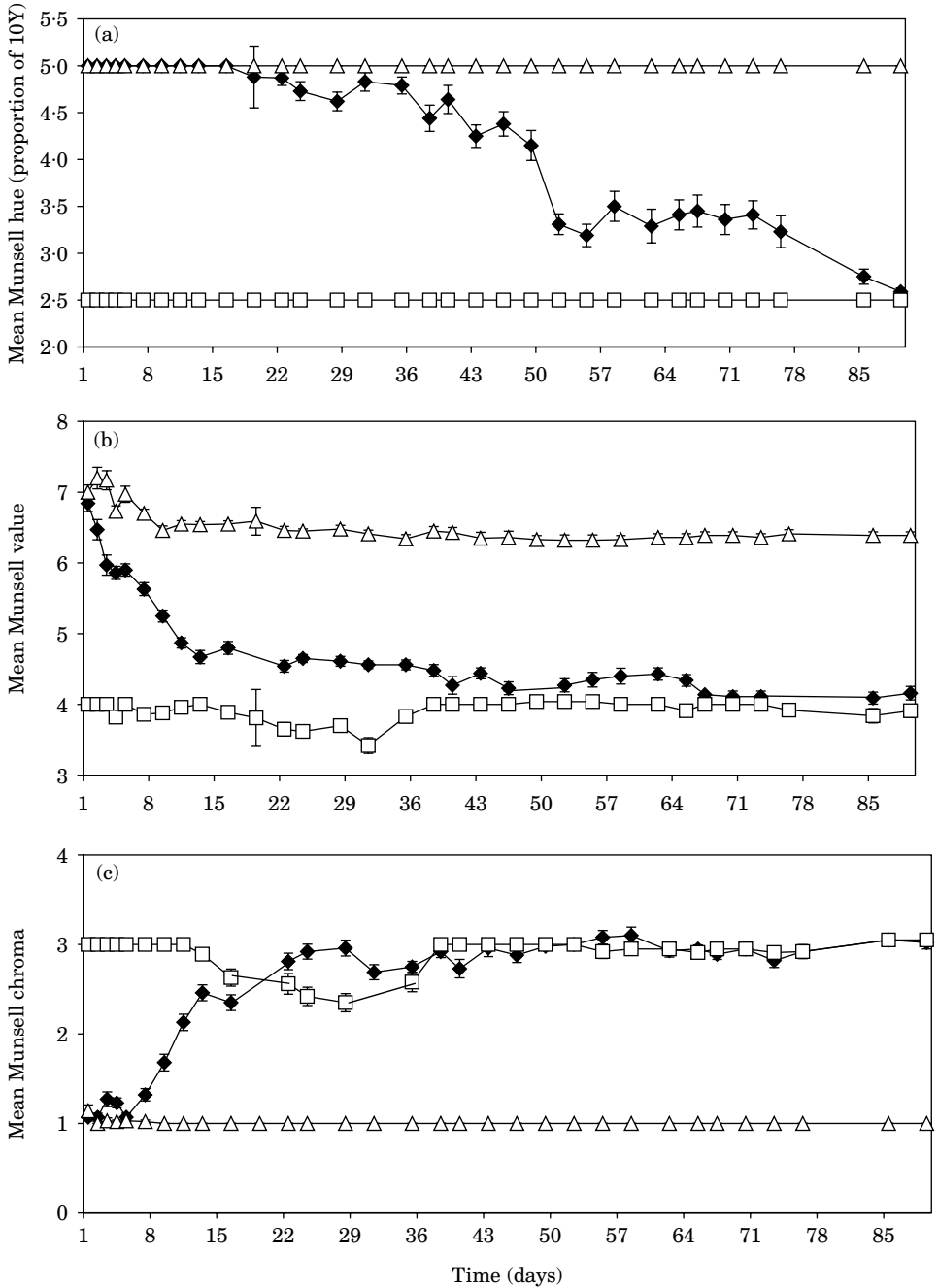


FIG. 2. Divergences in colour of cultured ( $\blacklozenge$ ) juvenile winter flounder from control fish colouration ( $\Delta$ ) to sediment colouration ( $\square$ ) after placement in aquaria containing sediment. Colour analyses are depicted as changes in mean  $\pm$  s.d. (a) hue, (b) value and (c) chroma.



and by day 16 had reached the chroma of the sediment (ANOVA, d.f. = 2 and 72,  $P < 0.001$ ; Tukey, d.f. = 82,  $P = 0.053$ ). At this time, however, the sediment had darkened due to anoxia and represented a false target for matching chroma. Between days 22 and 49 as the sediment became more anoxic and its chroma decreased, the chroma of the experimental fish fluctuated resulting in more variance in the data. From day 49 to the termination of the experiment, however, there were no further differences between the experimental and sediment chromas (ANOVA, d.f. = 2 and 62,  $P < 0.001$ ; Tukey, d.f. = 62,  $P \geq 0.474$ ).

#### *Experiment 3: sediment preference*

A total of 101 sediment preference trials were conducted for light coloured fish, however, not all five sampling intervals were observed in each trial. At each of the sampling periods, winter flounder consistently selected the naturally coloured, small grain size sediment [ $\chi^2$ ; 1 h: d.f. = 1,  $P < 0.01$ ; 2 h: d.f. = 1,  $P < 0.01$ ; 3 h: d.f. = 1,  $P < 0.01$ ; 4 h: d.f. = 1,  $P < 0.01$ ; 24 h: d.f. = 1,  $P < 0.01$ ; Fig. 3(a)]. There was no significant difference ( $\chi^2$ ; 1 h: d.f. = 2,  $P = 0.73$ ; 2 h: d.f. = 2,  $P = 0.43$ ; 3 h: d.f. = 2,  $P = 0.15$ ; 4 h: d.f. = 2,  $P = 0.05$ ; 24 h: d.f. = 2,  $P = 0.10$ ) between fish selection of the non-preferred sediments throughout the experiment. Water temperature ranged from 11.0 to 18.5°C, however, there was no difference between initial and final temperatures on any given day (ANOVA, d.f. = 1 and 6,  $P > 0.05$ ).

A total of 104 sediment preference trials were conducted for dark coloured fish, however, not all five sampling intervals were observed in each trial. At each of the sampling periods, winter flounder consistently selected the black coloured, small grain size sediment [ $\chi^2$ ; 1 h: d.f. = 1,  $P < 0.01$ ; 2 h: d.f. = 1,  $P < 0.01$ ; 3 h: d.f. = 1,  $P < 0.01$ ; 4 h: d.f. = 1,  $P < 0.01$ ; 24 h: d.f. = 1,  $P < 0.01$ ; Fig. 3(b)]. There was no significant difference between fish selection of the non-preferred sediments during 1–4 h ( $\chi^2$ ; 1 h: d.f. = 2,  $P = 0.14$ ; 2 h: d.f. = 2,  $P = 0.72$ ; 3 h: d.f. = 2,  $P = 0.24$ ; 4 h, d.f. = 2,  $P = 0.31$ ). At 24 h, however, more fish selected the natural coloured, small grain size sediment than either the natural coloured, large ( $\chi^2$ , d.f. = 1,  $P = 0.01$ ) or black coloured, large ( $\chi^2$ , d.f. = 1,  $P = 0.001$ ) sediments. Water temperature ranged from 12.3 to 15.5°C, however, there was no difference between initial and final temperatures on any given day (ANOVA, d.f. = 1 and 6,  $P > 0.05$ ).

## PREDATION OF CULTURED FISH

#### *Experiment 4: predation by birds of light and normal-coloured cultured fish*

More light-coloured than normal-coloured cultured winter flounder were eaten by birds ( $\chi^2$ , d.f. = 1,  $P = 0.018$ ; Table I) in all 10 trials. One hundred per cent of the light winter flounder were eaten by birds whereas only 58% of the normal winter flounder were consumed. Although cormorants and herons are the main avian predators of juvenile winter flounder (Pilon *et al.*, 1982; Birt *et al.*, 1987; Blackwell *et al.*, 1995), this experiment attracted mostly herring gulls *Larus argentatus* (Pontoppidan) and greater black-backed gulls *Larus marinus* (L.). Double-crested cormorants *Phalacrocorax auritus* (Lesson), green herons *Butorides virescens* (L.) and black-crowned night herons *Nycticorax nycticorax* (L.) were observed to a lesser extent near the pens.

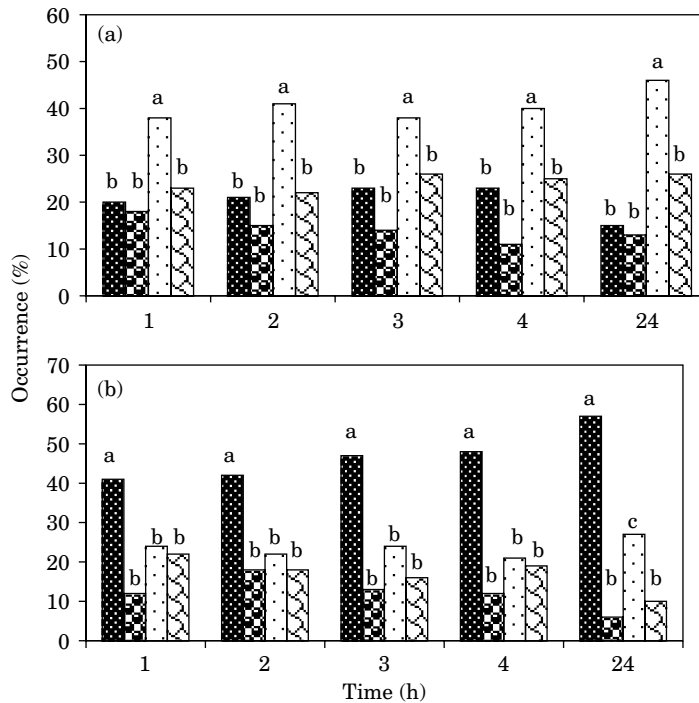


FIG. 3. Sediment preference of (a) light and (b) dark coloured cultured winter flounder (■, black small; ▨, black large; □, natural small; ▩, natural large). Columns with the same lower case letters within each time period are not significantly different ( $P > 0.05$ ).

#### Experiment 5: predation by birds of normal-coloured cultured and wild fish

More cultured than wild normal-coloured winter flounder were consumed by birds ( $\chi^2$ , d.f. = 1,  $P \leq 0.001$ ; Table I) in all 18 trials. Seventy-nine per cent of the cultured fish and 21% of the wild fish were consumed by birds.

### BEHAVIOUR OF CULTURED FISH

#### Experiment 6: comparison of cultured and wild winter flounder reactions to cues from sand shrimp

A total of 200 trials were run (Table II). Because there were no differences for all three variables tested between the predator and predator-prey treatments for

TABLE I. Summary of predation experiments

Predator	Available fish types	Vulnerable fish type	Number of trials	$\chi^2$	P-value
Birds	Cultured light-coloured v. cultured normal-coloured	Cultured light-coloured	10	2.79	0.018
Birds	Cultured normal-coloured v. wild normal-coloured	Cultured normal-coloured	18	15.02	<0.001

TABLE II. Summary of mean  $\pm$  s.d. bury time, per cent bury, distance, absolute distance and number of trials for the sand shrimp predator cue experiment. Different superscript letters in columns denote statistically significant ( $P < 0.05$ ) differences between fish types

Fish type	Bury time (s)	%Bury	Distance (cm)	Absolute distance (cm)	Number of trials
Wild	53.0 $\pm$ 119.4	77.7 $\pm$ 36.0 <sup>a</sup>	-9.4 $\pm$ 44.0	23.3 $\pm$ 38.3 <sup>c</sup>	55
Cultured	81.6 $\pm$ 127.1	55.8 $\pm$ 41.1 <sup>b</sup>	-2.8 $\pm$ 13.0	3.9 $\pm$ 12.7 <sup>d</sup>	55
Wild control	42.5 $\pm$ 76.1	75.1 $\pm$ 34.9 <sup>a</sup>	1.6 $\pm$ 21.6	6.9 $\pm$ 20.4 <sup>d</sup>	45
Cultured control	65.1 $\pm$ 94.4	45.9 $\pm$ 42.1 <sup>b</sup>	0.2 $\pm$ 4.8	1.6 $\pm$ 4.5 <sup>d</sup>	45

both cultured (ANOVA; bury time: d.f. = 1 and 32,  $P = 0.44$ ; %bury: d.f. = 1 and 33,  $P = 0.93$ ; distance: d.f. = 1 and 33,  $P = 0.94$ ) and wild (ANOVA; bury time: d.f. = 1 and 33,  $P = 0.145$ ; %bury: d.f. = 1 and 33,  $P = 0.31$ ; distance: d.f. = 1 and 33,  $P = 0.92$ ), these treatments were pooled together, and are from this point further referred to as the predator treatment. The resulting analyses then compared cultured and wild fish in predator and control treatments.

Forty of the 200 fish did not bury at all during the 15 min trial and as such were excluded from the bury time analyses. Of the remaining 160 fish, there were no statistical differences in the rate of burial (bury time) (ANOVA, d.f. = 3 and 156,  $P = 0.397$ ) between any of the treatments. Wild fish always buried more than cultured fish regardless of whether a predator was present (ANOVA, d.f. = 3 and 196,  $P < 0.001$ ; Tukey, d.f. = 196,  $P = 0.014$ ) or absent (Tukey, d.f. = 196,  $P = 0.002$ ).

There was no statistical difference in the distance fish moved when raw distance data were analysed (ANOVA, d.f. = 3 and 196,  $P = 0.156$ ), however, both wild and cultured fish moved towards the predator tank during predator trials, whereas, the fish moved away from the predator tank during control trials. When absdistance data were analysed so that direction was negated, wild fish moved further than cultured fish in the presence of a predator (ANOVA, d.f. = 3 and 196,  $P < 0.001$ ; Tukey, d.f. = 196,  $P \leq 0.003$ ). There was no difference in the distance that cultured fish travelled between predator and control trials (Tukey, d.f. = 196,  $P = 0.959$ ). Ten of the 55 wild fish (or 18%) initially buried, then after  $7.32 \pm 2.51$  min (mean  $\pm$  s.d.), uncovered themselves, and started swimming around the tank. Eight of these fish were located at -90 cm (next to the predator tank) at the end of the trial.

## DISCUSSION

### CRYPISIS OF CULTURED FISH

#### *Experiment 1: burying ability*

Burial in sediment increases the cryptic abilities of a winter flounder and may decrease predation, however, the ability for the fish to conceal itself in the sediment has been shown to be a learned response (Howell, 1994; Ellis *et al.*, 1997). Lack of sand during rearing probably led to the poor survival and high predation rate of released, cultured plaice (Blaxter, 1970). Juvenile sole *Solea solea* (L.)

showed an increased burial rate if they previously had been exposed to sediment than those which had not, indicating that a learning or acclimation period is necessary for burial (Howell, 1994; Ellis *et al.*, 1997). Initial burying attempts of cultured sole were less efficient than those of wild sole which were able to cover themselves with sand quicker and more thoroughly (Ellis *et al.*, 1997). In addition, it took the sole with no burying experience 5 days to achieve the same burial pattern as those fish that had been raised on sediment (B.R. Howell, pers. comm.).

It seems that this burial pattern is also true for cultured winter flounder. As time progressed, the amount of fish completely buried increased during 48 h. Japanese flounder showed a similar burial pattern to the winter flounder in this study in that an 18 h acclimation period was necessary for all fish to bury (Miyazaki *et al.*, 1997). According to Iglesias & Rodríguez-Ojea (1994), however, cultured turbot *Psetta maxima* (L.) buried immediately in sand upon release into the wild.

#### *Experiment 2: colour adaptation ability*

Even though this study lasted 89 days, the cultured winter flounder were not entirely colour adapted to the sediment at the end of the experiment. This lengthy colour transformation also occurred in Ellis *et al.*'s (1997) study on reared sole. Ninety-one days were required for cultured sole to match their yellow-red (YR) hue (initial hue *c.* 0YR) to that of wild sole (10YR). In this study, winter flounder changed hue from 5Y to 2.6Y in 89 days. At day 89, the hue of the fish was very close to, but not exactly, that of the sediment (2.5Y). The cultured sole value increased from 2.5 to 6.0 in only 7 days to match wild sole value. In contrast, winter flounder took 43 days to decrease the value from 7.0 to 4.0 to match the sediment value. Sole and winter flounder matched chromas in 33 and 31 days, respectively. Sole changed chroma from 1.0 to 4.0 while winter flounder changed from 1.0 to 3.0. Even though winter flounder required more time than sole to match colour, these results were more similar than those of other colour adaptation experiments.

Other camouflage studies have shown that flatfishes can adjust their pigment and pattern markings to match different sediments in less than 2 days. The eyed flounder *Bothus ocellatus* (Agassiz) matched different patterns repeatedly in under 10 s (Ramachandran *et al.*, 1996), while *Bothus podas* (Delaroche) and *Scophthalmus rhombus* (L.) exhibited colour and pattern changes in under 2 days (Sumner, 1911). Even winter flounder were capable of matching some patterns after 30 min (Saidel, 1988). Lanzing (1977) suggested that pattern matching in flatfishes is expedited by a preset, internal index of chromatophore arrangements in different patterns. The arrangement that most closely matches the background pattern is 'selected,' thus enabling a rapid change.

The time differential for colour adaptation in fishes depends on the nature of the colour change. There are two kinds of colour changes: physiological and morphological. Both are controlled by the nervous and endocrine systems and both occur within the chromatophores. Change in pigment distribution in the chromatophores causes a short-term or physiological change in skin colour (Fujii, 1993). A long-term or morphological change occurs when there is an actual change in the number of chromatophores or the amount of pigment contained within the chromatophores (Fujii, 1993).

Both morphological and physiological colour changes are brought about primarily by visual cues in response to light (Odiorne, 1957; de Groot *et al.*, 1969; Moyle & Cech, 1988). Flounders respond to the albedo or the per cent of incident light that the sediment reflects by using their specialized retinas (Sumner, 1940; Walls, 1963). Flatfish retinas are divided into two visual fields: the upper part of the retina views the sediment while the lower part sees the source of the natural light (Walls, 1963). The degree to which the fish changes its skin colour is a direct response to this ratio (Sumner, 1940).

In both Ellis *et al.*'s (1997) work and this experiment, colour adaptation was measured by matching colour tone rather than colour pattern. Since this adaptation required a morphological change in the chromatophores, the colour change was much longer than a physiological change would have been.

Morphological and physiological colour changes tend to happen simultaneously, although the latter is faster than the former. Odiorne (1957) found that when maintained on a dark background, fishes both increased the number of melanophores and the melanin content in the cells. In this study the winter flounder chroma and value changed drastically within the first week of the experiment, but hue was slow to differentiate. It is possible that the quick initial chroma and value changes to brighter but darker tones were physiological changes, while the more lengthy hue change from yellow to yellow-orange, and the final chroma and value changes were morphological. This theory is supported by a colour change study on juvenile lump suckers *Cyclopterus lumpus* L. Davenport & Bradshaw (1995) showed that lump suckers were able to disperse melanin quickly resulting in a colour value change (lightening or darkening of skin) while hue and chroma remained constant.

For a stock enhancement programme that is limited by time and money, lengthy morphological colour changes are probably not necessary prior to the release of cultured fishes. It appears that the quicker physiological colour changes are sufficient enough to camouflage winter flounders and reduce predation from visually searching predators. Rearing fish in tanks that simulate the release environment colouration would augment their colour adaptation ability.

### *Experiment 3: sediment preference*

In the field there is a well-known relationship between flatfish distribution and grain size and sediment type (Rogers, 1992; Walsh, 1992; Abookire & Norcross, 1998; Gibson & Robb, 2000), and this relationship has been corroborated further by sediment preference experiments in the laboratory (Tanda, 1990; Moles & Norcross, 1995; Gibson & Robb, 2000). Therefore, it is not surprising that the winter flounder selected small grain sized sediments in both sediment preference experiments. Gibson & Robb (1992) showed that fish <3.0 cm  $L_T$  are incapable of burying effectively in sediment sizes >1 mm, and Tanda (1990) showed that juvenile marbled sole *Pseudopleuronectes yokohamae* (Günther) and Japanese flounder preferred grain sizes in which they could most easily bury themselves. Moles & Norcross (1995) found that several juvenile flatfishes indigenous to Alaskan waters, actively selected sediments with grain sizes <500  $\mu\text{m}$ . In another laboratory study, Phelan *et al.* (2001) confirmed that winter flounder <70 mm  $L_T$  prefer sediments  $\leq 540 \mu\text{m}$ .

In addition to selecting the smaller grain size, winter flounder selected the colour sediment that best matched their body colour. This is a valuable point for rearing strategies for enhancement. In order for the winter flounder to be inconspicuous in the release site, there must be comparably coloured, small-grained sediment available to the fish. Alternatively, the winter flounder could adjust their colour to match the colour of the release sediment while still in the hatchery by introducing sediment to the rearing tanks. This would give the cultured fish the added benefit of acclimation to sediment and time to hone their burial skills (Gibson & Robb, 1992; Kellison *et al.*, 2000; B.R. Howell, pers. comm.).

## PREDATION OF CULTURED FISH

The predation vulnerability of dark cultured fish differed by 21% between the two bird predation experiments. Although the birds consumed a variable number of fish per day, they tended to consume fish that were easiest to capture. In the first bird predation experiment, obvious colour differences existed between the cultured fish. Given a choice, birds selected the most visible and easily captured fish, the light-coloured cultured winter flounder. In contrast, light-coloured cultured winter flounder juveniles were not statistically preyed upon any more than normal-coloured juveniles by sea ravens *Hemitrip-terus americanus* (Gmelin) in a laboratory study (Fairchild, 1998), however, the major visual predators of juvenile winter flounder are avian (Klein-MacPhee, 1978; Buckley, 1989), not piscivorous. Moreover, Tanaka *et al.* (1998) reported that pigmentation abnormalities frequently seen in hatchery-reared Japanese flounder were probably the most important contributor to predation immediately upon release.

The second prey choice in the first bird experiment was the normal-coloured cultured fish. Although their colour matched the sediment, it is likely that these cultured fish exhibited unnatural behaviour (*i.e.* increased movement and lack of burying) and as such, were still fairly conspicuous to birds, resulting in an overall total of  $7.9 \pm 1.4$  (mean  $\pm$  s.d.) fish eaten per trial. In the second bird predation experiment,  $5.0 \pm 2.6$  cultured fish per trial were eaten by birds. Neither fish type was more visible due to colour variation, thus, increasing the difficulty to detect and capture the fish. It is likely, however, that behavioural differences between the cultured and wild winter flounder increased the conspicuousness of the cultured fish to avian predators and, thus, increased their predation vulnerability. Wild winter flounder, probably were much more difficult for birds to capture so the cultured fish in this experiment were preyed on more heavily, especially compared to the normal-coloured cultured winter flounder in the first bird predation experiment.

Little research has been done on the extent of avian predation on flatfish populations. Although crustaceans and fishes have been the focus of most predation studies for flatfish populations, birds seem to be responsible for a large percentage of mortality for juvenile flatfishes. In the Dutch Wadden Sea, studies have shown that great cormorants *Phalacrocorax carbo* (L.) prey heavily on flatfish populations (Leopold *et al.*, 1998). Young-of-the-year (0 year group) flatfishes composed 73% of the species and 79% of the biomass of the cormorants' diet.

In the New Hampshire estuaries, there are large populations of diving ducks [mergansers *Mergus* spp. (L.)], double-crested cormorants and stalking predators like great blue herons *Ardea herodias* (L.), green herons, black-crowned night herons, and snowy *Egretta thula* (Molina) and great egrets *Ardea alba* (L.), most of which have been observed hunting and eating juvenile winter flounder (pers. obs.). While it has been acknowledged that great blue herons and cormorants are predators of young winter flounder (Pearcy, 1962; Tyler, 1971; Pilon *et al.*, 1982; Birt *et al.*, 1987; Blackwell *et al.*, 1995), the remainder of these piscivorous birds has not been documented as predators of winter flounder. Therefore, this avian predation pressure either has been radically underestimated or grossly overlooked. Both herring and great black-backed gulls are present in the New Hampshire estuaries and consume juvenile winter flounder as suggested by Howe *et al.* (1976) and determined by this study, but they probably are not the primary avian predators of flatfishes. Because gulls were constantly around the docks of the CML, they were the main predators in this experiment.

## BEHAVIOUR OF CULTURED FISH

The natural predator avoidance strategy of flatfishes is to bury into the sediment and remain motionless, and this strategy was observed initially in the behavioural experiment. As expected, wild fish buried more than cultured fish, regardless of the presence or absence of a predator, further corroborating the results of experiment 1 that demonstrated that sediment-naïve cultured fish need an acclimation period to hone their burial skills.

Because cultured and wild fish moved both towards (negative) and away from (positive) the predator tank, distance analyses yielded large s.d. Regardless of trial type, cultured and wild fish moved the same distance. When absolute distance was analysed, however, cultured fish in both predator and control treatments moved less than wild fish in predator treatments. It is known that sand shrimp prey on juvenile winter flounder (Witting & Able, 1993; Taylor, 2004) and can elicit a cortisol stress response in cultured juvenile winter flounder (Breves & Specker, 2002). This stress response may explain the cultured fish's lack of movement. Little is known, however, about stress responses of wild juvenile winter flounder. Since they were much older than those used in Breves & Specker's (2002) study, the fish in this study were less vulnerable to sand shrimp predation because of their larger size (Witting & Able, 1995). Due to their prior predator experiences in nature, it is possible that the wild winter flounder recognized that there was no immediate threat despite the presence of the sand shrimp, or even that the sand shrimp were potential prey. This hypothesis would help explain why 18% of the wild fish in the predator treatment 'unburied' and moved around the raceway after  $7.32 \pm 2.51$  min (mean  $\pm$  s.d.).

The use of predatory cues to compare cultured and wild fish behaviour originated from a coho salmon conditioning experiment by Olla & Davis (1989). Not only did the cultured fish exhibit different behaviour from the wild fish, this behaviour increased their vulnerability to predation. Once conditioned, however, predator-conditioned cultured fish were less vulnerable in subsequent predation experiments than predator-naïve cultured fish. Most recently this type of unnatural 'cultured fish behaviour' has been described for several other

salmonids, and predator conditioning studies have proved beneficial for their survival (Brown & Smith, 1998; Berejikian *et al.*, 1999; Hirvonen *et al.*, 2000; Mirza & Chivers, 2000). While this 'cultured fish behaviour' is easily identifiable in 'roundfish' (*e.g.* freezing and cessation of foraging and shift in habitat use), it only has been guessed at and not clearly described for flatfishes, with the exception of lengthy off-bottom behaviour displayed by cultured Japanese flounder (Tsukamoto *et al.*, 1997). Despite this lack of characterization in flatfishes, it is hypothesized that 'cultured fish behaviour' is one of the primary causes of low post-release survival of Japanese flounder (Furuta, 1996) and turbot (Iglesias *et al.*, 2003).

The condition of juvenile, cultured winter flounder needs to be evaluated prior to any enhancement effort. Cultured fish should be raised in tanks simulating the release environment colour and exposed to sediment, either in the hatchery prior to release or at the release site in acclimation cages, to augment their cryptic abilities upon release and thereby minimize mortality from visually hunting predators. It is imperative that the release site habitat contains appropriate sediment grain size so that the winter flounder are able to bury. Further, because cultured fish are more vulnerable to predation, regardless of colour, and they behave differently than their wild counterparts, future research should examine whether predator conditioning studies are effective for winter flounder.

We thank C. Bouchard, D. Goethel and J. Jurek who provided us each year with a wild-caught broodstock. We are especially grateful to the many students who assisted with these experiments including S. Abramson, C. Benton, E. Carver, C. Hill, N. Rennels, K. Reynolds, G. Rice, P. Rodgers, G. Smith, K. Sullivan, M. Toran and S. Willey. We wish to thank the anonymous reviewers for their time and helpful comments. This research was funded by the UNH Sea Grant College Program grant number R/FMD-158.

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