Comparative behaviour and survival of hatchery-reared versus wild summer flounder (*Paralichthys dentatus*)

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Abstract: We describe the use of laboratory trials to compare substrate-specific behaviour and susceptibility to predation of hatchery-reared (HR) versus wild summer flounder (*Paralichthys dentatus*) juveniles. HR fish spent significantly more time swimming in the water column than wild fish and took significantly longer to become cryptic on the benthos than wild fish, regardless of substrate type. In predation trials with a blue crab (*Callinectes sapidus*) predator, naïve HR fish were significantly more susceptible to predation than wild fish. Antipredator-conditioned HR fish were significantly less susceptible to predation than naïve HR fish but significantly more susceptible than wild fish, irrespective of substrate. The modified behavioural patterns and increased susceptibility to predation of HR individuals observed in this study indicate that flounder reared in psychosensory-deprived hatchery environments may be poorly equipped to survive in natural habitats; they also indicate that it may be possible to mitigate detrimental behavioural patterns by exposing naïve HR fish to natural stimuli before release into natural environments. These results have important implications for stock enhancement, suggesting that stocked organisms are more likely to achieve postrelease survival if they are conditioned with natural stimuli prior to release into the wild.

Résumé : Nous décrivons le recours à des essais de laboratoire pour comparer le comportement spécifique du substrat et la vulnérabilité à la prédation chez des cardeaux d'été (*Paralichthys dentatus*) juvéniles, d'élevage et sauvages. Les poissons d'élevage passaient nettement plus de temps que les poissons sauvages à nager dans la colonne d'eau, et prenaient nettement plus de temps pour se dissimuler dans le benthos, quel que soit le substrat. Dans des essais de prédation faisant appel au crabe bleu (*Callinectes sapidus*), les poissons d'élevage novices étaient nettement plus vulnérables à la prédation que les poissons sauvages. Les poissons d'élevage conditionnés à un comportement antiprédateurs étaient nettement moins vulnérables que les poissons d'élevage novices, mais nettement plus vulnérables que les poissons sauvages, quel que soit le substrat. Les changements dans le comportement et la plus grande vulnérabilité à la prédation chez les individus d'élevage observés dans notre étude indiquent que les cardeaux élevés dans le milieu appauvri sur le plan psychosensoriel que représente l'écloserie peuvent être mal armés pour survivre dans un habitat naturel; ils indiquent aussi qu'il est possible d'atténuer les patrons comportementaux nuisibles en exposant les poissons novices à des stimuli naturels avant de les lâcher dans le milieu naturel. Ces résultats ont des incidences importantes pour le rétablissement des stocks, car ils font ressortir que les organismes ensemencés sont plus susceptibles de survivre après le lâcher s'ils ont été conditionnés par des stimuli naturels avant leur libération dans le milieu naturel.

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Introduction

Commercially important marine fish and invertebrate populations are declining worldwide in response to over-exploitation and habitat degradation. One result of these declining fisheries stocks has been an effort to identify and impose effective management methods in impacted areas. For example, overexploited stocks may be rejuvenated numerically through stock enhancement, a process in which large numbers of organisms are reared under artificial conditions and subsequently released into the natural environment (Leber 1995). Stock enhancement is receiving increasing attention as a management option, partly because its implementation appeals to our reluctance to impose harsher and less popular management or conservation methods (Travis et al. 1998). Additionally, stock enhancement approaches may create employment opportunities related to the construction and operation of hatchery facilities (Thacker 1994; Karney et al. 1997). In the United States, stock enhancement programs have been evaluated or are currently being evaluated in Alaska, California, Florida, Hawaii, Maine, Maryland, Massachusetts, Oregon, South Carolina, Texas, and Washington.

Perhaps due to growing popular interest, stock enhancement efforts often progress before questions inherent to this management approach are addressed (Munro and Bell 1997). From a feasibility perspective, two critical questions should be answered before stock enhancement is considered as a management option for a given species. First, is it possible to produce hatchery-reared (HR) organisms that are capable

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of surviving in the wild? Second, if so, can enhancement goals be met in a manner that is economically feasible? If these questions can be answered in the affirmative, then it is reasonable to further consider stock enhancement as a management option. However, responsible managers must also address ecological concerns prior to implementation of enhancement programs. Increasing evidence suggests that the mass release of HR organisms into the wild may have negative ecological ramifications, such as alteration of the genetic structure of natural populations (Busack and Currens 1995), displacement of natural populations with more aggressive HR conspecifics (e.g., Neilsen 1994), and impacts on the abundance and diversity of wild predators, prey, and competitors (Leber 1995).

Nested within the management concerns of stock enhancement are questions regarding the likely behavioural deficits of HR fish and their ability to survive in the wild. For example, natural cues and selection pressures for survival skills (e.g., ability to avoid predation or forage efficiently) are often bypassed in psychosensory-deprived laboratory conditions in which HR fish are initially raised (Olla et al. 1994, 1998; Ellis et al. 1997). The lack of selection pressures in hatchery environments may result in HR organisms that exhibit anomalous behaviours compared with wild conspecifics. If these anomalous behaviours, or behavioural deficits, result in HR organisms that are not behaviourally equipped to survive in the wild environment, then enhancement efforts will be of minimum value at best and futile at worst. Therefore, it is critical to address both the behavioural quality of HR fish and the ability of HR fish to survive in the natural environment before stock enhancement efforts proceed.

If HR fish exhibit abnormal behavioural patterns that result in decreased survival, and the abnormal behavioural patterns are not genetically based, then it may be possible to modify behavioural patterns to increase survival of HR fish in the wild. This idea has most often been approached from a predator-avoidance perspective (but see Olla et al. 1994; Zaragoza et al. 1994) and has been explored in several species of HR fish (e.g., rainbow and steelhead trout (*Oncorhynchus mykiss*) (Berejikian 1995; Brown and Smith 1998), coho salmon (*Oncorhynchus kisutch*) (Olla et al. 1994), and Atlantic salmon (*Salmo salar*) (Jaervi and Uglem 1993)). Inherent in this "antipredator conditioning" approach is the idea that HR fish may not be behaviourally equipped to deal with predators in the wild because of the predator stimulus free environment in which they have been reared. If HR fish can somehow be "conditioned" to recognize predators prior to release, then postrelease survival may be significantly increased.

Although much effort has been put into addressing behaviour and survival patterns of HR fish, scant information exists on North American flatfish species. Intense commercial and recreational exploitation, coupled with dwindling nursery habitat quality and area, makes summer flounder (*Paralichthys dentatus*) an excellent candidate for stock enhancement oriented studies (Waters 1996). The goals of this study were to assess the substrate-specific behavioural and survival capabilities of HR versus wild summer flounder and the feasibility of predator conditioning as a method to decrease susceptibility of HR individuals to predation.

Materials and methods

This study was conducted from February to July 1998 at the National Marine Fisheries Service laboratory in Beaufort, N.C., U.S.A. Newly hatched HR summer flounder yolk sac larvae were obtained from Great Bay Aquafarms, Inc. (New Hampshire) and raised to early juvenile stage (about 30–90 mm total length (TL)) in the laboratory. Rearing conditions were as documented by Burke et al. (1999), with the exception of water temperature, which ranged from 18 to 23°C for the fish used in this study. Wild young-of-the-year juveniles (about 30–90 mm TL) were collected from nearby shallow-water tidal flats in Beaufort, N.C., with a 2-m beam trawl (0.32-cm cod end mesh size). Wild fish were maintained in laboratory tanks for a minimum of 2 weeks prior to use in experimental trials to allow for dissipation of tidal rhythms (Morgan and Cordiner 1994) and for adjustment to general laboratory conditions. Because juvenile summer flounder utilize a spectrum of habitats as nursery areas, ranging from relatively low-energy mud substrate marsh habitats to relatively high-energy sand substrate beach habitats (Burke et al. 1991; T. Kellison et al., unpublished data), all experimental trials in this study were replicated on sand and mud substrates.

Behaviour of HR versus wild fish
Paired behavioural observations were made to compare substrate-specific behaviour of HR (28–63 mm TL) versus wild (34–69 mm TL) fish. In each behavioural trial, a single fish was introduced into one of three observation aquaria (22 cm length × 15 cm width × 15 cm depth) containing sand or mud substrate and screened from outside movement by black plastic. Following introduction, fish were left undisturbed for 24 h to acclimate to aquarium conditions. Each replicate fish was observed for 1 h through small openings in the plastic (two aquaria) or with a low-light sensitive video camera – monitor system (one aquarium). Light levels for daytime observations (about 1300–1400 lx) were based on field measurements. Nighttime behavioural observations were made with the video camera – monitor system using low-intensity red light (about 4–5 lx). To achieve paired (by time of day) observations, HR and wild fish were observed in adjoining 1-h time periods (i.e., back to back) (aquaria viewed through plastic screens) or with a 24-h interval between observations (aquarium viewed with the video camera). Paired behavioural observations began at a randomly chosen hour when using the video camera system, whereas paired observations made through the plastic screens by an observer were restricted to randomly chosen daylight hours. Statistical analyses indicated no effect of observation method (visual versus video camera system) on time spent buried, stationary on the benthos, or swimming in the water column (analysis of variance (ANOVA), $P > 0.24$ in all cases). Thus, data from the two observation methods were pooled for analyses. Because our focus was to identify possible behavioural differences between HR and wild fish, we did not address possible differences between diurnal and nocturnal behavioural patterns (although time-lapse video observations provide no evidence of differential circadian behavioural patterns between HR and wild fish).

To quantify substrate-specific behaviour of HR versus wild fish, fish activity was divided into four mutually exclusive categories: (1) buried, (2) stationary (not buried) on the benthos, (3) moving on the benthos, and (4) swimming in the water column. Behaviour was quantified by recording the amount of time during the 1-h observation period that each fish spent performing each of the four behaviours. Following each 1-h observation period, time spent performing each behaviour was summed within behavioural categories to calculate a total time spent performing each behaviour. Response variables were then calculated by subtracting the totals of HR fish from those of wild fish, by pair, within each behavioural category. Time spent moving on the benthos was excluded from

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statistical analyses for two reasons: (*i*) including all four behavioural categories would have nullified the statistical independence of the first three analyses (the four behaviours were mutually exclusive; the sum of the percent time spent performing the four behaviours for any observation was necessarily equal to 100%) and (*ii*) moving on the bottom was generally the most rarely exhibited behaviour, and therefore may be the least important in terms of vulnerability to predation. A total of 36 paired behavioural observations were made, split equally among substrate type (sand (*n* = 18) and mud (*n* = 18)). Behavioural data were analyzed using nonparametric statistics due to nonnormality of the response variables. A two-tailed Wilcoxon two-sample test (also referred to as a Mann–Whitney test) was first employed to test for an effect of substrate on time spent performing each behaviour. If there was no significant effect (*P* > 0.05) of substrate on the behavioural response of interest, we pooled data across substrates and used a Wilcoxon signed-rank test to determine whether the difference in time spent performing each behaviour between the HR and wild fish was significantly different from zero. One-tailed tests were employed for analyses of time spent buried and swimming in the water column, with the direction of difference dependent on the behaviour being analyzed. For example, we hypothesized that HR summer flounder would spend more time swimming in the water column than wild conspecifics based on behavioural observations of HR and wild Japanese flounder (*Paralichthys olivaceus*) (Furuta 1996, 1998). Similarly, we hypothesized that HR summer flounder would spend less time buried than wild conspecifics based on behavioural observations of HR and wild sole (*Solea solea*) (Howell and Baynes 1993; Ellis et al. 1997).

Cryptic adaptation to substrate

Experiments were performed to assess the ability of HR (34–77 mm TL) versus wild (35–79 mm TL) fish to become cryptic on a newly encountered substrate. Twelve 1.2-L containers (14 cm height, 12 cm diameter) were filled with seawater and equipped with 2 cm of natural substrate (sand (*n* = 6) or mud (*n* = 6)). Containers were placed in a flow-through water bath to keep temperatures similar to natural water temperatures (19–22°C). A single fish was then placed into each container in random order within a given substrate so that treatment combinations were orthogonal (HR–sand = 3, HR–mud = 3, wild–sand = 3, wild–mud = 3). Fish were observed immediately after introduction to the containers and subsequently observed at 5-min intervals for a 60-min period, whereupon each fish was assigned a value in minutes (0–60) corresponding to the elapsed time between introduction to the container and successful cryptic response. The "cryptic response" of each fish was quantified by recording the amount of time between introduction into the container and the successful completion of one of two behaviours: burial or pigmentation change to match the substrate. Successful burial was determined to occur when greater than 90% of a fish's body was covered in sediment. Successful pigmentation change was determined to have occurred when, at first glance following each 5-min interval, a fish was difficult to discern against the substrate background. The pigmentation and burial responses were therefore somewhat subjective but were designed as a "blind" test (the status (HR versus wild) of each fish was unknown to the observer) to ensure that potential observer bias did not affect results. Individuals that did not exhibit cryptic behaviour by trial termination were assigned a value of 61 min under the conservative assumption that a cryptic response would have occurred in the minute following trial termination. A total of 168 1-h trials were conducted, split equally among the four possible treatment combinations (HR–sand (*n* = 42), HR–mud (*n* = 42), wild–sand (*n* = 42), and wild–mud (*n* = 42)). Results were analyzed to determine the effect of fish status (HR versus wild) and substrate (sand versus mud) on both the time to cryptic response and the tendency to

bury. After confirming homogeneity of variances, the time to cryptic response was analyzed using a two-way ANOVA with fish status (HR versus wild) and substrate (sand versus mud) as treatments. Although the data were not normally distributed, ANOVA is robust to deviations from normality, particularly as sample size increases (*n* = 42 in this case) (Zar 1984). Additionally, a two-way ANOVA on rank-transformed data (Akritas 1990) yielded *P* values nearly identical to those of the analysis of untransformed data, suggesting that the results of the parametric ANOVA were not compromised by the deviation from normality. A log-linear *G* test was used to determine whether fish status (HR versus wild) and substrate (sand versus mud) significantly affected the tendency of fish to bury. Comparisons between treatment combinations were determined with protected, lower-level chi square contrasts within the *G* test.

Antipredator conditioning and predation trials

The natural predator guilds of early juvenile (about 30–100 mm TL) summer flounder are relatively undocumented. Blue crabs (*Callinectes sapidus*) were chosen as the predator in this study because they are abundant in juvenile summer flounder nursery habitats (T. Kellison et al., unpublished data), they are documented omnivores, with piscivory often compiling a significant proportion of their diet (Stoner and Buchanan 1990) and they readily consumed summer flounder juveniles in the laboratory environment. Additionally, crabs are documented predators of several flatfish species (Ansell and Gibson 1993; Furuta 1996).

To test whether experience with predator cues could increase survival rates of HR fish, 53 of the 106 HR fish (46–72 mm TL) used in predation trials (see below) were exposed to a single caged blue crab (>100 mm carapace width) for 24 h prior to predation trials. The caged crab was placed in the center of a tank containing HR fish and was fed juvenile spot (*Leiostomus xanthurus*) once during the 24-h antipredator conditioning period. Fish that received the antipredator conditioning treatment are subsequently referred to as antipredator-conditioned HR (APCHR) fish as opposed to HR fish.

Predation trials were conducted to test whether fish status (HR versus APCHR versus wild) and substrate (sand versus mud) had a significant effect on summer flounder mortality due to predation. Predation trials were conducted in 12 individually aerated aquaria (46 cm length × 23 cm width × 25 cm depth) with flow-through seawater and either sand (*n* = 6) or mud (*n* = 6) substrate. Each aquarium was screened from outside movement with black plastic. Fish were randomly assigned to a tank (within substrate) and allowed to acclimate to tank conditions for 24 h. At the end of the 24-h acclimation period, a blue crab (80–110 mm carapace length) was placed in the tank. Tanks were then left undisturbed for a 24-h period, after which crabs were removed from each tank and fish from each trial classified as "eaten" or "not eaten." Predation trails were replicated within fish status treatment levels (HR versus APCHR versus wild) on both sand (*n* = 33 per treatment) and mud (*n* = 20 per treatment) for a total of 159 trails. Data were analyzed using a log-linear *G* test with fish status (HR versus APCHR versus wild) and substrate (sand versus mud) as categories.

Results

Behaviour

Paired behavioural trials

Behavioural patterns of HR fish generally differed from those of wild conspecifics (Fig. 1). HR fish spent significantly more time swimming in the water column than wild conspecifics (one-tailed Wilcoxon signed rank test; data pooled over substrate (see below): $T_{35} = -78$, $P = 0.011$)

Fig. 1. Mean percent time (±SE) spent (*a*) swimming in the water column, (*b*) buried, (*c*) stationary (not buried) on the benthos, and (*d*) moving on the benthos by wild (solid bars) and HR (open bars) juvenile summer flounder. Statistical analyses were based on paired observations (Wilcoxon signed-rank test: α = 0.05). Asterisks denote significant differences between the HR and wild responses, pooled over substrates; n = 18 paired observations for each behaviour divided equally between substrates (sand (n = 9) and mud (n = 9)). See text for significance levels.
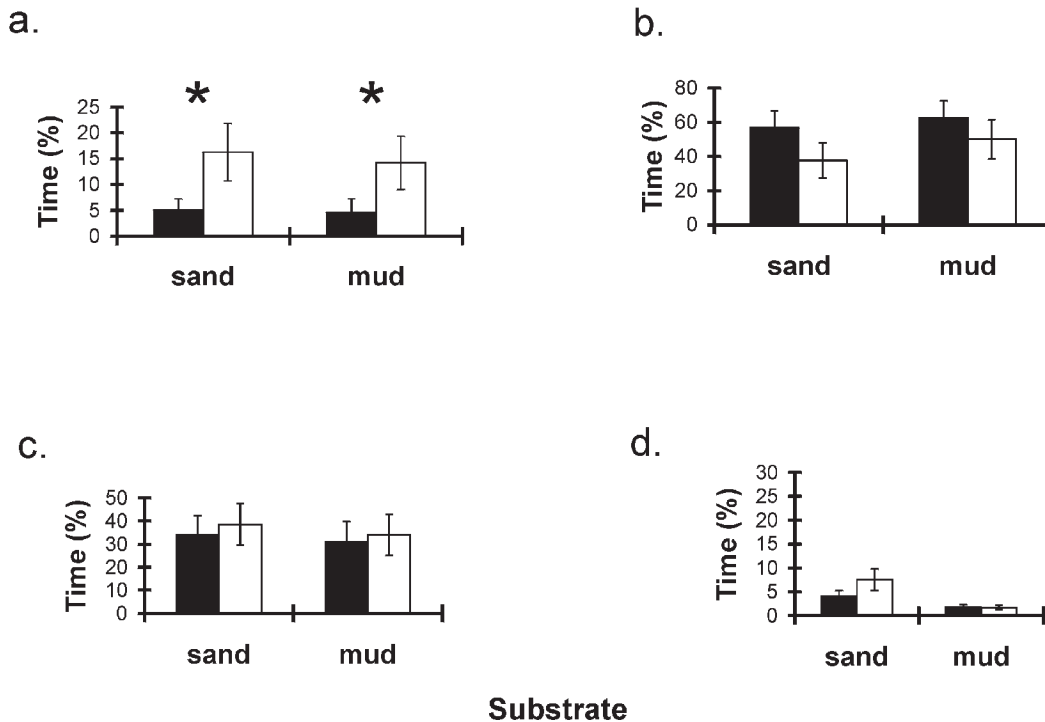
(Fig. 1*a*). HR fish spent less time buried than wild fish (Fig. 1*b*), although the mean difference in time spent buried between HR and wild fish was not statistically significant (one-tailed Wilcoxon signed rank test; data pooled over substrate: T_{35} = 46, P = 0.067). There was no significant difference in the amount of time spent stationary (not buried) on the benthos between HR and wild fish (one-tailed Wilcoxon signed rank test; data pooled over substrate: T_{35} = 1, P = 0.491) (Fig. 1*c*). Substrate (sand versus mud) had no significant effect on time spent by HR and wild fish swimming in the water column (Wilcoxon two-sample test: Z = 0.322, P = 0.747) (Fig. 1*a*), buried (Wilcoxon two-sample test: Z = 0.147, P = 0.883) (Fig. 1*b*), or stationary (not buried) (Wilcoxon two-sample test: Z = 0.095, P = 0.924) (Fig. 1*c*).

Cryptic adaptation to substrate

HR fish suffered an impaired ability to exhibit cryptic behaviours on a newly encountered substrate compared with wild conspecifics. HR fish took significantly longer than wild fish to become cryptic (burial or pigmentation response) on the benthos, regardless of substrate (two-factor ANOVA with interaction term; treatments = fish status (HR versus wild) and substrate (sand versus mud): $F_{1,164}$ = 42.91, P < 0.0001) (Fig. 2*a*). Time to cryptic response was significantly longer on mud than on sand substrate, regardless of fish status (two-factor ANOVA with interaction term; treatments = fish status and substrate: $F_{1,164}$ = 3.93, P = 0.049) (Fig. 2*a*). The fish status × substrate interaction was not significant (two-factor ANOVA with interaction term: $F_{1,164}$ = 0.43, P = 0.514). HR fish buried significantly less often than

wild fish, regardless of substrate (log-linear G test with interaction term; treatments = fish status and substrate: χ^2 = 8.9980, P = 0.003) (Fig. 2*b*). Additionally, substrate had a significant effect on the tendency of fish to bury. Both HR and wild fish buried significantly more often on sand than on mud (log-linear G test with interaction term; treatments = fish status and substrate: χ^2 = 23.8381, P < 0.0001) (Fig. 2*b*). The fish status × substrate interaction was not significant (log-linear G test with interaction term: χ^2 = 0.22, P = 0.636).

Predation and antipredator conditioning

HR fish exhibited greater susceptibility to predation than wild conspecifics. Increased vulnerability of HR fish to predation was partially mitigated by predator conditioning. Fish survival varied significantly according to fish status (HR versus APCHR versus wild) (log-linear G test with interaction term; treatments = fish status and substrate: χ^2 = 22.51, P < 0.0001). HR fish suffered significantly greater predation rates than wild fish (protected contrast within log-linear G test: χ^2 = 22.46, P < 0.0001) (Fig. 3). APCHR fish experienced significantly lower predation rates than HR fish (protected contrast within log-linear G test: χ^2 = 4.33, P = 0.038) but experienced significantly greater predation rates than wild fish (protected contrast within log-linear G test: χ^2 = 7.67, P = 0.006) (Fig. 3). Substrate had no significant effect on summer flounder mortality due to predation, regardless of fish status (log-linear G test with interaction term; treatments = fish status and substrate: χ^2 = 0.20, P = 0.658) (Fig. 3).

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Fig. 2. (*a*) Mean time (±SE) required to become cryptic (burial or cryptic pigmentation) on the benthos by wild (solid bars) and HR (open bars) summer flounder. Asterisks denote significant differences between the HR and wild responses, regardless of substrate (two-factor ANOVA with interaction term; treatments = fish status and substrate: α = 0.05, n = 180). (*b*) Proportion of wild (solid bars) and HR (open bars) fish that exhibited burying behaviour during the cryptic adaptation trials. Asterisks denote significant differences between the HR and wild responses, regardless of substrate (log-linear G test with interaction term; treatments = fish status and substrate: α = 0.05, n = 180).
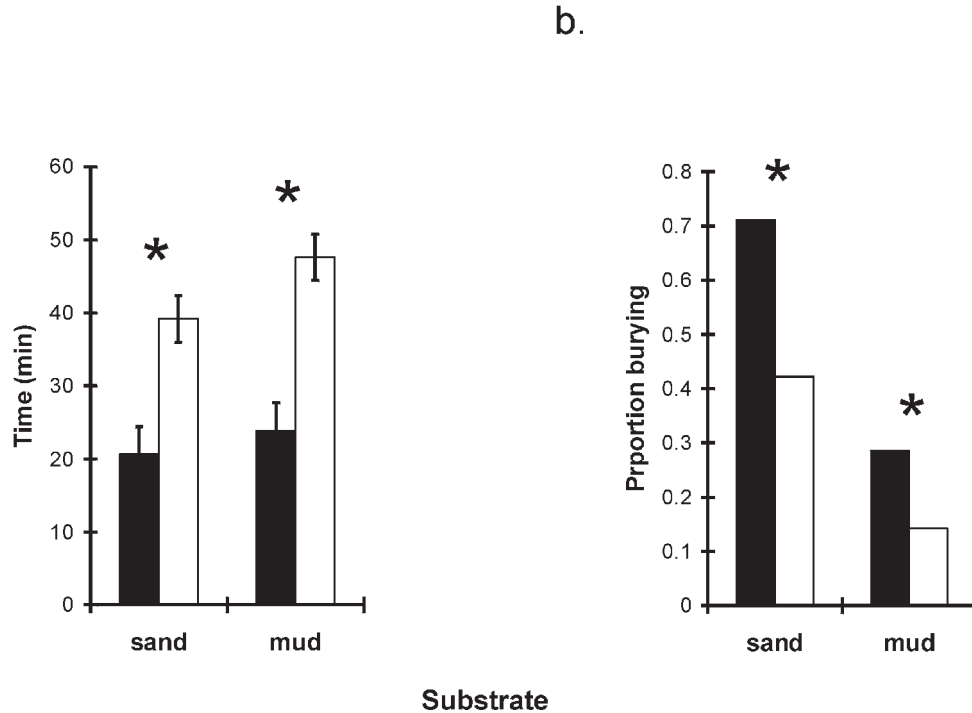
There was no significant fish status × substrate interaction (log-linear G test with interaction term: χ^2 = 4.26, P = 0.119).

Discussion

Behaviour and predation

The behavioural data in this study suggest that (*i*) HR summer flounder exhibit modified behavioural patterns relative to wild conspecifics, (*ii*) these modified behavioural patterns reduce survival in laboratory aquaria, and (*iii*) decreased survival of HR summer flounder can be partially mitigated by exposing HR fish to a predator stimulus prior to their exposure to a predator. Flatfish are evolutionarily specialized for a benthic existence. Their body form allows them to lie flat on the benthos, and natural behaviours such as burial and mimicry of the benthos are important to survival, increasing the ability to successfully ambush prey and elude the detection of potential predators (Ansell and Gibson 1993; Howell and Baynes 1993). HR fish in this study spent less time buried, were significantly less likely to bury, and took significantly longer amounts of time to become cryptic on the benthos than wild fish.

While the ability to bury and mimic benthic coloration and pattern are innate in HR summer flounder, such behaviours were poorly utilized compared with wild conspecifics and thus constitute "behavioural deficits" (Olla et al. 1994). Lack of experience on natural substrates may be a key factor causing behavioural deficits related to becoming cryptic on the benthos in this study. Previous research with flatfish has

suggested that behavioural deficits may diminish with exposure to natural conditions or stimuli. For example, both burying efficiency (the proportion of the ocular side covered by sand after a single burial attempt) and the ability to change pigmentation improved significantly with time spent on natural substrate in HR sole (Ellis et al. 1997). Other possibilities explaining the lack of ability to become cryptic on natural substrate by HR fish include hatchery diet deficiencies, which may preclude the development of normal pigmentation abilities (e.g., Seikai 1985), and stress, which may be a combination of many factors present in the hatchery environment, including diet deficiencies. If lack of experience on natural substrates is the main causative agent of the lack of ability of HR fish to become cryptic on natural substrates, then the use of rearing facilities that utilize natural substrate may be a critical step towards rearing hatchery fish that are behaviourally equipped to survive in the wild. Unfortunately, high-density culture techniques, such as those used in the rearing of summer flounder, necessitate high feeding rates and frequent cleaning of holding tanks, making use of natural substrates difficult and problematic. If additional factors, such as diet deficiencies, general stress, or selection for fish that are adapted to hatchery conditions, are the causes of the disability of HR fish to become cryptic on natural substrates, then alternative rearing strategies (e.g., improved diets or reduced-stress environments) must be employed or developed if HR fishes are to be equipped behaviourally to survive in the wild.

While cryptic behaviours such as burial and benthic mim-

icry are intuitively adaptive for flatfish, behaviours that make summer flounder more visible to predators, such as swimming in the water column, are intuitively maladaptive. The HR fish in this study spent significantly greater amounts of time swimming in the water column than wild conspecifics. This behaviour may be a result of hatchery feeding techniques, in which food pellets are dropped into rearing tanks. Fish that learn to swim towards the surface are rewarded with abundant food under such a feeding regime. Thus, swimming in the water column is rewarded (and therefore reinforced) and possibly selected for in the hatchery environment. Upon release, however, this behaviour would quickly become detrimental to survival. For example, laboratory experiments and field observations of Japanese flounder indicated that HR juveniles suffered high rates of predation-induced postrelease mortality due to increased amounts of time spent swimming in the water column as compared with wild fish (Furuta 1996). It is likely that increased activity, especially with regard to swimming in the water column, was a significant factor affecting the increased susceptibility of HR summer flounder to predation in this study (see below). If common hatchery feeding techniques (such as those employed in the rearing of the experimental HR fish used in this study) result in HR flatfish conditioned to swimming in the water column, then such aberrant behaviour may be altered by developing and utilizing alternative feeding techniques in which food is introduced on the bottom of rearing tanks (e.g., Berejikian et al. 1999).

HR fish in this study suffered significantly higher predation rates than wild conspecifics, regardless of substrate. It is probable that HR fish were more susceptible to predation due to a combination of modified behavioural patterns (increased time spent swimming in the water column, decreased time spent buried or stationary on the benthos, and decreased ability to exhibit cryptic behaviour on the benthos compared with wild conspecifics). Although predation by blue crabs is often regarded as dependent on tactile and chemical cues (Eggleston 1990; Weissburg and Zimmer-Faust 1994), predator–prey interactions observed in preliminary predation trials appeared to have been visually mediated (when a fish displayed movement, e.g., swimming into the water column, crabs immediately directed their attention toward the fish (chelipeds spread with chelae open) and subsequently pursued the fish). If visual cues are a significant contributor to the initiation of crab – summer flounder predator–prey interactions, then the behavioural deficits exhibited by HR fish in this study (more time spent swimming in the water column and less time cryptic on the benthos) are a plausible mechanism underlying the increased predation rates on HR summer flounder. If HR summer flounder are significantly more susceptible to blue crab predators than wild conspecifics, then it is likely that HR summer flounder would also suffer increased susceptibility to predation from other visual predators, such as piscivorous fish. Therefore, the aberrant behaviours exhibited by naïve HR summer flounder are likely to result in abnormally high rates of predation-induced mortality in the natural environment.

The APCHR fish experienced significantly lower predation rates than naïve HR fish but suffered significantly higher predation rates than wild fish in the predation trials. These results add to the growing evidence that deficiencies

Fig. 3. Proportion of summer flounder (wild, APCHR, and naïve HR) eaten by blue crabs in laboratory predation trials on sand (open bars) and mud (hatched bars) substrates. The asterisks bordered by lines denote significant differences between the wild, APCHR, and HR responses (log-linear G test with interaction term; treatments = fish status and substrate: $\alpha = 0.05$, $n = 159$).
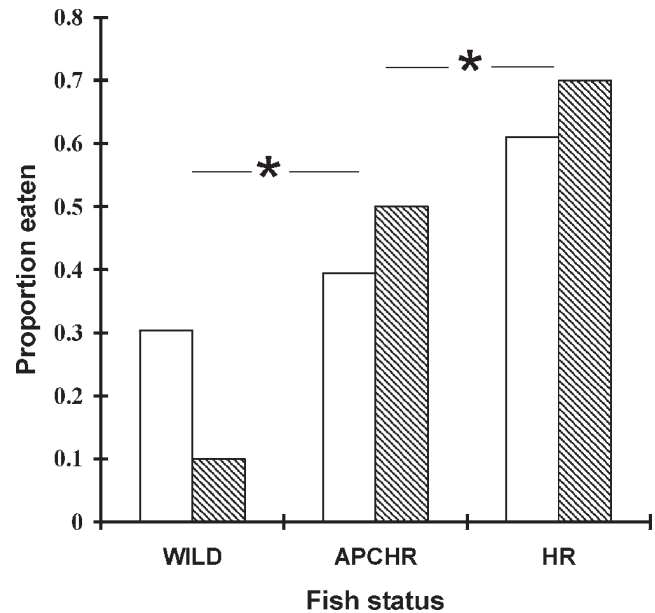
of HR fish, perhaps manifest in abnormal behavioural patterns, can be partially mitigated by exposing HR fish to natural predator cues prior to their release (antipredator conditioning) (Olla et al. 1994; Brown and Smith 1998). The mechanisms underlying the decrease in predation rates on APCHR fish compared with naïve HR fish are unknown. If the APCHR fish learned to recognize the blue crab to which they were being conditioned as a predator, they may have altered their behaviour by decreasing motion in the presence of a predator (i.e., during predation trials). Further research is required to determine: what combination of cues results in predator recognition and possibly antipredator behaviour and how antipredatory behaviour is manifest in summer flounder. Such research will aid in determining the best methods by which to condition HR summer flounder for release into natural environments.

Mechanisms underlying modified behavioural patterns

HR fish may exhibit modified behavioural patterns due to three nonmutually exclusive mechanisms: (*i*) genetic differences from wild populations (Reisenbichler and Brown 1995; Petersson et al. 1996), (*ii*) a lack of appropriate natural behavioural cues (Olla et al. 1994, 1998; Berejikian 1995), or (*iii*) a lack of natural selection pressures in the psychosensory-deprived hatchery environment (Berejikian 1995; Olla et al. 1998). The behavioural deficits documented in this study are most likely not genetically based, as the multiple broodstock were captured from the wild as adults. It is likely that a lack of appropriate natural behavioural cues contributed to the modified behavioural patterns exhibited by HR summer flounder in this study, as suggested by the fact that naïve HR fish were significantly more susceptible to predation than antipredator-conditioned fish. Additionally, a

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lack of natural selective pressures in the hatchery environment may have been an important factor affecting the increased susceptibility of HR fish to predation in this study. The wild fish with which the HR fish were compared in this study are assumed to have passed the "tests" of natural selection prior to collection. In such tests, wild fish that exhibit detrimental behavioural patterns (i.e., lack of ability to become cryptic on the benthos and a high proportion of time spent swimming in the water column) have presumably perished due to predation. Thus, the wild fish used in this study were "winners" in the game of natural selection thus far, while the HR fish, which have experienced no selective predation, remain a mixture of would-be winners and losers. From this viewpoint, the high postrelease mortality rates often suffered by HR fish are simply ridding the HR population of behaviourally deficient "losers." Such observations have prompted suggestions of including natural predators in hatchery-rearing programs (Independent Scientific Group 1999). Such novel approaches may be necessary to mitigate the effects of behavioural deficits, which may be responsible for the elevated levels of postrelease predation-induced mortality common to HR fishes (e.g., see Furuta 1996). Nevertheless, approaches designed to mitigate the effects of behavioural deficits on postrelease survival may add significant cost and effort to the rearing of HR organisms and should therefore be thoroughly examined in terms of economic and logistical feasibility prior to implementation.

Substrate

Previous research with estuarine-dependent fishes indicates that habitat is extremely important in determining growth and survival (Hoss and Thayer 1993). Thus, it may be critical to assess habitat-specific behaviour and survival of stock enhancement candidates to make informed decisions about optimal habitats for release. Substrate, representative of beach and marsh habitat in this study, was an important factor affecting the likelihood of fish to bury. Previous work with the closely related Japanese flounder suggests that flatfish exhibit increased burying behaviour on preferred substrate (Tanda 1990), which has been identified as sand for juvenile summer flounder (Keefe and Able 1994). In support of Tanda's (1990) suggestion of increased burial on preferred substrate, both HR and wild fish in this study took significantly less time to exhibit cryptic behaviour (pigmentation response or burial) and were significantly more likely to bury on sand than on mud substrate. These results also support the conclusions of Keefe and Able (1994), who reported that metamorphic (about 15 mm standard length) summer flounder buried significantly more often in sand than in mud. If HR fish exhibit increased burying activity in sand substrate habitats in the wild, and if burial enhances survival, then these results suggest that sand substrate habitats may support greater survival of juvenile summer flounder than mud substrate nursery habitats. This information has important implications for release of HR summer flounder in the wild (i.e., optimal release habitat) and should be tested in the field.

In all other cases, substrate had no significant effect on behaviour or susceptibility to predation. These results suggest that if differences exist in habitat-specific behaviour and survival, then, aside from cryptic abilities (pigmentation response or burial), these differences are not a result of substrate but of other habitat-specific factors. Such factors may include both biotic (prey availability and predator guilds) and abiotic (differences in temperature, salinity, dissolved oxygen, etc.) aspects of particular habitats. Habitat-specific biotic and abiotic factors likely interact at many levels to affect behaviour, growth, and survival of flatfish and other estuarine-dependent fishes (Keefe and Able 1994; Burke 1995).

In summary, the results of this study suggest that HR summer flounder, reared in the psychosensory-deprived hatchery environment, are not well equipped to survive in natural environments compared with wild conspecifics. The modified behavioural patterns (behavioural deficits) exhibited by HR fish in this study may be a mechanism underlying the reduced ability of HR fish to withstand predation in laboratory trials. Importantly, HR fish exposed to natural predator stimuli exhibited increased survival rates in this study, suggesting that behavioural deficits and, more importantly, increased susceptibility to predation can be mitigated through predator conditioning. The empirical research described herein may serve as a framework for evaluating the behavioural and survival capabilities and the ability to mitigate behavioural and survival deficits for other stock enhancement candidate species. The type of experimental approach used in this study, which specifically addresses stock enhancement feasibility, is an essential component of a responsible and well-rounded approach to stock enhancement (Leber 1995).

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