



SHORT COMMUNICATION

Effects of moving acclimation cages before release of cultured fish: alternate release strategies for a juvenile winter flounder *Pseudopleuronectes americanus* stock enhancement effort

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Biomass of winter flounder *Pseudopleuronectes americanus*, a target species of both recreational and commercial fisheries, is at an all-time low (Northeast Fisheries Science Center 2008), and due to new unprecedented regulations, the largest of the three stocks is closed to all fishing activities in federal waters (National Oceanic and Atmospheric Activities 2009). Winter flounder population recovery could be expedited by stock enhancement (Waters 1996; Le Francois, Lemieux & Blier 2002), and experimental stocking studies have been conducted since 1996 in New Hampshire. The goal of past projects has not been to initiate large-scale releases. Instead, 'responsible approach' (Blankenship & Leber 1995) has been applied to develop the processes needed to successfully enhance winter flounder by answering key questions in the event that large-scale stocking efforts occur. Much of the research has focused on optimal release strategies for juvenile winter flounder.

In many stock enhancement programmes, initial survival rates of newly released cultured fish are low (Pitman & Gutreuter 1993; Stottrup & Sparrevohn 2007; Sudo, Kajihara & Fujii 2008). These may be due, in part, to stress responses in hatchery-reared fish causing departures from behavioural norms which result in a higher probability of mortality (Schreck, Olla & Davis 1997). Developing release strategies

that minimize stress responses and reduce post-release mortality is essential to any enhancement effort and can be done with a combination of hatchery and field techniques. One such technique is using acclimation cages *in situ*. For many fish species, acclimation cages improve post-release survival, growth, and site fidelity (Koshiishi, Itano & Hirota 1991; Jonsson, Brannas & Lundqvist 1999; Kuwada, Masuda, Shiozawa, Kogane, Imaizumi & Tsukamoto 2000; Brennan, Darcy & Leber 2006; Sparrevohn & Stottrup 2007).

For juvenile winter flounder, predation by green crabs *Carcinus maenas* is of special concern (Fairchild & Howell 2000; Taylor 2005). Acclimation cages have been used at the release site under the assumption that these cages reduce immediate post-release mortality. Cultured flounder, stocked into these cages for 48 h, adjust to the release site, hone their burial skills, begin pigment change, recover from the stress of transport to the release site and maintain high site fidelity post release (Fairchild & Howell 2004; Sulikowski, Fairchild, Rennels, Howell & Tsang 2005, 2006; Fairchild, Rennels & Howell 2009). However, the cages also can be detrimental by attracting predators to the release site. In a recent study, Fairchild, Rennels and Howell (2008) compared green crab density when cages were absent, present but empty

and present and containing flounder. They found that crab density increased dramatically from 1.8 to 4.1 crabs 50 m^{-2} when empty cages were deployed. They hypothesized that the cages themselves provided desirable crab habitat on the relatively featureless, sandy, estuary bottom. When cages stocked with fish were deployed, crab density increased even higher to 6.35 crabs 50 m^{-2} , indicating that the dense assemblages of flounder further attracted this crustacean predator. Based on these findings, it is apparent that modification of the release strategy is necessary in order to offset this predator problem, and alternate release strategies are being investigated.

One suggested alternate release strategy is to stock and set acclimation cages at the release area for the required 48 h. Instead of releasing the fish in the same spot, the cages would be moved gently to a nearby secondary release site to offset any predatory crab aggregations, and the fish would be released there. However, this site transfer could negatively affect the fish by inducing more stress. The objective of this study was to determine if this alternate release strategy would be effective by evaluating the stress levels of flounder, as measured by cortisol concentrations, when stocked acclimation cages were moved between two sites.

The study was conducted in August 2006 at two sites (A and B) in the Hampton-Seabrook Estuary,

NH (Fig. 1). Site A is a well-studied location in the Hampton River that has been used as a release site for previous winter flounder stock enhancement studies. Site B is approximately 250 m downriver from Site A and has been examined as an alternate release site (Fairchild, Rennels *et al.* 2008). Both sites are sandy areas devoid of any macroalgae or structure, about 4 m deep at mean high water, have high salinity and dissolved oxygen, and naturally occurring wild juvenile winter flounder populations (Fairchild, Sulikowski, Rennels, Howell & Gurshin 2008). August bottom water temperature in these areas typically ranges from 12 to 21 °C (Fairchild, Sulikowski *et al.* 2008).

Two acclimation cages ($32 \times 32 \times 10 \text{ cm}$; bottom surface area = 0.1 m^2) constructed of 4 cm plastic-coated wire and lined with 3 mm nylon were lowered from the boat to the bottom at release site A. Each cage contained 50 cultured juvenile winter flounder (118 days post hatch; mean TL = $40 \pm 8 \text{ mm}$; 102% stocking density) reared at the University of New Hampshire's Coastal Marine Laboratory (per the methodology of Fairchild, Rennels, Howell & Wells 2007). Fish were allowed to acclimate over 2 days in the cages to simulate the normal flounder release strategy. After 48 h, one cage was hauled to the surface and the fish were immediately snap frozen on dry ice. The other cage was raised and tethered to the



Figure 1 The Hampton-Seabrook Estuary in New Hampshire. Winter flounder release studies have been conducted in the Hampton River at site (a). An alternate release site (b) was tested approximately 250 m downriver.

boat so that it was still submerged, and towed at ≤ 1 kt to release site B. There the cage was lowered to the bottom for 10 min to simulate a release, then raised, and all fish were snap frozen on dry ice.

Samples for whole body cortisol analyses were prepared according to Sulikowski *et al.* (2005, 2006). Individual juvenile flounder from each acclimation cage were thawed, weighed to the nearest gram, and dissected into smaller segments to facilitate the homogenization process. To ensure cortisol levels fell within detectable levels, five juvenile winter flounder were combined in a 50 mL test tube to yield approximately 5 g of tissue which was equivalent to an individual sample. In this scheme, each treatment (cage) consisted of 10 samples of pooled fish. The control values were obtained from previous studies by Sulikowski *et al.* (2005, 2006). Briefly, hatchery-reared fish (those that were not moved into or part of the acclimation cage experiment) were hand netted, immediately frozen on dry ice (-70°C), and stored at -20°C for later use. This procedure took about 10 s and yielded 5 ng g^{-1} wet weight samples that contained consistent and statistically similar (analysis of variance; $P > 0.05$) cortisol levels.

Individual samples (5 g of pooled tissue) were homogenized in ice-cold phosphate-buffered saline (PBS), centrifuged and the supernatant removed. Each sample was extracted three times with a three-fold volume of ether (anaesthesia grade) and the aqueous phase was frozen in an acetone/dry ice bath to facilitate removal of the organic phase. Following evaporation of the ether under a stream of nitrogen, the dried extracts were reconstituted in PBS with 0.1% gelatin. Approximately $1000\text{ counts min}^{-1}$ of tritiated cortisol were added to account and correct for procedural losses. The overall mean recoveries were 76%. Duplicate samples of cortisol were analysed by the Atlantic Veterinary College (Prince Edward Island, Canada) using standard radioimmunoassay techniques (e.g. Tsang & Callard 1987; Sulikowski, Tsang & Howell 2004). The intra- and inter-assay coefficients of variance were 4.4% and 5.1% respectively. Differences in cortisol concentrations between fish that remained in site A and those that were moved to site B were compared using a *t*-test. A probability (*P*) value of < 0.05 was considered statistically significant.

Despite very gently moving the caged fish to a nearby alternate release site, the fish experienced a significantly higher level of stress ($t(9) = -4.83$, $P < 0.001$), as measured by cortisol concentration, than the caged fish which were not moved. Caged fish

that remained in site A had a mean (± 1 SEM) cortisol concentration of $10.7 \pm 1.9\text{ ng g}^{-1}$ wet weight, whereas caged fish that were moved to site B had a mean cortisol concentration of $45.6 \pm 7.1\text{ ng g}^{-1}$ wet weight. Although there is potential for whole-body preparations to contain other extractable, free steroid metabolites that could cross-react with the cortisol antibody and contribute to the total measured hormone concentrations (i.e. King & Berlinsky 2006), this was unlikely in this study. Given the specificity of the antibody, the consistently low intra- and inter-assay coefficients of variance (4.4% and 5.1% respectively), and the results from two previous studies on juvenile winter flounder (Sulikowski *et al.* 2005, 2006), these results clearly denote an observed stress response produced by cage movement. In other studies using cultured juvenile winter flounder, cortisol concentrations under non-stressed conditions were similar ($0.8\text{--}9.6\text{ ng g}^{-1}$), but stressed conditions yielded much lower values ($7.2\text{--}15.5\text{ ng g}^{-1}$) compared with this study (Breves & Specker 2005; Sulikowski *et al.* 2005, 2006). Winter flounder which experience a sharp cortisol increase, such as the moved caged fish did, likely will have lower survival post release.

Generally when fishes are exposed to a stressor, primary stress responses are induced (Mazeaud & Mazeaud 1981). These include an increase in the release of the catecholamines adrenaline and noradrenaline, and the subsequent release of corticosteroid hormones, principally cortisol, from the interrenal tissue. The associated distress of this primary stress response can induce a cascade of secondary (physiological) stress responses that subsequently cause tertiary (whole animal) responses. For example, elevated cortisol levels can affect physiological parameters such as growth (Barton, Schreck & Barton 1987; Pickering, Pottinger, Sumpter, Carragher & Le Bail 1991), reproductive status (Pankhurst & Van Der Kraak 1997), as well as disturbances to the immune system (Pickering & Pottinger 1989). In addition, elevated cortisol levels can affect fish behaviour, which in turn can modify food acquisition, predator avoidance, aggression, learning, habitat selection (Olla, Davis & Schreck 1995) and social interactions with conspecifics (Gregory & Wood 1999).

Despite many of the positive attributes benthic acclimation cages afford cultured winter flounder, they are not effective since predatory green crabs are attracted to them (Fairchild, Rennels *et al.* 2008), and moving the cages adversely affects the fish. Because there are significant benefits to acclimating winter

flounder *in situ* before release, it is worthwhile to try other cage designs. One such design is a floating cage system so that non-swimming green crabs are unable to reach the flounder. These floating cages would allow the fish to acclimate to the abiotic conditions of the environment and recover from any stress incurred during transport. Unlike benthic acclimation cages, the fish would have no contact with the substrate; however, pre-release substrate conditioning could occur in the hatchery.

Because cultured winter flounder incur a high stress response when acclimation cages are moved to avoid predators pre-release, this release strategy does not seem viable. However, the upper stress limit for juvenile winter flounder is unknown, and further research in this area would be useful for quantifying post-release effects of different release strategies. Determining the formula for maximizing post-release survival using combinations of release strategies is essential for a successful enhancement effort. Evaluating the physiological effects of varying stress levels in cultured fish, and providing guidelines of cortisol levels that should not be exceeded in released cultured fish would be helpful for maximizing the cost effectiveness in all enhancement programmes.

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