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Predators Are Attracted to Acclimation Cages Used for Winter Flounder Stock Enhancement

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Acclimation cages are used for juvenile, cultured winter flounder Pseudopleuronectes americanus *so that the fish can adjust to their new environment, hone their burial skills, begin pigment change, and recover from the stress of transport to the release site, all in the absence of predation. However, there have been indications that the cages attract the predatory green crab,* Carcinus maenas. *Studies conducted at the release site in the Hampton-Seabrook Estuary, New Hampshire, USA, determined that green crab abundance was significantly higher (ANOVA,* $p < 0.01$*) on cages containing fish than on empty cages, proving that acclimation cages containing flounder do attract green crabs. In addition, when empty acclimation cages were deployed, crab densities significantly increased (ANOVA,* $p < 0.01$*) in the vicinity of the acclimation cages and continued to increase each day, indicating that green crabs are also attracted to empty cages. Thus, although acclimation cages are a necessary tool that allows the stocked fish to adjust to their new environment, they also may be a detriment if they attract predators to the site. Using active adaptive management, release strategies must be reevaluated to reduce such predator-prey encounters, thereby improving fish survival.*

Keywords survival, predator-prey dynamics, release strategy

INTRODUCTION

In many stock enhancement programs, initial survival rates of newly released cultured fish are low (Svåsand and Kristiansen, 1990; Pitman and Gutreuter, 1993; Leber and Arce, 1996; Tsukamoto et al., 1997; Tanaka et al., 1998; Iglesias et al., 2003). Developing release strategies that reduce this post-release mortality is essential to any enhancement effort and can be done with a combination of hatchery and field techniques. For example, reforming normal hatchery protocols to include elements that are more "natural" aids cultured fish in their adaptation to the wild (Maynard et al., 1996; Olla et al., 1998; Brown and Laland, 2001). In addition, predator training is useful for some cultured species to learn to recognize and avoid potential predators before release (Olla and Davis, 1989; Brown and Smith, 1998; Berejikian et al., 1999; Hossain et al., 2002; Kellison et al., 2003). In the field, predator-exclusion acclimation cages benefit cultured fish by allowing them to recover from stress associated with transport to the release site and to adjust to their new environment. Studies using several species have shown that post-release survival, growth, and site fidelity are greater in acclimated fish than in non-acclimated fish (Koshiishi et al., 1991; Jonssonn et al., 1999; Kuwada et al., 2000; Brennan et al., 2006; Sparrevohn and Støttrup, 2007).

Experimental stocking studies of winter flounder *Pseudopleuronectes americanus,* a potential stock enhancement candidate in the northeastern U.S., have been conducted since 1996 in New Hampshire (Fairchild and Howell, 2000, 2001, 2004; Fairchild et al., 2005; Fairchild et al., 2007; Sulikowski et al., 2005, 2006). One of the primary goals of the study has been to determine optimal release strategies for this species in the event that large-scale stocking efforts occur. Acclimation cages have been used at the release site under the assumption that these cages reduce immediate post-release mortality. Cultured flounder are stocked into the cages so that the fish can adjust to the release site, hone their burial skills, begin pigment change (Fairchild and Howell, 2004), and recover from the stress of transport to the release site (Sulikowski et al., 2005, 2006). After 48 hours, the fish are released into the wild and post-release surveys commence.

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Like other young flatfish, juvenile winter flounder are vulnerable to a suite of decapod predators; predation by green crabs *Carcinus maenas* is of special concern (Fairchild and Howell, 2000; Taylor, 2005). In a field study prior to the 2004 release of fish, the average green crab density at the release site and surrounding areas was 0.6 crabs/50 m^2 (Fairchild, unpublished data). Cultured flounder were stocked into acclimation cages, placed at the release site for two days, and then released from the cages. Within four days after the release, crab density increased 7-fold to 4.3 crabs/50 m^2 in the immediate area. Crab density returned to baseline densities quickly thereafter, but cultured winter flounder density also decreased quickly. No cultured flounder were recaptured outside of the immediate release site despite frequent sampling.

The sharp increase in crab density, combined with the sharp decrease in flounder, led us to hypothesize that crabs may be attracted to, and aggregate around, the acclimation cages containing the fish, and that crab predation may have been responsible for the decrease in the cultured flounder density. To validate that hypothesis, two studies were conducted at the release site. The objectives were to determine (1) if green crabs are attracted to the acclimation cages, and (2) if acclimation cages, even in the absence of fish within them, attract crabs.

METHODS

Study Area

The Hampton-Seabrook Estuary, located in the southeastern corner of New Hampshire is a small (approximately 192 hectares at mean high water), tidally dominated, shallow estuary (Figure 1). The estuary is comprised of several tidal rivers (Brown River, Blackwater River, and Taylor and Hampton Falls rivers which converge to form the Hampton River) and numerous tidal creeks. All tidal waters enter and exit through the sole harbor entrance. The majority of the estuary bottom is characterized by fine- to medium-grained sand (roughly >90%), with the remainder described as mud (Larry Ward, unpublished data). Water depth is relatively shallow in the estuary, ranging from <1 m at mean low water in the tidal creeks to >6 m at the harbor entrance, with 1–3 m of water in most channels at mean low tide (Jones, 2000). Over 88% of the water in the estuary is replaced on each tide, with the average tidal flow equaling 623,000 l/s (PSNH, 1973). Due to this strong tidal flushing, low dissolved oxygen does not seem to be a limiting factor in the estuary as shown by Jones (1997).

An approximate 1-km^2 area in the Hampton River in the Hampton-Seabrook Estuary was selected as our release site. It was chosen because of its favorable characteristics for juvenile winter flounder. These include a fine- to medium-grained sandy substrate (Phelan et al., 2001; Fairchild and Howell, 2004), shallow areas (<2 m) at low tide, bounded by a deep channel (Saucerman and Deegan, 1991), with relatively high salinities (28–30 ppt) and an appropriate thermal regime. There is also good access for sampling vessels and very few features that obstruct towed nets. Finally, it is close to a site that has been extensively sampled (>20 years) for winter flounder juveniles by a local utility company (NAI, 2005). This long and continuous database indicates that winter flounder juveniles are more abundant in this location than others in New Hampshire estuaries.

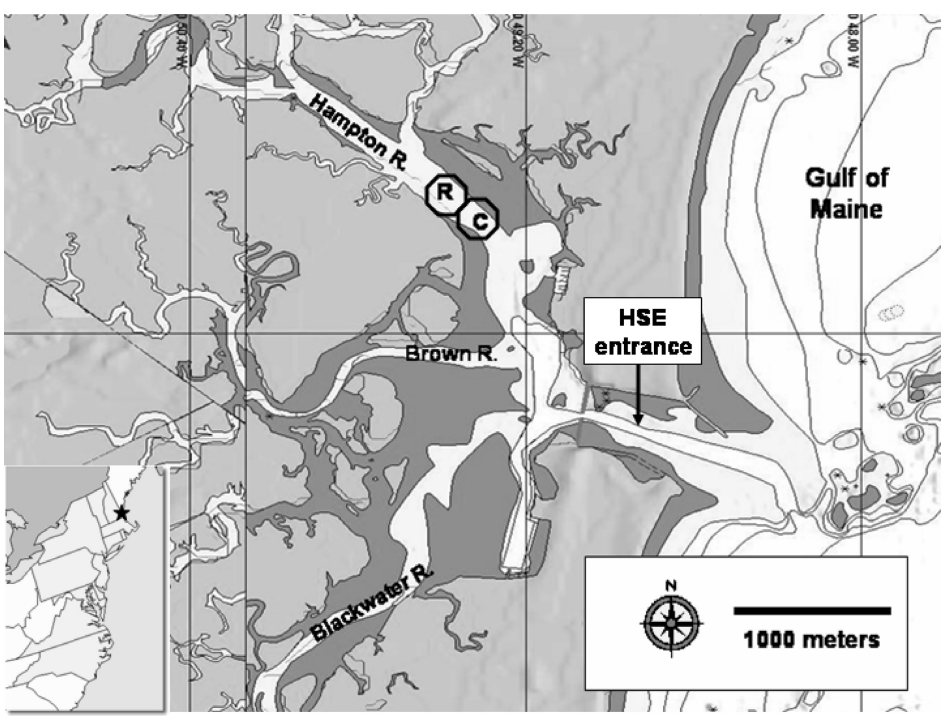

Figure 1 Hampton-Seabrook Estuary, New Hampshire, with inset showing location relative to the northeastern U.S. Winter flounder release studies (R) have been conducted in the Hampton River. A secondary site used as a control (C) was established approximately 250 m downriver.

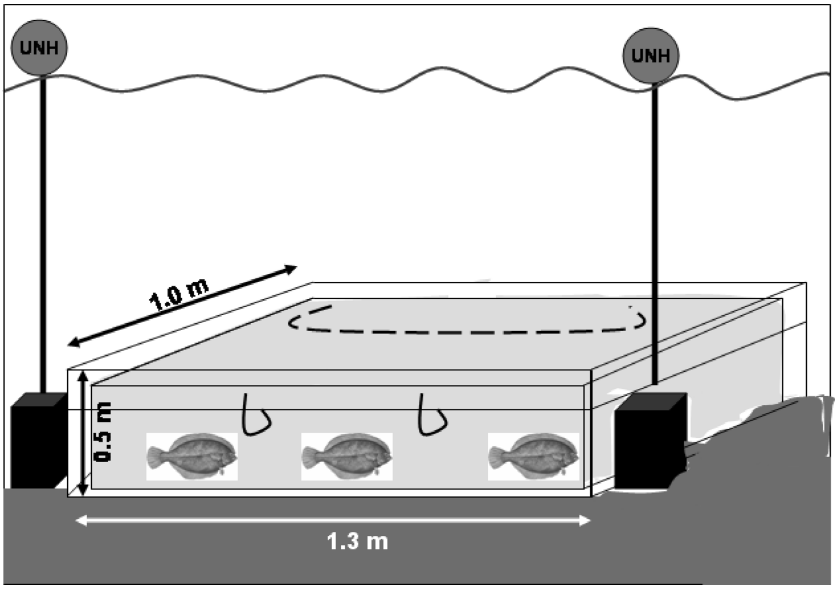

Figure 2 Fish are conditioned in plastic-coated wire acclimation cages lined with mesh and anchored on each end by cinder blocks tethered to buoys at the surface.

Use of Acclimation Cages

The acclimation cages (1.3 × 1.0 × 0.5 m) are constructed of 4-cm plastic-coated wire with a hinged lid that is secured shut by stretch cords (Figure 2). Inside the cage is a 3-mm nylon liner attached to the wire frame by cable ties. Attached to each end of the cage are two cinder blocks with buoy lines. During stocking, the cages are suspended off the stern of the research vessel so that they are partially submerged in water. Fish retrieved from live wells on board the boat are gently poured from buckets into the cage liner. Each cage is stocked with a known number of fish in order to standardize stocking densities, measured as the ratio of total fish ventral area to cage bottom area. When full, divers at the surface zip the liner shut, close the lid of the cage, and lower it to the bottom (typically 4–6 m). Once on the bottom, divers move the cages approximately 15 m apart and use 1-m long, 12-mm diameter rebar stakes to pin each cinder block to the bottom, which prevents the cages from moving with the tidal currents. For fish releases, divers descend to the cages, remove the stakes, open up the cages and liners, and gently turn the cages upside down to move the flounder out. All acclimation cage materials are then removed from the water.

Experimental Studies

To determine if green crabs are attracted to the acclimation cages, a study was conducted in August 2005 at the release site (Figure 1). Baseline crab densities were determined for 2 days before cage deployment using a 1-m beam trawl (4-mm mesh size) towed through the release site. Three replicate 50-m tows were done to calculate the mean green crab density, measured as number of crabs caught per 50 m^2. The following day, four acclimation cages were lowered from the boat to the bottom at the release site at high tide and secured by divers. Two cages were empty (control), while two cages contained cultured juvenile winter flounder reared at the University of New Hampshire's Coastal Marine Laboratory (per the methodology of Fairchild et al., 2007) and transported in live tanks aboard a research vessel to the research site. A total of 800 fish (mean TL = 42.3 ± 1.7 mm) were stocked into each cage (stocking density = 91%). Surveys of crab density within 5 m of each cage by SCUBA began approximately 15 min after deployment and continued daily at high tide for two more days. In this portion of the study, two divers simultaneously assessed each cage beginning with a count of green crabs found on the cage. Next, a 5-m line was clipped onto the middle of the cage and extended out to 2.5 m. The divers swam along this radius around the cage counting the crabs between the cage and 2.5 m. This technique was repeated for the 2.5- to 5-m area beyond the cage. Green crab counts were averaged to calculate a daily mean density (number crabs/50 m^2). The relationship between crab density and cage type (empty vs. with fish) over time was analyzed using ANOVA. Paired comparison t-tests were used to analyze crab density between cage treatments by day.

To determine if empty acclimation cages attract crabs, a second field study was conducted in September 2005. In this study, a second site (control) was established 250 m downriver from the release site (Figure 1). At both sites, baseline crab densities were determined by trawl surveys for two days as before. The following day, four empty acclimation cages were lowered from the boat to the bottom at the release site at high tide and secured by divers. No cages were deployed at the control site. Crab density surveys in both sites began approximately 15 min after cage deployment and continued daily at high tide for two more days. At the release site, these surveys were done by SCUBA, using

the same methods as in the first study, whereas at the control site, replicate beam trawl surveys were continued. Green crab counts were averaged to calculate a daily mean density (number crabs/50 m^2). The relationship between crab density and site (control vs. with cages) over time was analyzed using ANOVA.

RESULTS

Immediately after acclimation cages were deployed at the release site in the first study, green crab abundance increased from 2.2 to as much as 6.35 crabs/50 m^2 (Figure 3). Within one day after cage deployment, crab density was significantly higher (ANOVA, $p < 0.01$) on cages containing fish than on empty cages. However, before fish could be released from the cages the following day, green crabs gnawed through the nylon mesh cage liner, and the majority of the fish presumably escaped. Subsequently, crab density declined on these cages such that there was no statistical difference (t-test, $p = 0.06$) in crab density between treatments by day 5. Had the cage liners been crab-proof, crab density may have continued to increase. Despite this unanticipated difficulty, the acclimation cages containing flounder did attract more green crabs than empty cages.

If flounder were absent, green crabs still were attracted to the acclimation cages. Crab density significantly increased (ANOVA, $p < 0.01$) from 1.8 to 4.1 crabs/50 m^2 less than 15 min after empty cages were deployed in the second study (Figure 4). These cages continued to attract crabs at an exponential rate, and by the end of the study, crab density had increased to 10.8 crabs/50 m^2. Although the results indicate that more crabs were present at the release site than the control site after cages were deployed, two different gear types (visual dive surveys and beam trawl) were compared from day 3 to day 5. Because the relative efficiencies of these two survey methods are unknown for green crabs, these results are not definitive. However, for other taxa, like juvenile flatfish, small beam trawl efficiencies are known (Kuipers et al., 1992; Wennhage et al., 1997; Kellison et al., 2003; NUSCO 2006) and are lower than other methods. Considering that crabs move slower than flounders, more than likely, the gear efficiencies for this study are higher than in the flatfish studies.

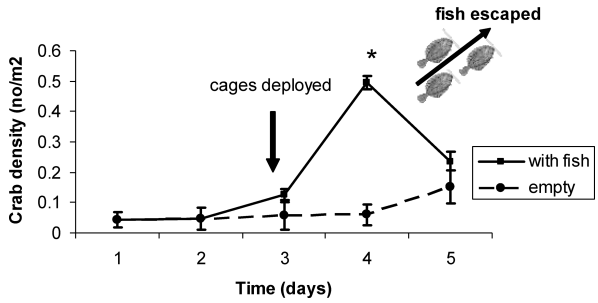

Figure 3 *Carcinus maenas* density before and after acclimation cage deployment. Two cages contained *Pseudopleuronctes americanus* and two were empty. Fish escaped from the cages after day 4. Asterisks (*) denote significant ($p \leq 0.05$) differences of crab density per day between treatments.

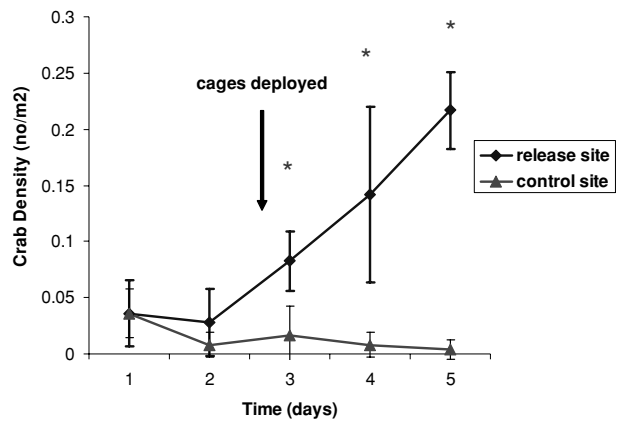

Figure 4 *Carcinus maenas* density at the release site (empty acclimation cages present) and a control site (acclimation cages absent). Asterisks (*) denote significant ($p \leq 0.05$) differences of crab density per day between treatments.

DISCUSSION

Since their introduction to the NW Atlantic coast, both green crab abundance and distribution have increased substantially (Glude, 1954; Audet et al., 2003; NAI, 2005; NUSCO, 2006). Although mean crab density in much of the Hampton-Seabrook Estuary is similar to that of other New England estuaries, as reported by Taylor (2005), crab density is lower at the Hampton River release site (see baseline densities, Figures 3 and 4). This, most likely, is due to the featureless landscape at the release site. The bottom type is sandy, and structures like macroalgae and rocks are absent. The only features are depressions created by burrowing animals (lobsters, crabs), several mooring blocks, and the occasional lobster trap. However, green crabs will migrate tens of meters to actively select favorable habitats (Moksnes, 2002); this phenomenon was observed in the second study when crab density increased 600% over five days (Figure 4), as presumably crabs moved into the release area from other areas within the estuary. Green crabs are attracted to the empty acclimation cages, and this is most likely because the cages provide shelter.

The predator-prey size relationship between green crabs and juvenile winter flounder has been evaluated in the laboratory (Fairchild and Howell, 2000). Cultured fish <20 mm TL were most susceptible to crab predation, though fish 21–70 mm TL were still killed at a rate of 4–8 %/day. Mortality rates decreased as the size ratio of fish to crab increased. In this study, mean flounder length was 42.3 ± 1.7 mm and, thus, beyond the most vulnerable size but still capable of being consumed by green crabs (Fairchild and Howell, 2000).

Fairchild and Howell's (2000) laboratory predator-prey study between green crabs and juvenile winter flounder was confirmed in the field by dietary analyses of green crabs collected from the Niantic River, CT (Taylor, 2005). Taylor (2005) found that green crabs consume 1–32% of the flounder year class and may be responsible for 0.4–8% of the daily mortality of young winter flounder. Since green crab densities are fairly low in New

England estuaries, Taylor (2005) believes that their effect on winter flounder populations is minimal and that other predators have a greater impact. However, compared to the Niantic River, diversity is low in the Hampton-Seabrook Estuary (Fairchild, unpublished data), and green crabs appear to be the dominant predator of young winter flounder. This predator could significantly reduce the impact of a localized winter flounder stocking effort since green crabs will congregate around acclimation cages, especially those containing flounder. Using active adaptive management (Blankenship and Leber, 1995), release strategies must be reevaluated to reduce such predator-prey encounters, thereby improving fish survival.

One way to lower crab-flounder interactions during releases is to release fish when crab density is lowest. Because crab foraging activity is highest during high tide (Hunter and Naylor, 1993; Gibson et al., 1998), fish survival may improve when released at low tide. In addition, green crabs are temperature sensitive (Glude, 1954; Ropes, 1969; Welch, 1969; Flach, 2003). In the Hampton-Seabrook Estuary, crab abundance increases throughout the warmer months and reaches a maximum in the fall, then decreases with the onset of cold winter water temperatures (NAI, 2005). Cultured fish could over-winter in the hatchery and then be released in early spring when green crab abundance is at its lowest. In addition, the fish would have a lower vulnerability to crab predation due to their larger size (>70 mm TL). A low-density crab zone could be created by luring crabs into traps or moving the acclimation cages to an alternate site right before release. Care must be taken, however, to ensure that fish are not stressed further by another move (Sulikowski et al., 2006). At a minimum, the cages must be redesigned so that crabs can not destroy the liner or get in.

The acclimation cages allow sediment-naïve, cultured winter flounder to hone their burial skills and undergo color changes to match the release site sediment (Fairchild and Howell, 2004). However, flounder-crab interactions might be reduced if the cages were used solely for the purpose of reducing transportation stress (Sulikowski et al., 2006) rather than for providing a semi-natural environment. In this scenario, the cages could be suspended off the bottom to prevent this non-swimming crab species from reaching the fish. Changes in hatchery protocols could be used to promote the needed burial skills and pigment adaptations before releases. In addition, preconditioning cultured winter flounder to green crabs may be possible; other flatfish species have shown increased survival after crustacean predator training (Kellison et al., 2000; Hossain et al., 2002). For example, juvenile Japanese flounder *Paralichthys olivaceus* had higher survival when they were conditioned to large caged or small (benign) sandy shore crabs *Matuta lunaris,* or they had experienced predation pressure by the crabs (Hossain et al., 2002). Similarly, cultured summer flounder *Paralichthys dentatus* survival was enhanced if fish were conditioned to blue crabs *Callinectes sapidus* (Kellison et al., 2000). Pre-release training will be essential to winter flounder, especially if they remain longer in the hatchery due to increased size-at-release strategies.

SUMMARY AND CONCLUSIONS

Although cages are necessary for acclimating cultured flounder (Fairchild and Howell, 2004; Sulikowski et al., 2005), they also are detrimental by attracting predators to the release site. Modification of the release strategy is necessary to offset this problem, and alternate release strategies must be investigated. Since the acclimation cages used thus far are crab attractants, at a minimum, the cages need to be modified. Using active adaptive management (Blankenship and Leber, 1995), release strategies must be reevaluated to improve fish survival. In addition, a more comprehensive understanding of the system slated for enhancement is necessary. In particular, the importance of predator-prey dynamics should not be overlooked.

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