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The influence of cage conditioning on the performance and behavior of Japanese flounder reared for stock enhancement: Burying, feeding, and threat response

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ABSTRACT

Flatfish reared for stock enhancement often exhibit irregular behavioral patterns compared with wild conspecifics. These "deficits", mostly attributed to the unnatural characteristics of the hatchery environment, are assumed to translate to increased predation risk. Initially releasing fish in predator-free conditioning cages may help flatfish adjust to the wild environment, establish burial skills, begin pigment change, recover from transport stress, and experience natural (live) food sources before full release into the wild. However, the impact of cage conditioning on the performance and behavior of flatfish has yet to be fully assessed. We conducted video trials with 10-cm, hatchery-reared Japanese flounder, *Paralichthys olivaceus*, in sand-bottomed aquaria to assess four treatments of flounder: (1) reared fish cage conditioned for 7 d in the shallow coast, (2) reared fish directly from hatchery tanks, (3) wild fish, and (4) reared fish released directly from hatchery tanks into the wild and then recaptured after 6 d at large. Burying ability, predation, and threat response to a model predator were examined. Wild fish buried most, followed by cage conditioned, and released-then-recaptured and non-conditioned (directly from tank) fish. Wild and conditioned fish revealed much lower variation in total movement duration, which corresponded with lower levels and variation in prey vertical movement. Fish of all condition types exhibited a lower number of attacks and off-bottom swimming events, and a lower movement duration when the model predator was in motion versus when it was still. This study is the first to evaluate the behavioral mechanisms of hatchery-reared flatfish that have been cage-conditioned or released-then-recaptured. In addition, we provide evidence that cage conditioning can enhance the performance of released flatfish.

1. Introduction

Stock enhancement, the spawning and rearing of organisms in captivity and releasing large numbers of young into the wild, is one of the few proactive strategies available to fisheries managers to restore, stabilize, or augment fish populations and thus fisheries catch. However, many stocked fish exhibit pronounced mortality immediately after release, attributed largely to behavioral deficiencies instilled by the unnatural hatchery environment (Flagg et al., 2000; Furuta, 1996, 1998; Hossain et al., 2002; Le Vay et al., 2007). For example, the higher incidence of off-bottom swimming behavior observed in hatchery-reared flatfish has been implicated as a leading cause of increased predation (Furuta, 1996, 1998; Kellison et al., 2000). In addition, released flatfish may take days to weeks before they begin feeding normally on wild prey (Fairchild, 2010; Furuta et al., 1997), and this short period of starvation can alter feeding behavior, which in turn may result in an even higher predation risk (Miyazaki et al., 2000). These behaviors (feeding and avoiding predation) thus are intricately linked.

These hatchery-induced behavioral deficiencies may be mitigated by providing some level of training or conditioning to reared flatfish, either in the hatchery or immediately before release in the wild. Examples of conditioning strategies that may be applied to flatfish in the hatchery include providing rearing tanks with sediments (Ellis et al., 1997; Fairchild, 2002; Fairchild and Howell, 2004; Miyazaki et al., 1997; Tanda, 1990), feeding fish live feeds (Furuta et al., 1997; Walsh et al., 2009), or introducing predator cues (Fairchild, 2002; Hossain et al., 2002; Kellison et al., 2000). Strategies that can be applied to ease the wild transition at, or near, the release site include conducting "operant conditioning" on fish to respond to light or sound cues for supplemental feed provision during the first few days/weeks post release (Anraku et al., 1998), or short-term release into predator-excluding cages before full release (Fairchild et al., 2008; Sparrevohn and Støttrup, 2007; Walsh et al., 2013). Cage conditioning allows hatchery fish to experience substrates and sediments, wild (live) food sources, and "safe" predator

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exposure (fish are able to see predators outside of cages and to detect olfactory predator cues) before actual release. In addition, the short period in the cage enables flatfishes to begin pigment change and recover from transport stress (Fairchild et al., 2008). Cage conditioning has shown to be effective in increasing post-release survival and recapture of flatfish species such as turbot, *Psetta maxima* (Sparrevohn and Støttrup, 2007), and Japanese flounder, *Paralichthys olivaceus* (Walsh et al., 2013).

Since 2008, Obama Laboratory, Japan Sea National Fisheries Research Institute, Fisheries Research Agency (JSNFRI-FRA), in Fukui, Japan, has been examining the effects of cage conditioning for Japanese flounder stock enhancement to establish if the strategy improves fitness of released individuals (i.e., to perform more like wild fish) or stocking success (i.e., the number of fish landed at market). From these releases, Walsh et al. (2013) assessed that recapture rates of conditioned fish caught by local fishermen were significantly greater than those of non-conditioned fish (i.e., fish released directly from hatchery tanks to the wild). In addition, laboratory experiments revealed that conditioned fish exhibited enhanced burying and feeding performance compared to non-conditioned fish. However, the degree to which behaviors refined during the cage experience contributed to enhancements in these performance measures remained unknown.

Video analyses allow the assessment of not only the end result of a performance measure (e.g., a buried fish; a full stomach), but also the means by which the end result was achieved (e.g., the sequence of behavioral events that lead to a buried fish or a full stomach). For example, in Walsh et al. (2013), burying ability was assessed by releasing recently conditioned and non-conditioned fish into tanks and returning after 5 min to quantify the number of fish buried. Likewise, feeding ability was assessed by providing tanks of conditioned and non-conditioned fish with prey and returning every 30 min to quantify how many prey remained. However, this form of experimental design did not allow observation of the behavioral mechanisms behind differences in performance.

Our objective was to assess not only whether cage conditioning enhances the performance of released juvenile flounder, but also to elucidate the behavioral mechanisms behind the performance. We approached this question by examining burying, feeding, and threat response behaviors, which we assessed by video-based experimental trials conducted in the laboratory immediately following the cage conditioning experience. We compared the performance and behavior of four fish types: (1) hatchery-reared “conditioned” fish that spent 7 d in a predator-free conditioning cage; (2) hatchery-reared “non-conditioned” fish directly from tanks; (3) “wild” fish; and (4) hatchery-reared, non-conditioned fish that were released and “recaptured” after 6 d at large in the wild.

2. Methods

2.1. Cage conditioning protocols and fish condition types

We based our experimental trials on the protocols instilled at Obama Laboratory, JSNFRI-FRA. Each of Obama Laboratory's 4 × 4 × 2 m cages holds between 2500–5000 fish depending on the year of release (H. Fujimoto, pers. comm.). We constructed conditioning cages on a smaller size scale (1 × 1 m × 0.5 m; 1/16th bottom surface area) of Obama Laboratory's cages (Fig. 1). Like Obama Laboratory's cages, the cage in the present study consisted of a metal-piped frame supporting a soft, 4-mm mesh enclosure on all sides. Since Japanese flounder are flatfish that primarily associate with the bottom, we focused on a cage density relative to bottom surface area that was similar to that of Obama Laboratory's 2010 Japanese flounder release in Obama Bay, Fukui, Japan; thus, approximately 150 fish from the hatchery were released into the predator-free enclosure.

Cages were erected in the shallow coast (1–2 m water depth) in a cove adjacent to Kyoto University's Maizuru Fisheries Research Station (MFRS), Maizuru, Kyoto, Japan (35°29′N latitude, 135°22′E longitude). To encourage burying and to mimic Obama Laboratory release procedures, approximately 5 cm of sand was distributed over the bottom of cages before fish introduction. Once introduced (June 24, 2010), fish were fed once per day with the hatchery-provided feed (formulated, commercially available pellets), as per Obama Laboratory protocol. Fish were conditioned in cages for 1 week before trial initiation.

Wild fish and (inadvertently) recaptured fish were seined from Kanzaki Beach, Maizuru, Japan, 1 to 2 d before trial initiation. Recaptured fish were identified by dark pigment spots located on the abocular side. These permanent markings (hypermelanosis) occur in over 95% of hatchery-reared fish but do not occur in wild fish, thus providing a “natural” marker (Tominaga and Watanabe, 1998). This evidence combined with a larger size compared to the local wild population, supported the assessment that these wild caught fish were hatchery reared. Investigation revealed that these wild-caught yet hatchery-reared fish were raised at Miyazu Laboratory, JSNFRI-FRA, and released by Kyoto Prefecture on June 23, 2010 (total length, TL [mean, range] of released fish = 10.3, 8.7 to 11.9 cm). Captured wild fish were smaller on average than all hatchery reared fish examined in trials (wild fish = 6.3, 5.0 to 8.0 cm TL; recaptured fish = 10.4, 8.8 to 12.5 cm TL; non-conditioned fish = 10.6, 9.4 to 12.3 cm TL; conditioned fish = 10.6, 9.9 to 11.8 cm TL); however, both 6-cm and 10-cm juveniles are within the size range of released seedlings for stock enhancement in Japan (Yamashita and Aritaki, 2010). Once collected, wild and recaptured fish were maintained together in a separate cage (identical to the conditioning cage) 1 to 2 m away from the conditioning cage in the shallow coast until trial initiation.

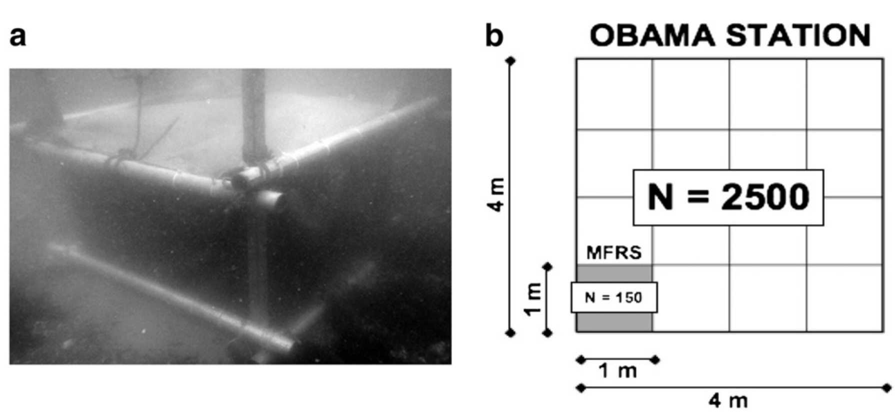

Fig. 1. Cage protocols at Maizuru Fisheries Research Station (MFRS) were smaller-scale representations based on Obama Laboratory protocols. Like those of Obama Laboratory, cages in the present study (a) consisted of a metal piped frame supporting a soft mesh enclosure on all sides. Cage density (b) matched that of Obama Laboratory's 2010 Japanese flounder release in Obama Bay, Fukui, Japan.