

Case Studies in Flatfish Stock Enhancement: A Multi-Year Collaborative Effort to Evaluate the Impact of Acclimation Cage Conditioning for Japanese Flounder, *Paralichthys olivaceus*, in Wakasa Bay, Japan

Michelle L. WALSH^{*1,3}, Hiroshi FUJIMOTO^{*2}, Takeo YAMAMOTO^{*2}, Tatsuya YAMADA^{*2},
Yoichi TAKAHASHI^{*2}, and Yoh YAMASHITA^{*3}

Abstract : Japan is the most active country in the world with respect to flatfish stock enhancement, both in the range of species and number of fish released. In Japan, Japanese flounder or hirame, *Paralichthys olivaceus*, is the primary species represented in the annual flatfish catch; thus, hirame has been a paramount choice for both aquaculture and stock enhancement for decades and is, in fact, the most important stocked marine finfish in Japan. A total of approximately 25 million Japanese flounder are released yearly from federal, prefectural, and local hatcheries throughout the country. Conditioning flatfish to the natural environment before release may increase successful recruitment to the fishery, as fish trained for “wild” conditions may transition more easily and successfully upon release. Since 2008, Obama Station, National Center for Stock Enhancement, has conducted pre-release experimental acclimation cage conditioning for Japanese flounder (N = 10,000-80,000) in both the Takahama and Obama portions of Wakasa Bay, Japan. Fish were reared via the “Hottoke shi-iku” method, a simplified rearing process that boosts cultivation efficiency of fingerlings by reducing rearing time and manpower. Recaptured fish were acquired through a cooperative effort between researchers and local fishermen (both commercial and recreational). To date, more conditioned fish have been recaptured via fishermen’s catch than non-conditioned fish. Initial observations suggest that non-feeding individuals captured near the release sites may be weaker and more likely to be caught by small boat beam trawl (towing speed 1-1.5 knots) than actively feeding, translocating fish. Thus, higher speed shrimp trawlers deeper in the bay (towing speed 3-3.5 knots) and set/fyke nets may be better, non-biased indicators of fitness and intermediate stocking success.

Key words : flatfish, hirame, *Paralichthys olivaceus*, Hottoke shi-iku, stock enhancement

Introduction

Flatfishes (flounder, halibut, sole) are among the most desirable and highly priced fishes sold for human consumption (Howell & Yamashita, 2005). Although flatfishes have supported valuable fisheries throughout the world for centuries, catches of many species have steadily declined. Many female marine fish species are capable of releasing hundreds of

thousands of eggs annually but because of the vulnerability of the small, early life-history stages, there is high natural mortality, and few survive to maturity. Spawning and rearing flatfish in captivity and releasing the young (i.e. stock enhancement) may help augment natural populations.

Over the past 10 years, researchers in the U.S. have conducted small-scale, experimental releases of winter flounder, *Pseudopleuronectes americanus*

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^{*1} University of New Hampshire, Department of Biological Sciences, Durham, New Hampshire 03824, USA
Email: michelle.walsh@unh.edu

^{*2} Obama Laboratory, Japan Sea National Fisheries Research Institute, Fisheries Research Agency, Obama, Fukui 917-0117, Japan

^{*3} Kyoto University Graduate School of Agriculture, Maizuru Fisheries Research Station, Maizuru, Kyoto 625-0086, Japan

(Fairchild, 2002; Potier, 2007), southern flounder, *Paralichthys lethostigma* (Tompkins, 2010; Sikes 2011), and summer flounder, *Paralichthys dentatus* (Kellison *et al.*, 2003) totaling approximately 46,000 fish. Japan, on the other hand, has been releasing flatfish as a fisheries management strategy for over 30 years. Releases of Japanese flounder (*Paralichthys olivaceus*), marbled flounder (*Pseudopleuronectes yokohamae*), barfin flounder (*Verasper moseri*), spotted halibut (*Verasper variegatus*), and at least four other species total over half a billion (Howell and Yamashita, 2005; Yamashita and Aritaki, 2010). Japanese flounder, or hirame, is the primary species represented in the annual flatfish catch; thus hirame has been a paramount choice for both aquaculture and stock enhancement for decades and is, in fact, the most important stocked marine finfish in Japan. A total of approximately 25 million Japanese flounder are released annually from federal, prefectural (state), local, and private hatcheries throughout the country (Tomiyama *et al.*, 2008).

To gain perspective on the feasibility of stocking flatfish as well as establishing effective strategies for large-scale releases, M. Walsh initiated collaboration with Y. Yamashita of Kyoto University's Maizuru Fisheries Research Station and Obama Station, National Center for Stock Enhancement, Fisheries Research Agency, in 2009 as part of her doctoral program at the University of New Hampshire. Our objective here is to present details of how and why we established this collaboration, as well as to share preliminary observations regarding cage conditioning for flounder stock enhancement.

Funding a US-Japan Collaboration

A number of funding opportunities (both US and Japan-based) exist for graduate students to engage in collaborations (Table 1). UJNR hosted a student exchange for aquaculture in 1998, however that funding opportunity has since been discontinued. The East Asia and Pacific Summer Institute (EAPSI), sponsored jointly by the National Science Foundation (NSF) and the Japan Society for the Promotion of Science (JSPS), offers an approximately three-month duration fellowship for U.S. graduate students to conduct summer research in Japan (as well as in other Asian-Pacific countries). Fulbright

offers highly competitive international scholarships lasting approximately 9-18 months (depending on host country). The Japanese Government offers longer-term (1-3 year) Monbukagakusho Scholarships for both undergraduate and graduate students, and the Japan Student Services Organization (JASSO) provides short-term opportunities (3 months-1 year) for foreign students to study or conduct research in Japan. NSF, JSPS, and Fulbright also provide opportunities for postdoctoral and professional level researchers to work in Japan, as well as for Japanese researchers and students to work in the U.S. The current collaboration with Obama Station was predominantly funded and initiated via EAPSI and Fulbright scholarships.

Acclimation Cage Conditioning for Flatfish Stock Enhancement

Hatchery reared flatfish exhibit irregular swimming, feeding, and cryptic (burial and color change) behavioral patterns compared with wild conspecifics, and these behavioral "deficits" are assumed to translate to increased predation risk once fish are released into nature (Furuta, 1996; Kellison *et al.*, 2000). Conditioning flatfish to natural stimuli before release can increase survival, and thus successful recruitment to the fishery, as fish trained for "wild" conditions may transition more easily and successfully upon release. (Kellison *et al.*, 2000; Sparrevohn and Støttrup, 2007). Predator-free acclimation cages may help flatfish adjust to the wild environment, establish burial skills, begin pigment change, recover from transport stress (Fairchild *et al.*, 2008a), and experience natural (live) food sources before full release into the wild. Obama Station has been examining the effects of acclimation cage conditioning for Japanese flounder since 2008 in order to establish whether the practice increases flatfish stocking success.

Methods

In brief, the main protocols were to raise flounder by the "Hottoke shi-iku" method — a low labor, high efficiency rearing technique (Takahashi, 1998), until they reached approximately 10-12 cm in late June/early July. Then, approximately half of the

Table 1. Graduate student funding opportunities for US-JPN collaboration

| AWARD | SPONSORING AGENCY | DURATION | WEBSITE |
|---|---|---|--|
| East Asia and Pacific Summer Institutes (EAPSI) | National Science Foundation (NSF) and the Japan Society for the Promotion of Science (JSPS) | 2 months (set dates) | www.nsf.org |
| JASSO International Student Scholarship for Short-term Study in Japan | Independent Administrative Institution Japan Student Support Services (JASSO) | 3 - 12 months (flexible dates) | www.jasso.go.jp/scholarship/short_term_e.html |
| Fulbright Graduate Research Fellow | Institute of International Education (IIE), U.S. Department of State | 12 - 18 months (flexible dates) | www.iie.org/fulbright |
| Monbukagakusho Scholarships | Japan Ministry of Education, Culture, Sports, Science and Technology (MEXT) | 1-5 yrs (semi-flexible dates in accordance w/academic year) | www.studyjapan.go.jp/en/toj/toj0302e.html |

reared fish were moved into 4 x 4 m acclimation cages at the release site for one week. Within 1-2 days, hatchery fish directly from tanks were released with conditioned fish from the acclimation cages. Researcher-initiated beam trawling (net size = width 2 m x height 20 cm x 8 mm² mesh; < 5 m depth) was conducted 1-2 times per week for the next month. Longer-term recaptures were supplied by fishermen (set [fyke] net, shrimp trawl and recreational fishermen [Fig.1]). All ethanol-preserved

recaptures were examined for markings, measured for growth, and subjected to dietary analyses (discussed elsewhere). Recapture rates, locations, and movements were documented.

Marking

Hatchery-reared Japanese flounder exhibit a high incidence (> 95%) of black, spotty malpigmentation on their blind (abocular) side (Tominaga and Watanabe, 1998), which acts as a natural marker of a

放流したヒラメを見つけて下さい

- ・12cmのヒラメ稚魚を甲ヶ崎へ放流しました。
- ・放流したヒラメは、囲い網の中で放流する環境に慣れさせた**囲い網群**と、飼育していた水槽でそのまま育成した**水槽育成群**の2群です。
- ・2群ともそれぞれ異なる焼き印標識をしています。

放流した場所もここです



囲い網群(背側焼き印 3点):8,200尾



・このような焼き印が付いたヒラメを見つけた方は下記までご連絡下さい

水槽育成群(腹側焼き印 3点):4,500尾



小浜栽培漁業センター :0770-52-2660 (担当 藤本)

Fig. 1. Poster distributed to recreational fishermen in eastern Obama Bay. Note the series of marks denoting conditioned (dorsal) and non-conditioned (ventral).

stocked fish. Condition status was determined using two methods: (1) a series of burn marks or brands inflicted on each fish's blind side (Fig. 1), and (2) by soaking fish en masse in an alizarin complexone

(ALC) dye bath during rearing. Two ALC ring marks on the otolith (i.e., via two immersions) denoted a conditioned fish, whereas one marked a non-conditioned fish.

Study Sites

Wakasa Bay is located in central Honshū along the north coasts of Fukui and Kyoto prefectures facing the Sea of Japan (Fig. 2). We focused releases in the western portion of Wakasa Bay. In 2008 and 2009, approximately 40,000 and 80,000 10-cm juveniles were released in Takahama Bay (35°N29' 38", 135°E32' 44"). In 2010, approximately 13,000 12-cm juveniles were released into the eastern portion of Obama Bay (35°N31' 59", 135°E45' 17"). Releases in Takahama Bay and Obama Bay occurred along the sandy coastline in waters 1-2 m deep. Both bays deepen into muddy-bottomed centers towards the mouth; Obama Bay depths increase to over 20 m providing appropriate habitat for a shrimp-trawl fishery.

Results

Fish Locations and Movement

In 2008, there were no researcher-initiated recapture attempts via beam trawl; recaptures were collected solely via local fishing (set-net) efforts and market landing data from the Takahama Bay fishermen's co-op. Nine set-nets were distributed coastally around Takahama Bay (Fig. 3a). Conditioned fish were released on July 3 and non-conditioned fish were released the following morning. The first recaptures (one conditioned and one non-conditioned fish) occurred four days after release. It took five

days for the first recaptured (conditioned) fish to move 4.5 km to the opposite (western) side of the bay. Within the first month of release, conditioned fish slightly led the advancement (by 1-2 days) towards the mouth of the bay. However, the mouth of Takahama Bay is wide, and we had no way to monitor the vast expanse of deeper waters in the middle of the bay. Our last confirmed fish location was of a no-conditioned fish at the mouth of the bay on October 13th.

In 2009, researcher-initiated beam trawling was conducted via local fishermen boat charter starting the day after all fish were released. Conditioned fish were released on June 29 and non-conditioned fish were released the following morning. The first recaptures (eight conditioned and 17 non-conditioned) occurred during initial beam trawl sampling on July 1. That year, both conditioned and non-conditioned fish appeared to disperse equally (Fig. 3b). After one week, both conditioned and non-conditioned fish were detected 3.5 km across the bay. Fish captured near the release site, which were mostly no-conditioned fish, showed very little in their stomachs even up to one month after release. Our last confirmed location was of a no-conditioned fish in the lower bay on November 12th.

In 2010, the release site was moved east to Obama Bay (Fig. 3c), closer to the hatchery. Unlike Takahama Bay, Obama Bay has a very tapered mouth, the deeper waters of which are utilized

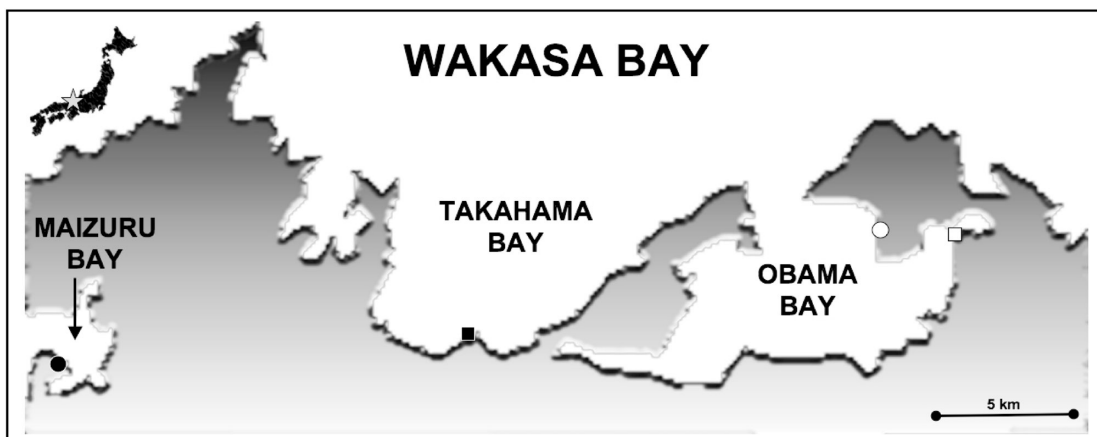


Fig. 2. Study sites in Wakasa Bay, central Honshū, Japan. Black square marks the release site in Takahama Bay (2008, 2009) and white square marks the release site in Obama Bay (2010). Black circle denotes Kyoto University's Maizuru Fisheries Research Station, and white circle denotes the National Center for Stock Enhancement, Obama Station.

by a substantial shrimp trawl fishery. Out of a fleet of 10 trawlers, one was hired to collect and preserve all Japanese flounder bycatch until the end of the season in November. In addition, since there are no set-net fishermen in Obama Bay, five stationary fish traps were set up near the release site. Researcher-initiated beam trawling began three days after release. All fish were released on July 6, and the first recapture (one conditioned fish) was collected the morning after release in the fish trap nearest to the release site. Two weeks after release, the first conditioned fish began appearing in shrimp trawls approximately 5.5 km away. Again, fish captured near the release site were mostly non-feeding, non-conditioned individuals. The number of recaptured conditioned fish (41) quickly surpassed the number of non-conditioned fish (13) captured via shrimp trawler. Our last confirmed location was of a conditioned fish caught by shrimp trawler on November 20th.

Recapture rates

If we examine the overall total recapture rate for all three years combined (including researcher-initiated beam trawling; Table 2), recapture rates for conditioned fish were slightly less than those for non-conditioned fish (0.0025 as compared with 0.0026). However, since the goal of Japan's flounder stocking effort is to overcome recruitment limitations by augmenting natural juvenile supply, and thus optimizing fishing harvest (Yamashita and Aritaki, 2010), total fishermen recapture rates provide a much more practical assessment of conditioning success. Focusing on fishermen's recapture efforts and market landing data, acclimation cage-conditioned fish were recaptured more than non-conditioned fish over all three years combined (recapture rate = 0.0020 conditioned, 0.0017 non-conditioned). In Takahama Bay in 2008, conditioned fish (0.0021) were recaptured more than non-conditioned fish (0.0018), but that trend was reversed in 2009 (0.0013 conditioned, 0.0015 non-conditioned). In 2010, recapture rates of conditioned fish almost doubled that of non-conditioned fish (0.0056 and 0.0031 for conditioned and non-conditioned). Market landing data will continue to be collected.

DISCUSSION

Conditioned fish exhibited higher overall performance than non-conditioned fish in terms of movement and fishermen's recapture rates. Sparrevojn and Støttrup (2007) investigated the effects of transferring turbot (*Psetta maxima*), to enclosures at the release site six days prior to release and found that conditioning had a positive effect on flatfish survival: mortality of cage conditioned fish was half that of non-conditioned fish. Kellison *et al.* (2003) found that hatchery-reared fish released in cages showed no difference in habitat-specific growth rates compared with wild fish. Released hatchery-reared flatfish have been shown to have lower residence time than their wild counterparts (Kellison *et al.*, 2003), but site fidelity can be increased by transferring fish to *in-situ* acclimation cages before release (Fairchild *et al.*, 2008b). Conditioned fish in our study moved more actively toward the mouths of the bays than non-conditioned fish, but this may be a result of (1) high coastal water temperatures (up to 30°C) in the shallows of the release area during the summer release time causing the fish to move toward cooler, deeper water, and (2) potentially higher concentrations of prey in the deeper waters of the bay, e.g. shrimp, which form the basis of the trawling industry in the middle of Obama Bay.

Given the empty stomachs of many of the fish recaptured near the release site, our work indicates that slow boat speed (< 1 knot) beam trawling efforts may target weak, non-feeding, unmoving fish. Non-conditioned fish, mostly non-feeding individuals, were caught more often by beam trawl than conditioned fish each year when researcher initiated recapture efforts were conducted. Similarly, Sparrevojn and Støttrup (2007) found that the catchability of non-conditioned turbot caught by beam trawl was 10% higher than that of cage conditioned fish. Efforts and money for recapture may be better spent with more local fishermen involvement, especially considering shrimp trawlers tow faster (approximately 3.5 knots), use a larger net (width 6 m x height 4 m x length 16 m, 18 mm² cod-end mesh), and are capable of surveying deeper (> 20 m) waters.

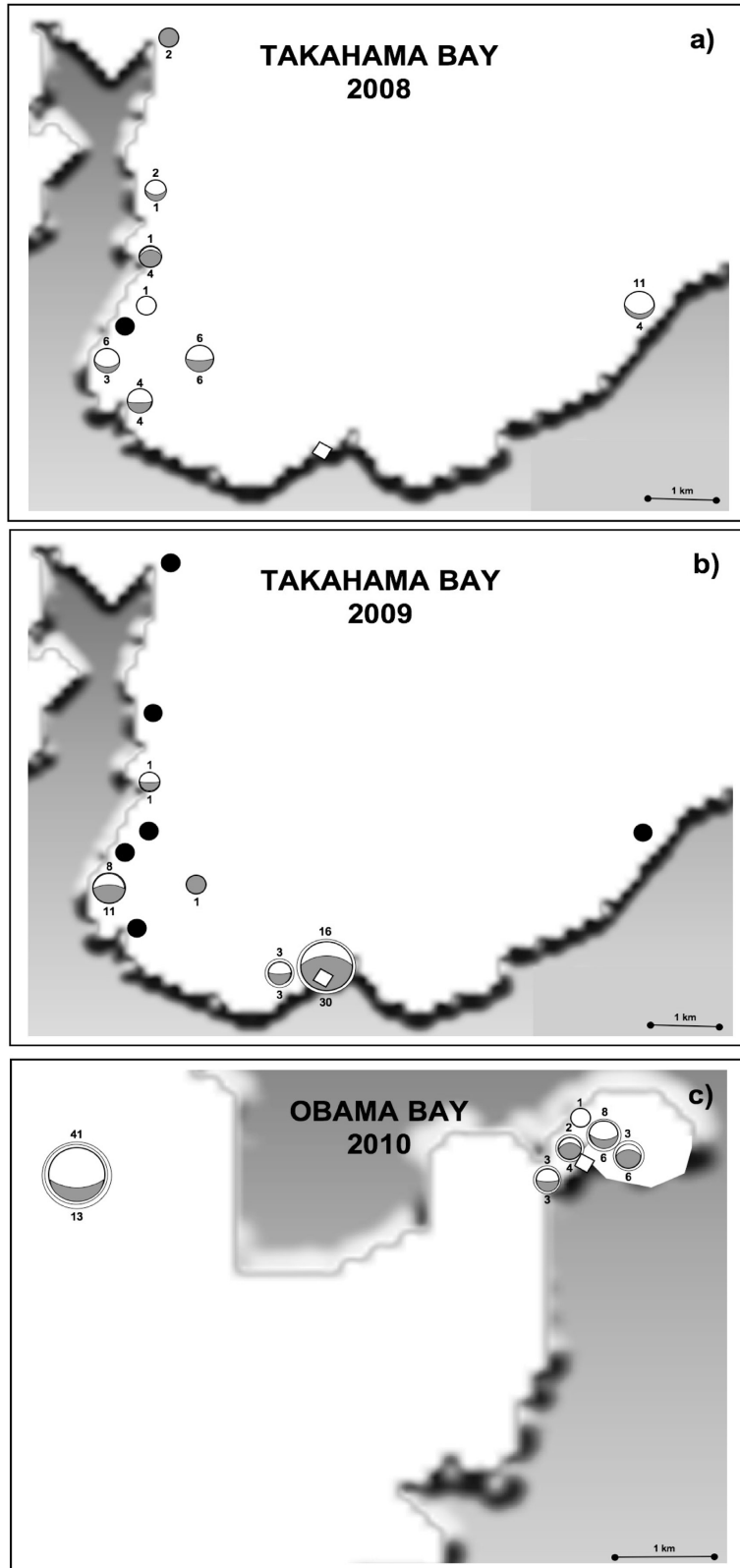


Fig. 3. Recapture locations of hatchery released Japanese flounder in (a) Takahama Bay in 2008 and (b) 2009 and (c) Obama Bay in 2010. Single-ringed circles denote fish recaptured by set-nets or traps, double-ringed circles by beam trawl, and triple-ring circles by shrimp trawler (with the exception of one fish caught in the trap nearest to the release area in 2010, which was included in the beam trawl tally nearest to the release site). Size of the circle reflects number of fish recaptured at each location. Degree of shading of the circle (white = conditioned; gray = non-conditioned) reflects the ratio of conditioned to non-conditioned fish recaptured at each location with numbers on the tops and bottoms of circles indicating actual numbers of conditioned and non-conditioned fish recaptured, respectively.

Table 2. Release/recapture data for acclimation cage Japanese flounder stocking efforts 2008-2010 (last updated December 31, 2010). Total recapture includes researcher-initiated beam trawling. Total fishermen recapture includes all fishing and passive trap recaptures (includes market landing data), but does not include researcher initiated beam trawling

| YEAR | CONDITION STATUS | RELEASE | | TOTAL RECAPTURE | | | FISHERMEN RECAPTURE | | | LAST RECAP DATE |
|---------|------------------|-----------|-----------|--------------------------------------|---------------|----------------|--|---------------------|----------------------|-----------------|
| | | # release | % release | TOT # (includ. research beam trawls) | TOT # recap % | TOT recap rate | TOT # recap (setnet, trap, shrimp trawl, rod&reel, market) | TOT # recap fshmn % | TOT recap fshmn rate | |
| 2008 | conditioned | 23,400 | 52.35 | 49 | 56.32 | 0.0021 | 49 | 56.32 | 0.0021 | 08/04/10 |
| | Non-conditioned | 21,300 | 47.65 | 38 | 43.68 | 0.0018 | 38 | 43.68 | 0.0018 | 10/14/09 |
| | total | 44,700 | | 87 | | 0.0019 | 87 | | 0.0019 | |
| 2009 | conditioned | 40,500 | 52.39 | 71 | 44.38 | 0.0018 | 52 | 48.15 | 0.0013 | 12/26/10 |
| | Non-conditioned | 36,800 | 47.61 | 89 | 55.63 | 0.0024 | 56 | 51.85 | 0.0015 | 12/26/10 |
| | total | 77,300 | | 160 | | 0.0021 | 108 | | 0.0014 | |
| 2010 | conditioned | 8,200 | 64.57 | 59 | 64.13 | 0.0072 | 46 | 76.67 | 0.0056 | 11/20/10 |
| | Non-conditioned | 4,500 | 35.43 | 33 | 35.87 | 0.0073 | 14 | 23.33 | 0.0031 | 10/19/10 |
| | total | 12,700 | | 92 | | 0.0072 | 60 | | 0.0047 | |
| OVERALL | conditioned | 72,100 | 53.53 | 179 | 52.80 | 0.0025 | 147 | 57.65 | 0.0020 | 12/26/10 |
| | Non-conditioned | 62,600 | 46.47 | 160 | 47.20 | 0.0026 | 108 | 42.35 | 0.0017 | 12/26/10 |
| | total | 134,700 | | 339 | | 0.0025 | 255 | | 0.0019 | |

In 2009, 80,000 fish were released, the highest number of all three years, yet that year was the worst with respect to fishermen recapture rate. Successful stocking endeavors require post-release densities below the carrying capacity of the release environment (Munro and Bell, 1997). The large number of fish released into Takahama Bay at one location may have strained the immediate carrying capacity of the environment, resulting in low overall recapture. Indeed, both Tanaka *et al.* (2005) and Sparrevohn and Støttrup (2007) found that during years of higher release numbers, the fraction of recaptured flatfish with food items in their stomachs

was lower than in years when fewer numbers were released. The sudden increase in the number of flatfish predators at the release site may cause short-term, density-dependent ecological changes in the dynamics of the prey species, e.g., prey numbers as well as the behavior of prey (Sparrevohn and Støttrup, 2007), and those changes may affect the overall success of the stocking effort.

Acclimation conditioning cages may provide flatfish released for stock enhancement with time for wild behavioral adjustment, which may increase burying and feeding ability and decrease predator mortality (Sparrevohn and Støttrup, 2007).

Nevertheless, conditioning cages in themselves may attract predators simply by providing structure to a barren bottom (Fairchild *et al.*, 2008a). Swimming crabs (*Portunus gladiator*), confirmed predators of juvenile Japanese flounder (Saitoh *et al.*, 2003), were observed crawling on the outside of conditioning cages in Obama Bay and around the release site in Takahama Bay; however, the number of crabs observed was much fewer than that of the green crabs (*Carcinus maenas*), observed in Fairchild's study (pers. obs).

Japan affords invaluable opportunities for flatfish stock enhancement research that at this time is not yet feasible in the U.S. The size and scope of Japanese releases far surpasses U.S. experimental releases, which do not provide a large enough release or recapture sample for full detailed analyses of the success of an *in-situ* stocking or conditioning effort. U.S. fisheries managers and scientists, therefore, can regard Japanese flatfish stocking efforts as case studies from which to model and base their own developing flatfish stocking programs.

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- Yamashita Y. and Aritaki M., 2010: Stock enhancement of Japanese flounder in Japan. in Daniels H. V. and Watanabe W. O. (Eds.), *Practical Flatfish Culture and Stock Enhancement*. Blackwell Publishing, pp. 237-255.
- This review takes a step back and regards the overall impact of stocking Japanese flounder in Japan. The authors compile data from many individual Japan-wide releases and compose "big picture" analyses of statistics such as market return rate and annual catch change rate.

Annotated References

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