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Successful stocking of a depleted species, spotted halibut *Verasper variegatus*, in Miyako Bay, Japan: evaluation from post-release surveys and landings

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ABSTRACT: Post-release adaptation and stocking effectiveness of a highly depleted pleuronectid flatfish, spotted halibut *Verasper variegatus*, were assessed in Miyako Bay, Japan. In early July 2004 and 2005, 22 000 and 5000 hatchery-reared juveniles (mean 81.2 and 89.8 mm total length, respectively) were released in shallow waters in the inner bay area. Results from surveys with a 2 m beam-trawl conducted over a 2 mo post-release period, laboratory predation experiments using potential predators (age-1 Japanese flounder), and a market census continued until December 2008, suggested that released juveniles, which had a size refuge from predators, could quickly adapt to brackish water habitats where there were suitable physical conditions (temperature, salinity and sediments) and abundant prey organisms. Juveniles selectively preyed on epibenthic gammarids and cumaceans, and had full stomachs 1 wk after release. Some released fish grew to >40 cm by age-1+, feeding exclusively on larger crustaceans (e.g. isopods, carideans and brachyurans). Market census revealed that released fish showed >99% contribution to catches, and recapture rates for fish released in 2004 (7.6%) and 2005 (15.7%) were higher than for releases in 2000 to 2003 (0.7 to 5.0%), possibly due to the development and application of improved release strategies (i.e. release habitat, size and season was appropriate in 2004 and 2005). Our study demonstrates that (1) local depleted stocks can be effectively restored by hatchery releases and that (2) development of release strategies based on the analysis of ecological interactions between released juveniles and the target ecosystem can largely contribute to the successful restocking of depleted species.

KEY WORDS: Restocking · Depleted species · Optimal release strategy · Post-release adaptation · Feeding ecology · Predation experiments · Market census · *Verasper variegatus*

INTRODUCTION

Marine stock enhancement and restocking, in which mass hatchery-reared juveniles are released in the wild to help enhance/restore depleted stocks, have been refined as one of the management tools of declining fisheries (Bell et al. 2006, 2008). The practical aspects and potential impacts of stocking practices, as well as integrated approaches, have been recently documented (Blaxter 2000, Bell et al. 2008, Lorenzen 2008).

Commercially important coastal flatfishes have been key species for stock enhancement (Blaxter 2000), possibly due to their less migratory and sedentary lifestyle (Able et al. 2005, Gibson 2005), and criteria for stocking have been defined (Støttrup & Sparrevohn 2007).

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One of the criteria is that there should be a recruitment limitation at a life stage. However, regardless of recruitment levels in natural populations, over 20 million hatchery-reared Japanese flounder *Paralichthys olivaceus* have been released annually along the entire coast of Japan (Kitada & Kishino 2006). While these extensive stocking programs have contributed to stabilize the catch of *P. olivaceus* (Yamashita & Aritaki 2010), reduced effects of stocking have been found (e.g. decline in contribution rate, recapture rate and market price), especially when a dominant year-class occurs (Tomiyama et al. 2008). However, clear and direct effects of release trials have been reported for smaller, more localized releases of highly depleted species, where stock size of wild populations were small but releases were closely followed by increased landings (Howell & Yamashita 2005).

Spotted halibut *Verasper variegatus* is a pleuronectid flatfish distributed in coastal waters of northeastern Asia, growing to 65 cm total length (Chen et al. 1992). *V. variegatus* is a target species for 'restocking' (1 of 3 categories of juvenile release practice aimed at restoring severely depleted spawning biomass; Bell et al. 2008) because of their depleted stock conditions, high commercial value and high growth performance (Wada et al. 2006, Shimamura et al. 2007). Commercial landings decreased during the 1980s (Nemoto et al. 1999), alongside the isolation of small local populations off northeastern Honshu, Seto Inland Sea and western Kyushu (Ortega-Villaizán Romo et al. 2006; see Fig. 1a). Specific habitat requirements during settlement include shallow tidal flats (Wada et al. 2006) or protected sandy shores (Noichi et al. 2006); recent degradation and/or loss of these key habitats is considered to have contributed to reductions in recruitment of wild fish (Wada 2007). Numbers of spotted halibut juveniles released have increased from 1000 in 1993 to >300 000 in 2006, with increased landings in some prefectures. For example, in the Fukushima Prefecture, catch increased from 0.8 t and 0% hatchery contribution in 1993 to 2.5 t and 62% hatchery contribution in 1996 (Nemoto et al. 1999). However, recapture rates vary largely between years, and release protocols have been developed by trial and error, due largely to the lack of basic ecological data (e.g. feeding, growth and distribution) of wild juveniles in each locality, which are the indices of release habitat suitability (Tanaka et al. 2005, 2006, Sparrevohn & Støttrup 2008).

Recent studies have revealed that poorly developed behavioral skills in hatchery-reared juveniles, such as feeding or burying ability and escape responses from predators (Furuta 1996, Fairchild & Howell 2004), could lead to high predation-induced mortality just after release (Furuta et al. 1997, Tanaka et al. 2006). Although conditioning and training before release have been reported to mitigate such mortality (Hossain et al. 2002, Sparrevohn & Støttrup 2007), the key to better survival of released juveniles is to optimize the release strategy (i.e. release habitat, season, juvenile size and number released in relation to the nursery area); this should be based on the ecology of wild juveniles and the population dynamics of predators and prey (Furuta 1996, Yamashita & Yamada 1999). Therefore, a more comprehensive approach should be developed for the release strategy of highly depleted species for which ecological data of wild counterparts is often lacking.

We aimed to develop appropriate release protocols for spotted halibut through the assessment of (1) adaptation processes in released juveniles from distribution, growth, feeding and prey selectivity, (2) adequate refuge size for juveniles through laboratory experiments and evaluation of predator densities in the wild, and (3) commercial recapture data of release cohorts from 2000 to 2005. On the basis of these analyses, the important points for establishing the optimal release strategy of this depleted species are clarified and potential and future perspectives of spotted halibut restocking programs are discussed.

MATERIALS AND METHODS

Study area. This study was performed in Miyako Bay, Iwate Prefecture, located in northeastern Japan (Fig. 1). Miyako Bay is a semi-enclosed bay area which has a wide variety of environments such as rocky shores, sandy beaches and tidal flats. The point at which the Tsugaruishi River flows into the bay provides suitable nursery habitats for many coastal fishes. However, no collection record of spotted halibut juvenile exist and wild adults were rarely caught in this bay (e.g. only 5 wild halibut were landed in 1998). Gillnet, set net and longline fisheries all operate in Miyako Bay (Okouchi et al. 2004). This protected bay is appropriate for assessing stocking effectiveness as 90% of commercial landings are sold through the local Miyako Fish Market, where a market investigator collects data of recaptured fish on all business days (Okouchi et al. 1999).

Hanoki and Akamae were selected as release sites (Fig. 1b, Table 1), both of which are located at the southeastern part of the bay. Hanoki, a small beach located 1 km north of Akamae, has coarse bottom sediments (median diameter: 2.33 mm; silt and clay fractions: 8.0%) surrounded by dense seagrass beds (163 stems m^{-2}); Akamae had muddy-sand sediment composition (median diameter: 0.20 mm; silt and clay fractions: 9.0%) and significantly lower seagrass densi-

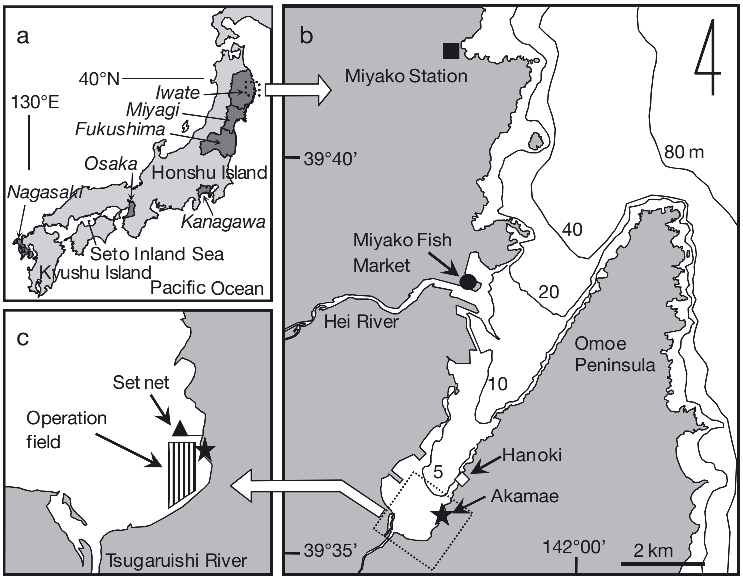

Fig. 1. Study area. (a) Dark grey areas show the prefectures which have released spotted halibut during the period from 2005 to 2008; (b) geographic features of Miyako Bay: (■) Miyako Station, (●) Miyako Fish Market, (◇) Hanoki release area, (★) Akamae release area; (c) sampling area. Hatched area: operation field, (▲) location of set net

ties (11 stems m^{-2}, Mann-Whitney test, $p < 0.01$). Bottom water temperature of the inner part of the bay is lowest (ca. 7°C) from January to March and highest (ca. 23°C) from August to September, and was comparable to another semi-enclosed bay in Iwate Prefecture (Yamashita et al. 1994).

Release protocol of hatchery-reared juveniles. Seedlings for the release of spotted halibut in 2000 to 2005 were produced in the Miyako Station of National Center for Stock Enhancement (Fig. 1b). Almost all the broodstock maintained in the station originated from wild fish landed at the Ofunato Fish Market located 60 km south of Miyako Bay. Rearing techniques for juveniles generally followed those detailed in Aritaki et al. (2001). Cohorts of fish released during this study are described in Table 1, which includes releases conducted both before (2000 to 2003) and after (2004 and 2005) optimization of release strategies.

All juveniles released in Hanoki and Akamae were tagged with single or multiple alizarin complexone (ALC) immersion to distinguish release cohorts (Yamashita et al. 1994); these were complemented by 2 external brands (2004: dorsal side; 2005: ventral side) to facilitate rapid detection of release cohort in the market (Okouchi et al. 2004; Table 1). All juveniles were transported by truck in 1000 l containers from the Miyako station to the inner part of Miyako Bay and released from buckets at each site (Fig. 1b).

Post-release surveys. Post-release surveys of released juveniles in Akamae were conducted in 2004 and 2005 using a 2.0 m beam trawl (0.2 m height, 3 mm mesh: see Table 2), with the exception of the first post-release survey in 2005 (1.5 m push net, 0.3 m height, 3 mm mesh; see Table 2). The trapezoid field of operation (200 and 300 m parallel sides at depths of 0.6 to 2.8 m) was set 40 to 200 m offshore from the release point (Fig. 1c). Trawls were towed for 100 m at 0.6 m s^{-1} parallel to the shore within the field of operation, while the push net was operated at 0.3 m sec^{-1} in areas 40 to 100 m from the shore and at depths of less than 1 m within the field.

Table 1. *Verasper variegatus*. Detailed release protocols, estimated returns and recapture rates of spotted halibut released in Miyako Bay from 2000 to 2005

Year	Date	Site	Size (mm)	No. released	Tags Internal[a]	Tags External	Number of returns[b] 1+	Number of returns[b] 2+	Number of returns[b] ≥3+	Total return	Recapture rate (%)
2000	25 Apr	Hanoki	25.9	10100	ALC (1)	–	70	146	50	266	2.6
	17 Jul	Hanoki	57.0	5200	ALC (1)	–	27	182	43	252	4.8
2001	21 May	Hanoki	22.8	8100	ALC (2)	–	19	24	11	54	0.7
	30 Jul	Hanoki	65.0	6200	ALC (2)	–	35	165	111	311	5.0
2002	15 Jul	Akamae	70.7	19200	ALC (1)	–	23	114	101	238	1.2
2003	6 Jul	Akamae	76.7	6300	ALC (2)	–	28	48	102	178	2.8
2004	9 Jul	Akamae	81.2	22000[c]	ALC (1)	Brand (dorsal side)	240	1098	344	1682	7.6
2005	12 Jul	Akamae	89.8	5000	ALC (1)	Brand (ventral side)	112	512	160	784	15.7

[a] Number in parentheses shows the number of alizarin complexone (ALC) rings in otolith
[b] Hatch date was estimated to be 1 January
[c] Includes 3000 individuals with no internal/external tags released on the opposite side of Miyako Bay from Akamae

The prey assemblage at the release site was surveyed through 20 to 50 m hauls using a sledge net (0.6 m width, 0.5 m height, 0.7 mm mesh, operation distance: 20 to 50 m), 7 times from 29 May to 6 September in 2004 and 6 times from 28 May to 30 August in 2005. All samples were collected during the daytime and distances of all tows and hauls were measured using a laser range finder (Laser 400, Nikon Vision). Bottom water temperature and salinity were logged at 20 min intervals at Akamae during the study period (Compact-CT, Alec Electronics). Dissolved oxygen (DO) and pH were recorded during each post-release survey using a multi-parameter water quality meter (Horiba Japan). Gear efficiency was not considered in the density estimations for juveniles and prey items.

Market surveys followed the procedures described in Okouchi et al. (2004) and were continued until December 2008. Briefly, surveys were conducted ~260 d yr^{-1} to identify total spotted halibut landings, to check for pigmentation and brand marks and to measure total length. All the released spotted halibut could be clearly distinguished from wild ones by the occurrence of permanent abnormal pigmentation patterns (hypermelanosis) on the blind side, which wild fish lack. Each month, a sample (n = 3 to 11) of the landed fish were purchased and measured for total length, total weight, sex, age (by counting the translucent zones of the sagittal otolith) and to determine the presence of an ALC mark in the sagitta. Stomach content analysis was also performed as described below.

The total contribution of recaptured fish in each year class was estimated on a monthly basis using established methods (Okouchi et al. 1998). These estimates defined normal distributions of the mean and the standard deviation of total length measurements for ALC-tagged and brand-marked fish in different year classes; these length distributions were fitted to the entire sampled population (Okouchi et al. 2004).

The von Bertalanffy growth curve was fitted to the age-length data of brand-marked fish (2004 and 2005 release cohorts) and the *F*-test was examined to compare the statistical difference of growth performance between the 2 release cohorts following the method described in Akamine (2009). Since the main spawning season for spotted halibut is late December to January (Yamaguchi et al. 2001), 1 January was assumed to be the date of hatching.

Sample processing. Collected spotted halibut juveniles and prey items were immediately cooled by ice in the field. In the laboratory, total length (0.1 mm) and weight (mg) of juveniles were measured, and dissected stomachs and collected prey items were fixed in 10% formalin. The stomach contents of juveniles and prey items were sorted to the lowest possible taxonomic level, counted and weighed (mg).

The stomach contents index (SCI) was defined as the percentage weight of the stomach contents per somatic weight (total weight less stomach contents weight), and was calculated individually to compare the feeding intensity of juveniles. Further, the index of relative importance (IRI) for each prey item in the diet was calculated as:

$$\text{IRI} = (\%N + \%W) \times \%F \tag{1}$$

where N is the number of prey consumed, W is the weight of prey consumed and F is the frequency of fish consuming the prey for each sampling date (Hyslop 1980). The %IRI (Cortés 1997) of each prey item (i) was then calculated for juveniles collected on each date as:

$$\%\text{IRI}_i = \frac{100 \times \text{IRI}_i}{\sum_{i=1}^{n} \text{IRI}} \tag{2}$$

Gammaridean amphipods, the main food items for spotted halibut juveniles (Noichi et al. 2006, Wada 2007), were classified into 4 types in terms of microhabitat (Sudo & Azeta 2001): (1) epifauna (species living on or above the bottom surface); (2) shallow burrowers (species burrowing into the superficial bottom sediment); (3) infaunal tube dwellers (species living in a tube formed in the bottom sediment); and (4) deep burrowers (species burrowing deep into the bottom sediment).

Selectivity by juveniles for 4 major prey categories (epifaunal gammarids, infaunal tube dwelling gammarids, cumaceans and mysids) was calculated for individuals by using Manly's α (Manly 1974, Chesson 1978). Selectivity (α_i) was calculated as:

$$\alpha_i = \frac{r_i}{n_i} \times \frac{1}{\sum_{j=1}^{m} \frac{r_j}{n_j}}, \; i = 1, \ldots m \tag{3}$$

where r_i and n_i are the proportions by number of prey items in category i in the diet and in the environment, respectively, and m is the number of prey types. This index ranges from 0 to 1 (complete avoidance to preference). When $\alpha = 1/m$, a given prey is consumed in proportion to its availability (neutral preference, 0.25 for $m = 4$ in this paper). In the calculations, data of prey items collected on 10 July, 24 July and 9 August 2004 were applied to the diets of juveniles collected in early July, late July and early August 2004, respectively; those collected on 13 July, 19 July and 8 August 2005 were applied to the diets of juveniles collected in early July, late July and early August 2005, respectively.

Total lengths of juveniles for each sampling date in each year were compared using a parametric 1-way ANOVA followed by Tukey's multiple comparison test; SCIs of juveniles were compared using a nonparametric Kruskal-Wallis test followed by Scheffe's multiple comparison test, because Bartlett's test for homogeneity of variances detected heteroscedastic variances in SCI data (Zar 2009). The Spearman rank correlation between days after release and total length of juveniles was also tested (Zar 2009).

Assessment of potential predators. To assess potential predators, qualitative sampling was conducted in Akamae from June to August 2005 using a set net equipped with a fence net (100 m length, 80 mm mesh) and a bag net (10 m width, 30 m length, 50 mm mesh) deployed at a depth of 2.8 m (Fig. 1c). Depending on the wave and weather conditions, the set net fisheries were conducted either daily or every few days; all the samples were collected on 18, 28 June; 8, 13, 15, 19, 29 July; and 8, 18, 30 August. Samples were identified, measured and weighed; the mean percent by number (%*N*) and by weight (%*W*) was calculated by month for each species.

Laboratory predation experiments. Laboratory predation experiments were conducted in June 2005 at the Miyako Station to examine the size refuge from predation. Age-1+ Japanese flounder (mean ± SD: 187.7 ± 20.8 mm total length) were used based on their prevalence in the release area. First, 3 hatchery-reared age-1 Japanese flounders were put in each of 6 replicate 500 l tanks for 3 d. The tank was supplied with running seawater (mean 15.6°C, salinity of 33.3 psu) under moderate aeration. Six spotted halibut juveniles (ranging from 47.6 to 108.4 mm) were then transferred to each replicate tank. To determine size-dependent predation, size variations in the 3 Japanese flounder in each tank were small (CV < 8.2%) and mean total lengths in the 6 replicate tanks ranged from 165.7 to 208.7 mm. No food was supplied during the experiments. Survival of juvenile spotted halibut was observed for 7 d and results were divided into 3 categories: survived, bitten or consumed. The latter 2 indicated that the juveniles died from predation. Total length of spotted halibut juveniles bitten by Japanese flounder (TL_{bitten}) and mean total lengths of Japanese flounder (TL_f) in the 6 tanks were fitted to a simple linear regression. Maximum total length of juveniles consumed by Japanese flounder ($TL_{consumed}$) and TL_f were fitted to a linear regression. Statistical test for significance of the regressions were conducted (Zar 2009).

RESULTS

Physical conditions and prey abundances

Water quality measurements are shown in Fig. 2. Mean (±SD) water temperature and salinity were 22.0 ± 1.9°C and 28.0 ± 4.1 psu during the survey period in 2004, while those in 2005 were 21.1 ± 2.6°C and 27.8 ± 4.9 psu, respectively, with larger drops in salinity and temperature coinciding with freshwater inflows to Akamae. DO and pH recorded at each sampling ranged between 6.5 to 9.5 mg l^{-1} and pH 7.8 to 8.3 in 2004, and 6.9 to 10.1 mg l^{-1} and pH 8.1 to 8.7 in 2005, respectively.

Seasonal changes of abundance of prey items (gammarids, mysids and cumaceans) are shown in Fig. 3. Although total amounts of prey items were larger in 2004 than those in 2005, seasonal changes showed similar patterns in both years: i.e. prey abundances were relatively low in May, peaked in early July and then decreased by early September.

More than 12 species of gammarids were identified. Prey species consisted of 7 epifaunal species (*Pontogeneia rostrata*, *Ampithoe valida*, *Ampithoe lacertosa*, *Jassa* sp., *Paradexamine marlie*, *Aoroides* sp., *Guernea* sp.), 2 species of shallow burrowers (*Synchelidium lenorostralum*, *Orchomene* sp.), 2 species of infaunal tube dwellers (*Corophium insidiosum*, *C. acherusicum*) and 1 species of deep burrower (*Harpiniopsis* sp.). The 2 species belonging to the genus *Corophium* were analyzed as *Corophium* spp. because of the difficulty in

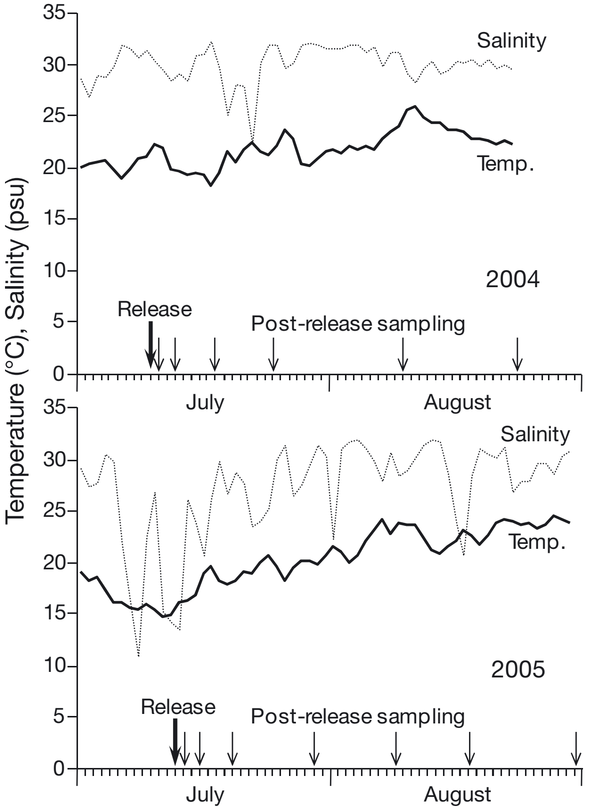

Fig. 2. Seasonal changes of daily mean water temperature and salinity at Akamae during the survey periods in 2004 and 2005. Thick and dotted lines denote water temperature and salinity, respectively. Thick and thin arrows indicate dates of release and dates of sampling, respectively

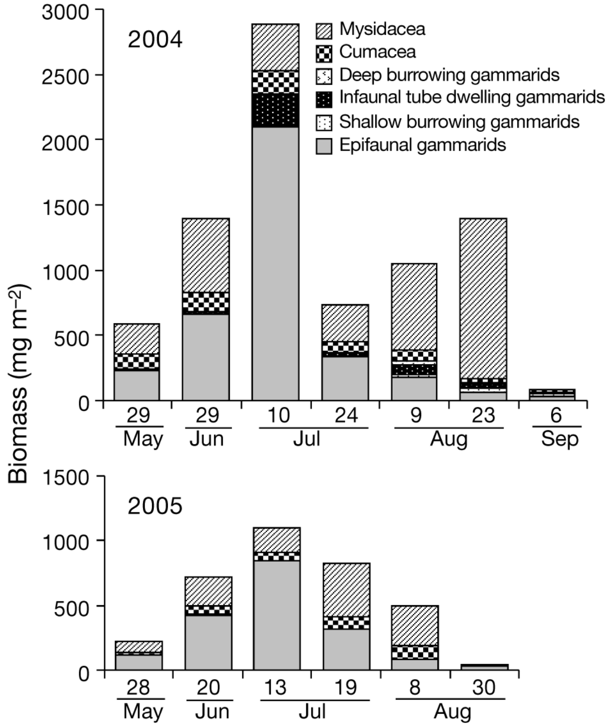

Fig. 3. Seasonal changes of prey abundance shown by biomass (mg wet weight) in 2004 and 2005. Gammarids were described by 4 microhabitat types

identifying the small specimens in the juvenile stage. Epifaunal gammarids, which showed the marked increase in early July (Fig. 3), accounted for the highest abundance of total gammarid biomass (by weight, 88.1% in 2004 and 99.1% in 2005), while infaunal tube dwelling gammarids (9.4% and 0.7%), shallow burrowing gammarids (1.7% and 0.1%) and deep burrowing gammarids (0.8% and 0.1%) showed lower abundances during the survey periods. At the species level, 3 epifaunal species *A. valida* (50.4%), *P. rostrata* (24.4%) and *A. lacertosa* (10.3%) and infaunal tube dwelling species *Corophium.* spp (9.4%) showed higher abundances in 2004, while *A. valida* (82.6%) and *P. rostrata* (13.0%) accounted for >95% of total gammarid biomass in 2005. Almost all the collected mysids consisted of the brackish water species, *Neomysis awatschensis* (99.6% in 2004 and 100% in 2005). Biomass of cumaceans was the smallest among the prey categories (Fig. 3).

Density and feeding of recaptured juveniles

A total of 116 and 35 juveniles were collected in 2004 and 2005 respectively (Table 2). In 2004, juvenile density peaked at 3 d after release and then markedly decreased, and was relatively low during the survey period in 2005 (Table 2). In 2004, mean total lengths remained at the same level in July and then significantly increased in August (Tukey's post hoc test, $p < 0.05$; Fig. 4). In contrast, no significant differences were detected between any sampling dates in 2005 (ANOVA, $p = 0.30$); however, significantly positive Spearman correlation coefficient ($r_s = 0.201$, $p = 0.02$) indicated the increasing trend in total length during the survey periods.

Stomach contents index (SCI) was lowest (0.39) at 3 d after release, but that became significantly higher (over 2.2) 8 d after release in 2004 (Fig. 5). Percent empty stomach was 16% at 1 d after release, peaked at 3 d (37%) and decreased to 0% at 8 d and thereafter. In 2005, SCI gradually increased and became significantly higher only at 7 d after release (Fig. 5). Percent empty stomach peaked at 1 d after release (58%) and quickly decreased to 0% at 3 d and thereafter. In both 2004 and 2005, approximately 90% of gut contents consisted of cumaceans and gammarids (Fig. 6). In particular, epifaunal gammarids (mainly *Ampithoe valida* and *Pontogeneia rostrata*) accounted for 48.3 and 55.5% of total stomach contents in 2004 and 2005, respectively, while infaunal tube dwelling gammarids (1.0 and 0.2%, respectively) and shallow burrowing gammarids (0.4 and 0%, respectively) accounted for lower abundance. In addition to gammarids and cumaceans, larger crus-

Table 2. *Verasper variegatus.* Operation record of post-release surveys and number and densities of spotted halibut juveniles collected at Akamae in 2004 and 2005

Sampling date	Days after release	Sampling gear	Swept area (m^2)	No. of juveniles collected	Density (ind. 1000 m^{-2})
2004					
10 Jul	1	Beam trawl	2400	25	10.4
12 Jul	3	Beam trawl	1056	51	48.3
17 Jul	8	Beam trawl	2900	23	7.9
24 Jul	15	Beam trawl	5560	14	2.5
9 Aug	31	Beam trawl	1460	3	2.1
23 Aug	45	Beam trawl	1240	0	0.0
2005					
13 Jul	1	Push net	2400	11	4.6
15 Jul	3	Beam trawl	2430	11	4.5
19 Jul	7	Beam trawl	2120	5	2.4
29 Jul	17	Beam trawl	2000	1	0.5
8 Aug	27	Beam trawl	1400	3	2.1
17 Aug	36	Beam trawl	1380	3	2.2
30 Aug	49	Beam trawl	1400	1	0.7

taceans such as isopods (Flabellifera and Valvifera) and caridean shrimps accounted for higher percentages in August, when total length of juveniles increased in both years (Fig. 4).

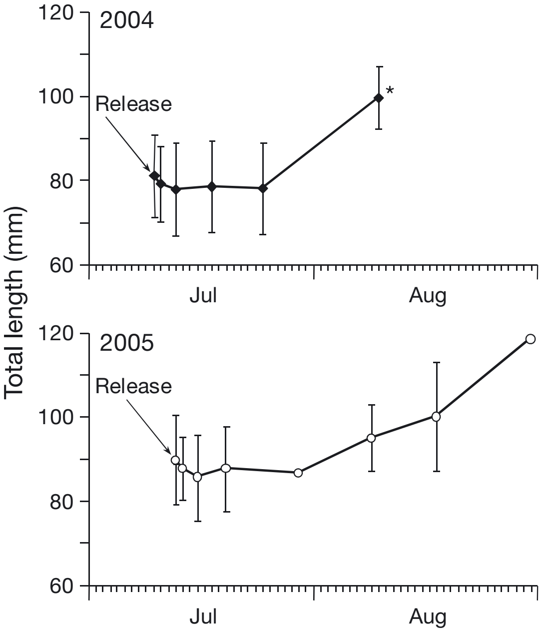

Fig. 4. *Verasper variegatus*. Post-release changes in total length (±SD) of spotted halibut in 2004 and 2005. *Significant difference among different sampling dates

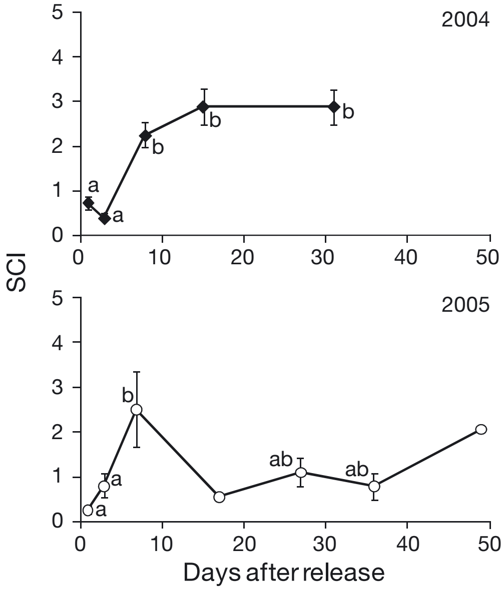

Fig. 5. *Verasper variegatus*. Post-release changes of mean (±SE) stomach contents index (SCI) of spotted halibut in 2004 and 2005. Different letters represent significant differences among different sampling dates in each year. No letter is shown for the points where statistical analyses could not be applied (n ≤ 2)

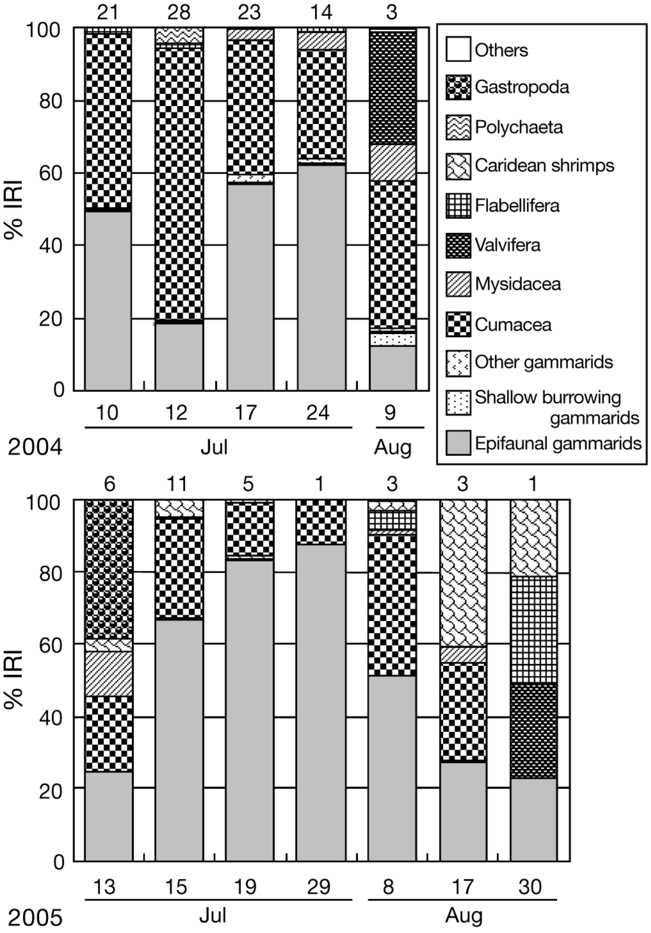

Fig. 6. *Verasper variegatus*. Post-release changes in stomach contents composition (index of relative importance, %IRI) of spotted halibut in 2004 (upper) and 2005 (lower). Numeral above each column shows the number of individuals analyzed

The patterns of selectivity of the 4 prey categories by released juveniles were similar between the 2 years (Fig. 7). Epifaunal gammarids were positively selected 3 d after release in both years, and this pattern was clearer in 2005. Cumaceans were selectively consumed in 2004; in 2005 they were positively selected within 10 d after release, and then decreased to slightly negative selection. Mysids and infaunal tube dwelling gammarids were negatively selected throughout the survey periods, even in 2004 when the biomass and densities of these prey items were relatively high in the environment (Fig. 3).

Potential predators and size refuge from predation

A total of 3655 individuals from juvenile to adult stages of at least 34 fish species were collected in the set net samples. Large crustaceans such as swimming crabs (Sudo et al. 2008), sandy shore crabs (Hossain et al. 2002, Kellison et al. 2002) and cuttlefishes (Saitoh et al. 2003), known as potential nocturnal predators of juvenile Japanese flounder, were not collected. The species which showed the largest percentage by number and weight, respectively, were Pacific herring (44.9 %*N*; mean total length ± SD: 58.0 ± 7.3 mm) and Japanese flounder (24.5 % *W*; 208.6 ± 77.7 mm) in June, Japanese anchovy *Engraulis japonicus* (76.2 %*N*; 35.1 ± 9.9 mm) and black rockfish *Sebastes shlegelii* (32.6 % *W*; 183.8 ± 49.7 mm) in July, and Japanese smelt *Hypomesus japonicus* (72.3 %*N*; 54.0 ± 6.7 mm) and greater amberjack *Seriola dumerili* (57.2 % *W*; 295.8 ± 24.9 mm) in August. Japanese flounder showed the largest maximum size (450 mm) and median size (188 mm) among the 3 benthic piscivores (Japanese flounder, black rockfish and frog sculpin *Myoxocephalus stelleri*) and was considered the most important potential predator of juvenile spotted halibut.

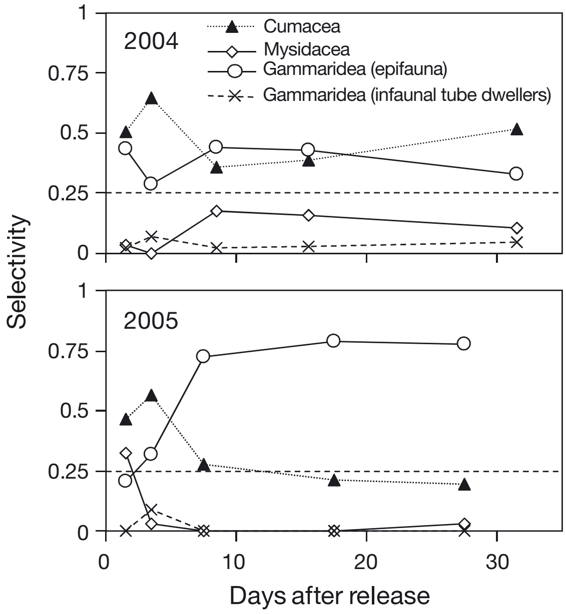

Fig. 7. *Verasper variegatus*. Mean selectivity by released spotted halibut for each prey category in 2004 and 2005 as calculated in terms of Manly's α. The horizontal dotted line represents neutral preference (α = 0.25)

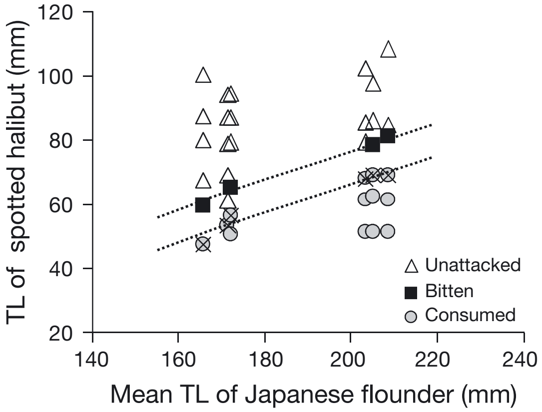

Fig. 8. *Verasper variegatus* and *Paralichthys olivaceus*. Relationship between total lengths (TL) of spotted halibut consumed, bitten and unattacked by 3 Japanese flounder in a tank and mean TL of 3 Japanese flounder in 6 replicated tanks. (×) Maximum TL of spotted halibut consumed by Japanese flounder in each tank. Upper dotted line indicates linear regression of TL of bitten spotted halibut and mean TL of Japanese flounder, while lower indicates linear regression of maximum TL of spotted halibut consumed by Japanese flounder and mean TL of Japanese flounder in each tank

In the laboratory predation experiments, spotted halibut juveniles were consumed in all the trials, and bitten juveniles were observed in 4 tanks (Fig. 8). The relationship between TL_{bitten} and TL_f was expressed as the following linear regression: $TL_{bitten} = 0.467\ TL_f - 16.5$; $r^2 = 0.9851$, $p < 0.01$. The relationship between $TL_{consumed}$ and TL_f was expressed as the following linear regression: $TL_{consumed} = 0.423TL_f - 18.3$; $r^2 = 0.9741$, $p < 0.01$. These significant linear regressions indicated the size-dependent predator–prey relationships between Japanese flounder and spotted halibut juveniles and demonstrated that the refuge from predation would be attained for individuals >80 mm for this predator size range (Fig. 8).

Growth, feeding and recapture rates of landed fish

The age-length relationships of landed spotted halibut released in 2004 and 2005 are shown in Fig. 9. In both years, released fish showed strong growth, reaching >40 cm by 1 yr of age and >50 cm by 2 yr of age. Released fish were recruited to the local fishery approximately 1 yr after release (mean TL > 25 cm). The von Bertalanffy growth curve fitted to age-length data in 2004 and 2005 was expressed as follows: $L_t = 54.54(1 - e^{-0.3883(t\ -0.0972)})$; 2004 release cohort, n = 1228) and $L_t = 54.04(1 - e^{-0.4682(t\ -0.1384)})$; 2005 release cohort, n = 620). Statistically significant differences were detected between these growth curves ($F_{(3,\ 1842)} = 51.81$, $p < 0.001$). Tag retention rate of the brand mark was estimated as 85 % in the 2004 release cohort and 79 % in the 2005 release cohort.

Market recapture rates of fish released in 2004 and 2005 were 7.6 and 15.7 %, respectively, and exceeded the recapture rates of fish released from 2000 to 2003 (range: 0.7 to 5.0 %; weighted mean: 2.4 %: Table 1). Approximately 80 % of 2004 and 2005 year class fish were recaptured by 2 yr of age. Only 7 and 2 wild spotted halibut were landed in 2007 and 2008, respectively, indicating that the contribution rate (proportion of reared fish in the landings) was >99 % in Miyako Bay during the survey periods (100 % in 2000–2006, 99.1 % in 2007, and 99.6 % in 2008).

Gut contents of recaptured fish from fishery-independent and fishery-dependent surveys mainly consisted of a variety of crustaceans, but the relative importance of each prey item was quite different in each size class (Fig. 10). Gammarids comprised over 50 % of the gut contents of fish <15 cm and were the most important prey. Gammarids were hardly consumed by fish >20 cm, and a variety of larger crustaceans (valviferans, carideans, thalassinideans, anomurans and brachyurans) comprised approximately 80 % of the gut contents of fish between 15 and 25 cm. The percentage of valviferans decreased to 30 % while brachyurans accounted for over 50 % in fish >25 cm. Brachyurans, caridean shrimps and thalassinideans accounted for approximately 70, 20 and 10 % of the gut contents of fish >30 cm, respectively. Polychaetes consumed by fish <20 cm were identified as anterior tips of the *Diopatra* sp. which protruded from the sedimentary tubes in tidal flats.

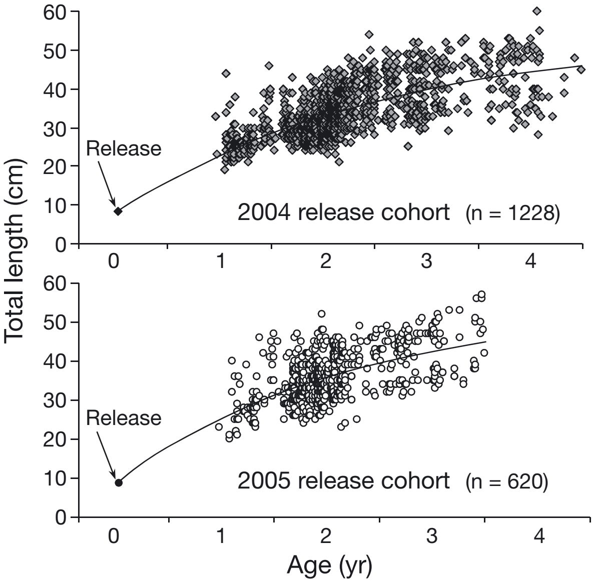

Fig. 9. *Verasper variegatus*. Age–length relationships of landed brand-marked spotted halibut released in 2004 and 2005. (◆) Mean total length of juveniles at release in 2004; (●) mean total length of juveniles at release in 2005. The von Bertalanffy growth curve fitted to age–length data in 2004 and 2005 release cohorts is shown

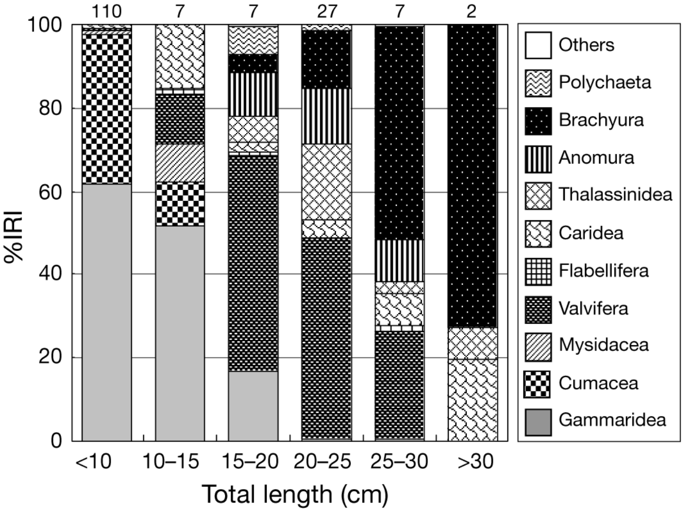

Fig. 10. *Verasper variegatus.* Ontogenetic change of composition (index of relavive importance, %IRI) of prey items of spotted halibut recaptured by post-release surveys (n = 117, 5.1 to 11.8 cm) and fishery (n = 43, 16.5 to 46.6 cm) in every 5 cm size class. Numeral above each column shows the number of individuals analyzed

DISCUSSION

Adaptation processes of juveniles

The most important concept for the development of release techniques is to minimize juvenile mortalities just after release (Yamashita & Yamada 1999). Therefore detailed understanding of the post-release adaptation, dispersion and mortality processes of juveniles are essential for the establishment of an optimal release strategy (Furuta 1996, Tanaka et al. 2006, Sparrevohn & Støttrup 2007). In the present study, post-release adaptation/survival processes of spotted halibut juveniles were estimated through the following approaches: comparison of prey abundances and feeding patterns of juveniles in association with physical parameters, assessment of potential predators in the field and estimation of the size refuge from predation through laboratory experiments.

For released juveniles, beginning feeding soon after release, achieving high growth, and obtaining size refuges are critical for preventing mass post-release mortality (Yamashita et al. 1994, Furuta 1996, Støttrup et al. 2002). In our study, the peak abundances of prey items were matched with the timing of release in 2004 and 2005 (Fig. 3). Detailed comparisons between gut contents of spotted halibut juveniles and prey items in the environment revealed that juveniles could adapt to the released area approximately 1 wk after release (Fig. 5) by selectively feeding on the epifaunal gammarids and cumaceans while showing negative selectivity for preying on pelagic mysids or infaunal tube dwelling gammarids (Figs. 6 & 7). Tsuruta & Omori (1976) revealed that the oral morphology of spotted halibut is characterized by small curvatures of the premaxilla and dentary, and relatively symmetric jaws between the ocular and blind sides. These characteristics are quite different from those of infaunal feeders (e.g. marbled flounder *Pseudopleuronectes yokohamae*) which have a strongly curved premaxilla and dentary on the blind side, and a toothless jaw on the ocular side. They also found that spotted halibut slowly approach prey items at a constant speed (~1 cm s^{-1}) and suddenly swallow them on the bottom, while Japanese flounder, which have a wider mouth gape and canine-like teeth on both jaws, approach foods whilst accelerating (5 to 30 cm s^{-1}) and prey on them in the water column. The oral morphologies and feeding behavior of spotted halibut are an adaptation for feeding on epifaunal prey items. The differences in feeding behavior and feeding ecology of spotted halibut and Japanese flounder might lead to different adaptation processes in released juveniles: spotted halibut can select wider ranges of prey animals and adapt to the release environments relatively quickly as shown in our study, while Japanese flounder sometimes take a few weeks to adapt because feeding incidences of juveniles largely depend on mysid abundance (Furuta et al. 1997, Tanaka et al. 2006).

Not only were prey availabilities suitable for juvenile spotted halibut in the present study, but so too were physical parameters (temperature, salinity and sediment compositions). Laboratory rearing at various water temperatures (18, 21, 24 and 27°C) indicated that higher juvenile growth performances were attained at 18 to 24°C (maximum: 21°C) while decreased growth and survival rates, and an increase in metabolic costs, were observed at 27°C (Murata et al. 1998). Water temperatures 1 mo after release were within the suitable range for juvenile growth in the 2 years studied (Fig. 2). Further, spotted halibut have been shown to have a strong tolerance to low salinity conditions (≥2 psu) based on a well developed physiological ability for osmoregulation, and juveniles even grew better in the low salinity conditions (8, 16 psu) in the laboratory (Wada et al. 2004, 2007). Therefore the brackish water conditions in the release area (Fig. 2) would be preferable for juveniles. Also, the similar composition of bottom sediments in Akamae (median diameter: 0.20 mm) and that of the natural habitat in western Japan (median diameter: 0.21 mm, Wada et al. 2006) would facilitate sediment-naive juveniles to bury quickly, as shown in hatchery-reared Japanese flounder, marbled flounder (Tanda 1990) and winter flounder *Pseudopleuronectes americanus* (Fairchild & Howell 2004). The presence of these suitable abiotic conditions would help juveniles to adapt to the natural environment.

Recent studies have demonstrated that conditioning or learning of reared fish before release could improve behavioral skills such as feeding, burying and predator avoidance (Hossain et al. 2002, Fairchild & Howell 2004, Arai et al. 2007), resulting in higher survival of juveniles just after release (Sparrevohn & Støttrup 2007). However, some negative impacts of conditioning processes have been observed, such as attraction of predators during on-site acclimation using cages (Fairchild et al. 2008). Although conditioning prior to release may improve the slightly delayed feeding of spotted halibut juveniles just after release, the fact that juveniles could adapt to the release habitat within 1 wk after release, and subsequently undergo a high incidence of recruitment to the fishery, might indicate that conditioning before release may not be necessary under near-optimal biotic/abiotic release conditions.

Although juveniles showed a high feeding incidence soon after release (Fig. 5), total lengths of juveniles remained the same (Fig. 4) as at release whilst juvenile densities decreased during the 2 wk after release (Table 2). Recent studies have indicated that predation would be the main cause of juvenile mortality just after release (Yamashita et al. 1994, Furuta 1996, Sudo et al. 2008). However, in our study, predation-induced mortality was not considered a major cause of the decrease in juvenile density: there were a small number of large potential benthic predators in the release area, and laboratory experiments indicated that a size refuge from predation could be attained at 80 mm (Fig. 8), which is smaller than the mean release sizes for the 2 years studied. Therefore, the lack of change in total lengths was not thought to be due to a lack of growth in juveniles unable to adapt to the environmental conditions, but was possibly due to the dispersal of larger juveniles. Although it is difficult to investigate the mortality/survival processes of juveniles that dispersed from the field of operation, mass mortalities were not considered to have occurred because of the relatively high recapture rates of 2004 and 2005 release cohorts (Table 1). Since it was reported that Japanese flounder >30 cm distribute in the central to outer part of Miyako Bay (Okouchi et al. 2004), post-release changes in the spatial distribution of juvenile spotted halibut and other potential causes of mortality should be clarified.

Growth and recruitment to local fishery

Market surveys were important to understand the growth and recruitment patterns of released fish, check the validity of release protocols and evaluate the effectiveness of stocking (Okouchi et al. 1999, Nakagawa et al. 2007, Tomiyama et al. 2008). Detailed market surveys revealed that spotted halibut remained in Miyako Bay for at least 3 yr and showed high growth performance, attaining a maximum total length of 43 cm at age 1+ and 54 cm at age 2+, recruiting to local fisheries approximately 1 yr after release (Fig. 9).

Growth patterns of spotted halibut during the 2 yr after release were almost equal to those of Japanese flounder (Paralichthyidae: Okouchi et al. 1999) and exceeded those of other pleuronectid flatfishes distributed in the northwestern Pacific (Chen et al. 1992). The observation that young and adult spotted halibut exclusively preyed on crustaceans, which probably matched their mouth gapes (Fig. 10), indicated that interspecific competition between spotted halibut and piscivorous Japanese flounder or other infaunal feeding flatfishes (Tsuruta & Omori 1976) hardly occurred from juvenile to adult stages. Further, the growth of released spotted halibut in Miyako Bay (Fig. 9) was comparable to those of the wild fish in southwestern Japan (Wada et al. 2006), suggesting that prey conditions were suitable in Miyako Bay.

Male and female spotted halibut in Fukushima Prefecture mainly recruit to spawning stocks on the continental shelf (~120 m depth) in winter, at age-2 and age-3, respectively (Shimamura et al. 2007). A tag-recapture survey of age-1 spotted halibut released in Miyako Bay in 1999 (n = 3000; mean: 136 mm) revealed that >90 % of recaptured fish (n = 582) were collected in Miyako Bay, but some individuals moved southward (max. 220 km south from the bay; M. Aritaki et al. unpubl. data). Hence, it is suggested that mature spotted halibut would migrate from Miyako Bay to the southern/deeper waters to recruit to local spawning stocks. As the next step, it is necessary to elucidate the differences in growth and migration patterns between sexes and to detect the spawning grounds. Long-term market censuses are necessary to examine whether release practices could enhance wild recruits or not.

Optimal release strategies for depleted species

Establishment of optimal release strategies is essential for successful stocking of a target species (Yamashita & Yamada 1999). The higher recapture rates of fish released in 2004 and 2005 than those in 2000 to 2003 (Table 1) were probably attributable to the improved release protocols in recent release cohorts, i.e. sufficiently large juveniles to allow a size refuge from predation were released in the estuarine area with suitable physical conditions and abundant prey organisms. Although detailed analyses of juvenile adaptation and prey/predator abundance were not performed in 2000 to 2003, lower recapture rates of these release cohorts might be attributable to inappropriate release sizes and/or release seasons: small juveniles (2000: 25.9 mm; 2001: 22.8 mm) were released in a sub-optimal season (April 2000, May 2001) when water temperature (<10°C) and prey abundances (Fig. 3) were low; others were released in an appropriate season (July 2000 to 2003), but sizes at release were too small (57.0 to 76.7 mm; weighted mean: 68.8 mm; Table 1).

The recapture rate of fish released in 2005 was over twice that of fish released in 2004 (Table 1). It is difficult to evaluate the relationship between carrying capacity and stocking magnitude, but the significantly higher growth performance in the 2005 year class fish (Fig. 9) implied that the smaller number of juveniles released in 2005 might lead to the higher recapture rate. Also, the larger size at release in 2005 may explain some causes in the different recapture rates. Since the optimal release site (Akamae), season (July) and size (> 80 mm) were determined in the present study, it will be important to examine the optimal release magnitude in Miyako Bay, and then to investigate more strategic release protocols. For instance, a mass release of smaller juveniles may be the best size at release for economic efficiency (net income/hatchery production and release costs), which sometimes differs from the ecologically optimal size at release (Yamashita & Yamada 1999).

The trial and error release histories of spotted halibut in Miyako Bay (Table 1) revealed that a comprehensive understanding of the ecological interaction between released juveniles and the target ecosystem, which was supported by complementary laboratory experiments for predation and juvenile eco-physiology, are important in developing effective stocking technology for highly depleted species; biological information of their wild counterparts in each locality were often limited.

Potential and future perspectives

Our study shows a clear addition to the emerging evidence that depleted stocks and capture fisheries can be effectively enhanced if appropriate target species are selected and the optimal release strategy of hatchery-reared juveniles is applied (Blaxter 2000). As a next step, it will be necessary to continue careful and responsible release practices considering the genetic variations of seedlings (Blankenship & Leber 1995, Ward 2006), because ecological and genetic influences of mass release of seedlings on the wild population are potentially large in highly depleted species (Ortega-Villaizán Romo et al. 2006). Restocking programs of spotted halibut should be promoted based on the concepts of conservation and restoration of coastal environments aimed at improving the recruitment of wild fish, with particular care to ensure genetic diversity based on the careful management of locally derived broodstock.

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