

Influence of seagrass leaf density and height on recruitment of the cardinalfish *Cheilodipterus quinquelineatus* in tropical seagrass beds: an experimental study using artificial seagrass units

Yohei NAKAMURA*, Masahiro HORINOUCHI** and Mitsuhiko SANO*

Abstract : To clarify the effects of differences in seagrass leaf density and height on the appearance of newly-recruited fishes, a field experiment using artificial seagrass units (ASUs) was undertaken at Iriomote Island, the Ryukyu Islands, Japan. Four types of small-scale, structurally-different ASUs, including (1) long dense leaves (D-units), (2) long leaves thinned to about 40% of D-unit density (T-units), (3) dense leaves shortened to 40% of D-unit height (S-units), and (4) no leaves (control; C-units), were used. Dividing observations were made to count recruited fishes on each ASU daily over 14 consecutive days. A total of 10 species in 7 families were recruited to the ASUs. The cardinalfish, *Cheilodipterus quinquelineatus*, dominated (85% of cumulative total number of fishes). The mean number of *C. quinquelineatus* recruits per replicate on D-units was significantly greater than that on the other unit types, indicating that difference in leaf density and height affected the recruitment of the juvenile of this fish species.

Keywords : *recruitment, seagrass leaf density, seagrass leaf height, Cheilodipterus quinquelineatus.*

1. Introduction

Leaf density and height in seagrass habitats could be important as fish appearance and abundance, because complexity as habitat can provide shelters (HECK and ORTH, 1980; BELL and POLLARD, 1989), microhabitat availability (HECK and ORTH, 1980), and diverse and abundant prey (ORTH *et al.*, 1984; CONNOLLY and BUTLER, 1996; JENKINS *et al.*, 2002). Such complexity in habitat structured by seagrass leaves strongly affects juvenile growth rates and mortality especially for vulnerable juveniles (HECK and ORTH, 1980; BELL and POLLARD, 1989; LEVIN *et al.*, 1997). However, relationships between seagrass structural attributes (leaf den-

sity and height) and fish abundance patterns are not fully understood (WILLIAMS and HECK, 2001), in spite of several attempts. While some studies have found that greater leaf density and/or height support larger numbers of fish species and individuals (BELL and WESTOBY, 1986a, b), others have found little or no relationship among these factors (BELL and WESTOBY, 1986c; BELL *et al.*, 1987; CONNOLLY, 1994; HORINOUCHI and SANO, 1999, 2001).

Use of artificial seagrass has been effective in manipulative experiments to clarify the relationships between habitat structure and organisms in seagrass habitats (BELL *et al.*, 1987; LEE *et al.*, 2001; JENKINS *et al.*, 2002). BELL *et al.* (1985), for example, found that artificial seagrass effectively attracted juvenile fish and macro-invertebrates typical of natural seagrass. In the present study, artificial seagrass units (ASUs) were used to simulate natural reef-associated seagrass patches so as to clarify the influence of seagrass leaf density and

*Department of Global Agricultural Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi, Bunkyo, Tokyo 113-8657, Japan

**Research Center for Coastal Lagoon Environments, Shimane University, 1060 Nishikawatsu-cho, Matsue, Shimane 690-8504, Japan

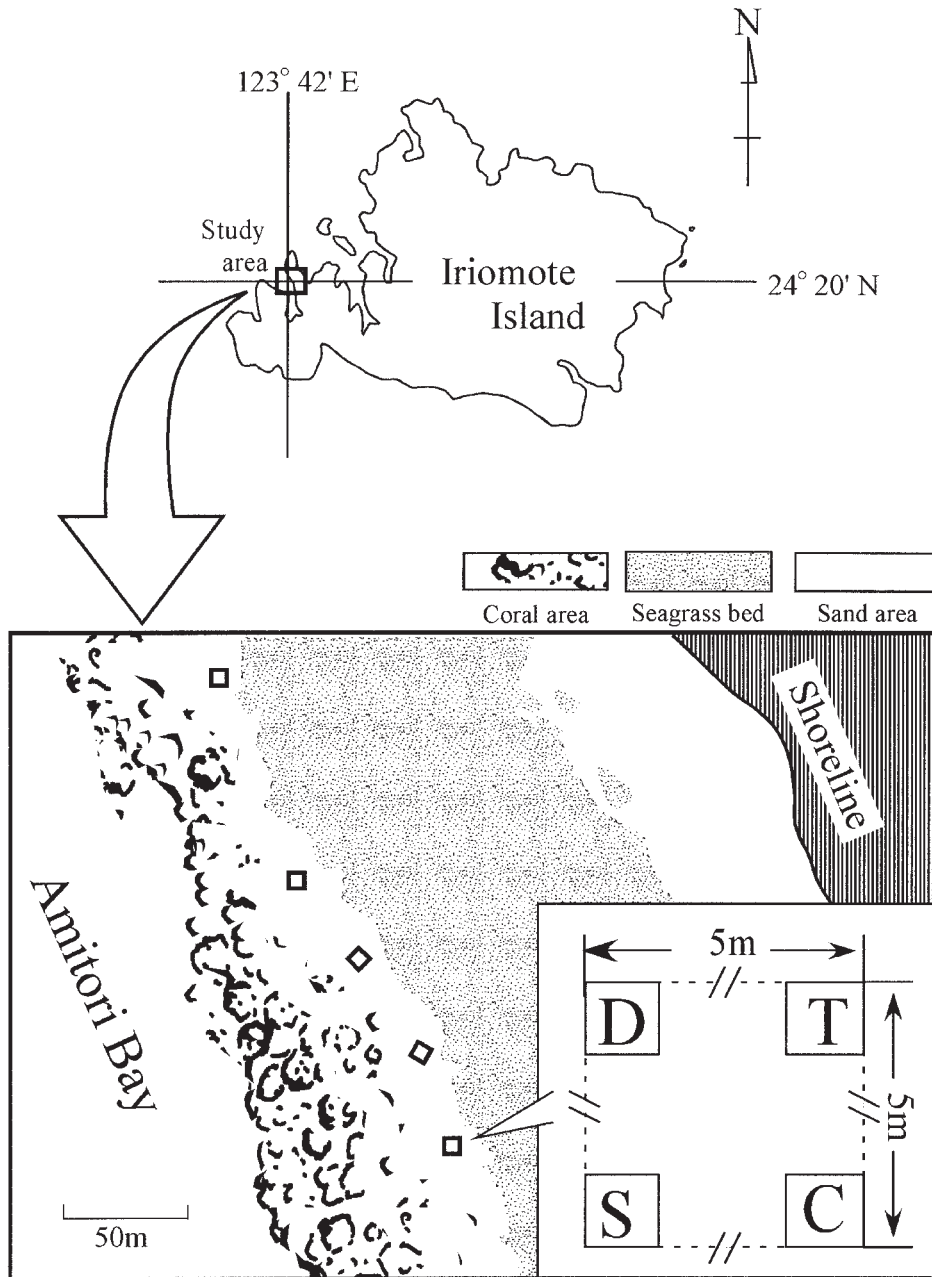


Fig. 1 Map of the study site at Iriomote Island, Ryukyu Islands, Japan, showing the locations of the five experimental square plots. D, T, S and C on each corner of the plot indicate four artificial seagrass treatments. For explanation of each artificial seagrass treatment, see text.

height on the abundance of newly-recruited fishes. Specifically, the recruitment of cardinalfish, *Cheilodipterus quinquelineatus*, which was dominant in the seagrass habitats

was examined in ASUs with different seagrass leaf density and height.

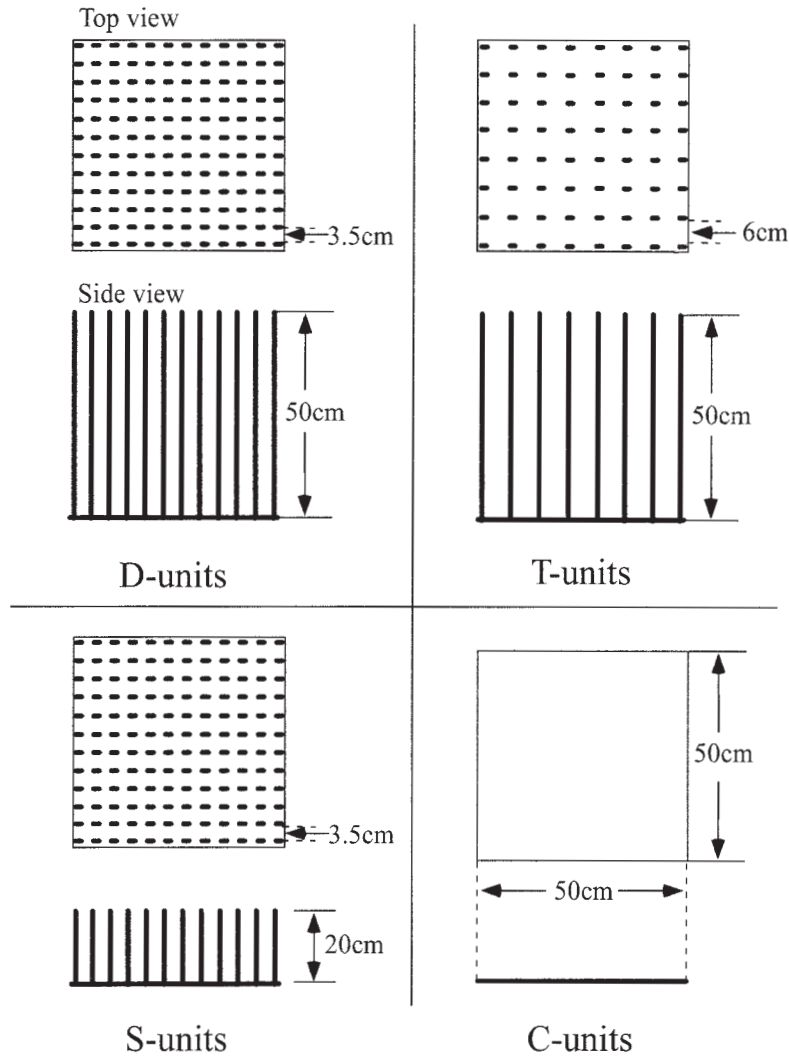


Fig. 2 Design of four different types of artificial seagrass units (D-, T-, S-, and C-units).

2. Materials and Methods

2.1 Study site

The study was carried out on a fringing coral reef in Amitori Bay ($24^{\circ}20' N$, $123^{\circ}42' E$), western side of Iriomote Island, the Ryukyu Islands, southern Japan, in May, 2003 (Fig. 1). Next to a coral-dominated area, a seagrass bed formed an extensive belt along the shore. Vegetation in the bed was dominated by *Enhalus acoroides*, having a mean shoot density of $122.4 \pm 18.9(\text{SD})\text{m}^{-2}$ ($n=6$), and mean height of $50.6 \pm 10.4\text{cm}$ ($n=30$). The experiment was conducted in a flat and sandy-rubble area adjacent

to the edge of the seagrass bed. In this area, some coral and seagrass patches occurred at a low-tide depth of about 1 m.

2.2 Experimental design

Each ASU was constructed of green polypropylene leaves (10mm in width and 1mm in thickness) tied to a 50cm square green steel mesh base. The basic experimental design involved five replicates of each of four artificial seagrass treatments (Fig. 2) : (1) long dense leaves (50cm in height, 12×12 rows = 144 leaves per unit, D-units), (2) long leaves thinned to

about 40% of D-unit density (50cm in height, 8×8 rows=64 leaves, T-units), (3) dense leaves shortened to 40% of D-unit height (20cm in height, 12×12 rows=144 leaves, S-units) and (4) base without leaves (control, C-units).

At the study site, five 5 m square plots, separated from one another by at least 30 m, were established randomly at least 5 m distant from natural seagrass and coral patches (Fig. 1). Each ASU treatment was randomly assigned to each corner of each plot (i.e. 4 ASU treatments per plot). The ASUs were set on May 14, with deliberate efforts made not to disturb fishes.

2.3 Fish censuses

All individuals of each fish species in the ASUs were visually counted using SCUBA, between 1000 and 1600 h everyday from May 15 to 29. Individuals maintaining a position in open areas close to the outer edge of the units were also regarded as residents of the units and counted. During the underwater observations, the total length (TL) of each individual was estimated to the nearest millimeter with a transparent plastic ruler (SANO, 1997). Such observations were conducted very carefully, in order not to disturb the fishes.

Cheilodipterus quinquelineatus was selected as the target species of the study, because it recruited on the ASUs more abundantly than other species (see Results). We defined recruits as settled juveniles surviving until the final census date (JONES, 1990; LEVIN, 1994). In Amitori Bay, juvenile *C. quinquelineatus* commonly inhabit seagrass beds and coral patches on reef flats, whereas adults are abundant on reef slopes (KAGAWA, 2003). Newly-recruited individuals (<25mm TL; FINN and KINGSFORD, 1996) are found mostly in May and June (KAGAWA, 2003), feeding mainly on calanoid copepods (93 % of total food volume) (NAKAMURA, unpubl. data).

2.4 Data analysis

Cheilodipterus quinquelineatus recruit numbers in the ASUs were compared among the four treatments. Because the fish censuses were repeated without any removal of fish from the ASUs, the number of individuals counted at each census included both new and previously

observed recruits. Under this sampling design, the data set obtained on each census date is not independent. Therefore, single-factor repeated-measures analysis of variance (ANOVA) was applied to test whether or not the density of *C. quinquelineatus* recruits differed among ASU types, and a post hoc Student-Newman-Keuls multiple comparison test was conducted to examine the difference in the mean values. Prior to the analyses, data were transformed to $\sqrt{x+0.5}$, owing to fish being absent on some ASUs during the study period (ZAR, 1999). C-units were excluded from this analysis, because no fish were observed on those units throughout the study period.

3. Results

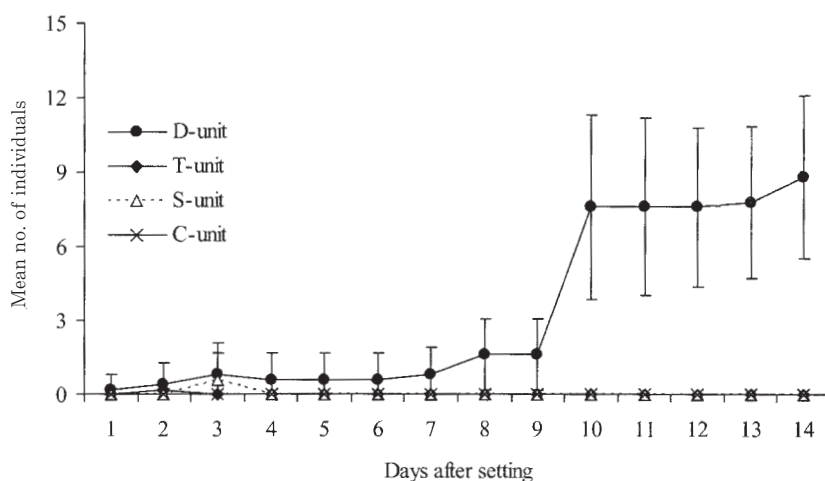
A total of 10 species in 7 families were recorded in the ASUs (Table 1). *Cheilodipterus quinquelineatus* recruits accounted for 85% of the cumulative total number of all species (Table 1). The size range of this species was 11 to 25mm TL (Table 1).

The repeated-measures ANOVA demonstrated a highly significant difference in the mean number of *C. quinquelineatus* recruits per replicate, among the three types (D-, T-, and S-units) ($F_{2,207} = 21.4$, $P < 0.001$; Fig. 3). The Student-Newman-Keuls multiple comparison test indicated that density of recruits on the D-unit was significantly greater than those on the T- and S-units (D->T=S-unit, $P < 0.05$). *C. quinquelineatus* recruits were always observed on the D-units, from the first day after setting the ASUs until the last day of the experiment (Fig. 3). Although the mean numbers of recruits in the D-units were low (0.2–1.6 individuals) during the first to ninth days, many recruits (> 8.8 individuals) were found from the tenth to fourteenth days. In the T- and S-units, on the other hand, the few individuals observed on the second and third days after setting subsequently disappeared (Fig. 3). No recruits were observed in the C-units.

In the D-units, most *C. quinquelineatus* recruits occurred along the outer wall of the units (about 90% of the cumulative total number of fish counted), although some individuals were found inside the units.

Table 1 Cumulative number of individuals and size range (total length) of fish species which occurred on the ASUs during the study period

Family	Species	No. of individuals	Total length range (mm)
Pomacentridae	<i>Dischistodus prosopotaenia</i>	21	15
Labridae	<i>Coris batuensis</i>	1	25
	<i>Oxycheilinus bimaculatus</i>	2	40
Scaridae	<i>Calotomus spinidens</i>	2	25,40
Apogonidae	<i>Apogon ishigakiensis</i>	1	11
	<i>Apogon properuptus</i>	2	12
	<i>Cheilodipterus quinquelineatus</i>	232	11-25
Pinguipedidae	<i>Parapercis cylindrica</i>	3	40
Blenniidae	<i>Meiacanthus grammistes</i>	9	15-18
Monacanthidae	<i>Acreichthys tomentosus</i>	1	20

Fig. 3 Mean number (\pm SE) of *Cheilodipterus quinquelineatus* recruits per replicate ($n=5$) on each type of artificial seagrass unit (D-, T-, S-, and C-units) over 2 weeks.

4. Discussion

All of the fish species occurred in the ASUs were observed in adjacent natural seagrass habitats (NAKAMURA *et al.*, 2003; NAKAMURA and SANO, 2004). In addition, a large number of *Cheilodipterus quinquelineatus* recruits appeared in the ASUs simultaneously with their occurrence in adjacent natural seagrass habitats. This indicates that the ASUs in the present study were effective in investigating the effects of seagrass leaf height and density on fish recruitment patterns.

D-units with long dense seagrass leaves supported a number of *C. quinquelineatus* recruits, whereas few or no recruits appeared on the other unit types with shorter or sparser leaves, throughout the study period. This suggests a preference by recruits for some factors

associated with long dense seagrass.

Most recruits on the D-units were positioned in open areas close to the outer limit of the reefs. Similarly, recruit abundance of *C. quinquelineatus* at the outer limits of natural seagrass microhabitat is significantly greater than that inside such habitats (NAKAMURA, unpubl. data).

Several possible explanations for such microhabitat use can be offered. Hydrodynamics sometimes plays an important role in affecting the distribution patterns of small fishes. BREITBURG *et al.* (1995), for example, found that larvae of *Gobiosoma bosc* actively selected a low flow microhabitat associated with the downcurrent sides of structures. In the present study, the greater abundance of *C. quinquelineatus* in open areas close to the

"walls" of seagrass leaves indicate their preference for low-flow microhabitats, owing to possible wave attenuation at such sites (GAMBI *et al.* 1990). On the other hand, the occurrence of few recruits inside the units may be due to the oscillation of the leaves with water motion. At the present study site, flapping and undulating movements of seagrass leaves were regularly observed, sometimes having high amplitude. Because such movements can physically disturb small fish among the seagrass leaves, the juveniles would prefer the outer edge (HORINOUCI and SANO, 1999).

For such small-sized fish such as the *C. quinquelineatus* vulnerable to predation, availability and accessibility of shelters against predators can affect the distribution of recruits. Denser and higher seagrass leaves may provide more effective shelters, compared with sparser or shorter seagrass leaves (HECK and ORTH, 1980; BELL and POLLARD, 1989). Since some predators such as *Caranx sexfasciatus* often observed, it is likely that the recruits preferred the long and dense leaves of the D-units as shelters. Open areas along the outer edge of the D-units in which most recruits appeared can be a safe zone from which they can easily flee to escape from approaching danger.

In conclusion, the appearance patterns of *C. quinquelineatus* recruits reflected their preference for weak flow microhabitats and effective shelters created by long dense seagrass leaves.

Acknowledgments

We are grateful to Hiroyoshi KOHNO, Ken SAKIHARA, Nagahiro NAKAZATO, Jun ISHIDA, and the Okinawa Regional Research Center (ORRC), Tokai University, for assistance in the fieldwork. Constructive comments on the manuscript from Hisashi KUROKURA, Hiroyuki KAWASAKI, Manabu KAGAWA, Graham HARDY, and two anonymous reviewers were much appreciated. This study was supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science, Sports and Culture (No. 14560141), and is a contribution from the Okinawa Regional Research Center.

References

- BELL, J.D. and D.A. POLLARD (1989) : Ecology of fish assemblages and fisheries associated with seagrasses. *In* Biology of seagrasses. LARKUM, A.W.D., A.J. MCCOMB and S.A. SHEPHERD (eds.), Elsevier, Amsterdam, p. 565-609.
- BELL, J.D., A.S. STEFFE and M. WESTOBY (1985) : Artificial seagrass: how useful is it for field experiments on fish and macroinvertebrates? *J. Exp. Mar. Biol. Ecol.*, **90**, 171-177.
- BELL, J.D. and M. WESTOBY (1986a) : Importance of local changes in leaf height and density to fish and decapods associated with seagrasses. *J. Exp. Mar. Biol. Ecol.*, **104**, 249-274.
- BELL, J.D. and M. WESTOBY (1986b) : Abundance of macrofauna in dense seagrass is due to habitat preference, not predation. *Oecologia*, **68**, 205-209.
- BELL, J.D. and M. WESTOBY (1986c) : Variation in seagrass height and density over a wide spatial scale: effects on common fish and decapods. *J. Exp. Mar. Biol. Ecol.*, **104**, 275-295.
- BELL, J.D., M. WESTOBY and A.S. STEFFE (1987) : Fish larvae setting in seagrass: do they discriminate between beds of different leaf density? *J. Exp. Mar. Biol. Ecol.*, **111**, 133-144.
- BREITBURG, D.L., M.A. PALMER and T. LOHER (1995) : Larval distributions and the spatial patterns of settlement of an oyster reef fish: responses to flow and structure. *Mar. Ecol. Prog. Ser.*, **125**, 45-60.
- CONNOLLY, R.M. (1994) : Removal of seagrass canopy: effects on small fish and their prey. *J. Exp. Mar. Biol. Ecol.*, **184**, 99-110.
- CONNOLLY, R.M. and A.J. BUTLER (1996) : The effects of altering seagrass canopy height on small, motile invertebrates of shallow Mediterranean embayments. *P.S. Z. N. I: Mar. Ecol.*, **17**, 637-652.
- FINN, M.D. and M.J. KINGSFORD (1996) : Two-phase recruitment of apogonids (Pisces) on the Great Barrier Reef. *Mar. Freshwater Res.*, **47**, 423-432.
- GAMBI, M.C., A.R.M. NOWELL and P.A. JUMARS (1990) : Flume observations on flow dynamics in *Zostera marina* (eelgrass) beds. *Mar. Ecol. Prog. Ser.*, **61**, 159-169.
- HECK, K.L. JR. and R.J. ORTH (1980) : Seagrass habitats: The roles of habitat complexity, competition and predation in structuring associated fish and motile macroinvertebrate assemblages. *In* Estuarine perspectives. KENNEDY, V.S. (ed), Academic Press, New York, p. 449-464.
- HORINOUCI, M. and M. SANO (1999) : Effects of changes in seagrass shoot density and leaf height on abundances and distribution patterns of juveniles of three gobiid fishes in a *Zostera marina* bed. *Mar. Ecol. Prog. Ser.*, **183**, 87-94.
- HORINOUCI, M. and M. SANO (2001) : Effects of changes in seagrass shoot density and leaf

- height on the abundance of juveniles of *Acentrogobius pflaumii* in a *Zostera marina* bed. Ichthyol. Res., **48**, 179–185.
- JENKINS, G.P., G.K. WALKER-SMITH and P.A. HAMER (2002) : Elements of habitat complexity that influence harpacticoid copepods associated with seagrass beds in a temperate bay. Oecologia, **131**: 598–605.
- JONES, G.P. (1990) : The importance of recruitment to the dynamics of a coral reef fish population. Ecology, **71**, 1691–1698.
- KAGAWA, M. (2003) : Breeding behavior, early life history, and ecology of the cardinalfish genus *Cheilodipterus* (Perciformes: Apogonidae). Msc Thesis, Tokai University, Shimizu. 57pp., 6 tables, 67 figs. (in Japanese).
- LEE, S.Y., C.W. FONG and R.S.S. WU (2001) : The effects of seagrass (*Zostera japonica*) canopy structure on associated fauna: a study using artificial seagrass units and sampling natural beds. J. Exp. Mar. Biol. Ecol., **259**, 23–50.
- LEVIN, P.S. (1994) : Fine-scale temporal variation in recruitment of a temperate demersal fish: the importance of settlement versus post-settlement loss. Oecologia, **97**, 124–133.
- LEVIN, P.S., R. PETRIK and J. MALONE (1997) : Interactive effects of habitat selection, food supply and predation on recruitment of an estuarine fish. Oecologia, **112**, 55–63.
- NAKAMURA, Y., M. HORINOCHI, T. NAKAI and M. SANO (2003) : Food habits of fishes in a seagrass bed on a fringing coral reef at Iriomote Island, southern Japan. Ichthyol. Res., **50**, 15–22.
- NAKAMURA, Y. and M. SANO (2004) : Comparison between community structures of fishes in *Enhalus acoroides*- and *Thalassia hemprichii*-dominated seagrass beds on fringing coral reefs in the Ryukyu Islands, Japan. Ichthyol. Res., **51**, 38–45.
- ORTH, R.J., K.L. HECK JR and J. VAN MONTFRANS (1984) : Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator-prey relationships. Estuaries, **7**, 339–350.
- SANO, M. (1997) : Temporal variation in density dependence: recruitment and postrecruitment demography of a temperate zone sand goby. J. Exp. Mar. Biol. Ecol., **214**, 67–84.
- WILLIAMS, S.L. and K.L. HECK, JR. (2001) : Seagrass community ecology. In Marine community ecology. BARTNESS, M.D., S.D. GAINES and M.E. HAY (eds). Sinauer Associates, Sunderland, p.317–337.
- ZAR, J.H. (1999) : Biostatistical analysis, 4th edn. Prentice Hall International, London, 663pp.

Received January 13, 2004

Accepted April 12, 2004