

Spatial and temporal patterns of juvenile stone flounder *Kareius bicoloratus* growth rates during and after settlement

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ABSTRACT: Spatial and temporal differences in habitat characteristics of coastal nursery grounds can have a large impact on growth rate, survival, and subsequent recruitment of species with estuarine-dependent early life history stages. Stone flounder *Kareius bicoloratus* is a temperate North Pacific flat-fish species characterized by large recruitment variability and an estuarine-dependent juvenile stage. Post-larvae settle from mid-January to early April in inshore and estuarine nursery grounds, and juveniles subsequently move farther inshore using selective tidal stream transport. Laboratory studies were conducted to determine the sensitivity of juvenile stone flounder growth rates to changes in temperature and feeding rates, at conditions common during settlement (8°C) and post-settlement (12°C). The relationship between RNA:DNA ratio and growth rate was developed in the laboratory, and was used to measure *in situ* growth rates of juveniles from 5 different habitats during settlement (March) and after (April). Juvenile stone flounder in the laboratory grew relatively quickly feeding *ad libitum* at 8°C (4.1% body weight d⁻¹) and 12°C (7.0% bw d⁻¹), but growth rate was very sensitive to changes in feeding rate at both temperatures. Growth rate of starved juveniles was not significantly different between 8 and 12°C and averaged -1.3% bw d⁻¹. RNA:DNA ratios were good predictors of growth rate ($R^2 = 0.88$), with temperature as a covariate. *In situ* growth rates at all 5 stations (1 estuary, 1 seagrass bed, and 3 deeper inshore settlement areas) were higher in April than in March. *In situ* growth rates were highest during both months at the low-salinity estuarine station where fish had highest gut fullnesses. Growth rates were also high at a deeper inshore station near a sewage treatment outfall characterized by higher prey abundances than those typically found in open water areas of Sendai Bay, Japan. Growth rates were consistently lowest at the vegetated (*Zostera* spp. bed) habitat, although retention in this habitat between March and April was high. Spatial differences in sediment grain size and prey abundances may be the primary factors responsible for the large variability in habitat-specific growth rates. Temperature-corrected comparisons of growth limitation suggest that discrete habitats maintain their relative values as nursery grounds over time, although the magnitude of growth limitation was much greater during settlement (March) than post-settlement (April).

KEY WORDS: Stone flounder *Kareius bicoloratus* Growth Habitat RNA:DNA Recruitment

INTRODUCTION

Variability in growth and survival rates of early life history stages is considered one of the primary mechanisms controlling annual recruitment and therefore adult population sizes in fishes (Cushing 1975, Sis-

senwine 1984). Annual recruitment can vary considerably, exerting a strong influence on the fluctuations observed in marine fish population sizes. Such dramatic fluctuations in recruitment are thought to be caused by relatively subtle changes in factors affecting the growth and survival rates of early life history stages (May 1974, Houde 1987, 1989). Specifically, small spatial and temporal changes in abiotic factors (such as temperature, salinity, turbidity), biotic factors (predator and prey population sizes), and habitat qual-

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ity (presence of vegetation, sediment characteristics, current patterns) can greatly affect the growth and mortality rates of larval and juvenile fishes. Although mortality rates are generally lower for juveniles than for larvae, the juvenile stage is usually more prolonged and therefore total mortality of juveniles can equal or exceed that during the larval stage (Smith 1985, Folkvord & Hunter 1986, Kaeding & Osmundson 1988). Additionally, the juvenile stages of many species are dependent on inshore estuaries, which naturally vary both spatially and temporally in their value as nursery habitats and are extremely vulnerable to anthropogenic modification.

The primary cause of mortality during the juvenile stage is thought to be predation (Cushing 1974, Houde 1987, Bailey & Houde 1989). Rapid growth is vital for juvenile fishes to reduce their vulnerability to predation, which has been shown to decrease with increasing size in many species (e.g. Werner et al. 1983, Post & Evans 1989). Juvenile fishes also increase foraging efficiency with growth as a result of increased swimming speeds, larger mouth gapes, and an increased choice of appropriate-sized prey (e.g. Bindoo 1992, Ghan & Sprules 1993).

Therefore, the availability of juvenile nursery grounds suitable for optimal foraging and avoiding predation has a large impact on growth and mortality rates and subsequent recruitment. Assessment of those characteristics of optimal nursery habitats is critical to understanding how spatial and temporal fluctuations in environmental variables can affect recruitment and therefore adult population sizes. Several habitat characteristics have been found to be important to growth and survival of juvenile fishes, including temperature, salinity, turbidity, sediment grain size, prey availability, and the presence of vegetation (Burke 1991, Burke et al. 1991, Malloy & Targett 1991, Sogard 1992, Lankford & Targett 1994). Many studies have documented the importance of habitat characteristics, particularly submerged vegetation, to successful foraging, predator avoidance and survival of juvenile fishes (Orth & Heck 1980, Rozas & Odum 1988, Sogard 1992).

Stone flounder *Kareius bicoloratus* is a temperate North Pacific pleuronectid that supports important commercial and recreational fisheries throughout the coastal waters of Japan. Recent decreases in adult population size are thought to be the result of overfishing and highly variable annual recruitment (Tsuruta 1991). Adults spawn offshore (20 to 50 m depth) during December and January, larvae (10 to 15 mm total length, TL) settle during February and March in coastal waters, and subsequently immigrate to inshore nursery grounds (Tsuruta 1978, 1991). Although juveniles are found at highest densities in shallow (≤ 2 m depth) brackish water 8 to 12°C on

fine to coarse sand (Omori et al. 1976, Yusa et al. 1977), they occur in a wide variety of habitats, including inshore seagrass beds, mudflats, as well as deeper (10 to 15 m) areas with relatively low benthic productivity (Yusa et al. 1977, Yamashita et al. in press). Juvenile stone flounder mortality rates are extremely high immediately following settlement (Yusa et al. 1977, Yusa 1981) and may have a substantial impact on annual recruitment. In addition to a high mortality rate, absolute mortality of juveniles is probably substantial because estuarine residence lasts for about 5 mo (Omori & Tsuruta 1988).

Because stone flounder settle in heterogeneous habitats when temperatures are low, spatial and temporal changes in temperature and prey availability may have a large impact on post-settlement survival and subsequent recruitment. However, there are no data describing the sensitivity of growth rates in this species to different temperatures and variable feeding rates. Additionally, specific habitats may differ in their value as nursery areas, and an understanding of which habitats contain fast-growing individuals may reveal the critical components of stone flounder nurseries. Some nursery grounds contain dense submerged vegetation (primarily *Zostera* spp.), which may provide important predation refuges as well as microhabitats for prey items. In spite of the reported value of vegetated habitats to other juvenile fishes, their importance as flatfish nurseries is not clear. Non-vegetated habitats may be better nurseries for flatfish due to their benthic habits and requirement for suitable burying substrate.

This study examined the spatial and temporal patterns of growth and feeding of juvenile stone flounder in 3 coastal settlement areas and 2 estuarine nursery habitats. The relative importance of temperature and food availability to growth rates of newly settled and juvenile stone flounder was determined in laboratory experiments. RNA:DNA ratios of fish were developed as correlates of growth rates in laboratory experiments, and used to determine *in situ* growth rates of juveniles from the 5 different habitats in and around Sendai Bay, Japan, during March and April 1992. Spatial and temporal patterns in the degree of growth limitation were used to develop hypotheses regarding important habitat characteristics of stone flounder nursery grounds and post-settlement distribution patterns.

MATERIALS AND METHODS

Laboratory experiments. Newly settled juvenile stone flounder were collected with a push net (79 × 59 cm) from Stn 1 (see Table 1, Fig. 1 for description of sampling stations) on March 3, 1992 (19 to 28 mm TL, water temperature 6 to 8°C, salinity 15‰) for the 8°C

Table 1 Station characteristics, sample sizes and densities of stone flounder *Kareius bicoloratus* used to determine *in situ* RNA:DNA ratios and growth rates in and around Sendai Bay, Japan. Salinity is range for March–April on the bottom. N: number of samples analyzed for RNA:DNA ratios. ΔD : instantaneous rate of density decrease (defined in 'Materials and methods')

Station	Depth	Vegetation	Salinity (‰)	Collection dates (1992)	Temp (°C)	N	Fish density (1000 m ⁻²)	ΔD (d ⁻¹)	<i>Crangon affinis</i> density (10 m ⁻²)
1 (Gamou)	10–50 cm	None	3–16	15 Mar 92	10.8	12	856.2		0.39
				24 Apr 92	16.5	20	218.8	0.0332	
2 (Matsushima)	10–50 cm	<i>Zostera</i> spp.	25–31	18 Mar 92	8.0	9	1022.3		
				22 Apr 92	12.5	8	88.7	0.0698	0.75
3 (Matsushima)	1–3 m	None	30–32	18 Mar 92	6.8	20	93.8		
				22 Apr 92	10.5	4	4.5	0.0868	1.1
4 (Airport)	10 m	None	29–33	23 Mar 92	7.2	17	1635.2		
				14 Apr 92	8.5	21	50.6	0.1580	6.1
5 (Off Torinoumi)	10 m	None	29–33	23 Mar 92	7.0	16	442.9		
				14 Apr 92	8.5	16	10.2	0.1714	8.7

experiment, and on April 16, 1992 (20 to 38 mm TL; water temperature 9 to 10.5°C, salinity 16‰) for the 12°C experiment. The size ranges used represent typical size ranges of fish that would experience the experimental temperature in the field (Y Yamashita pers. obs.). Fish were returned to the laboratory and maintained in 7 l of static, aerated, 1 μ m filtered seawater (31‰) at 8 or 12 (± 0.5)°C. Fish were fed wild zooplankton (primarily calanoid and harpacticoid copepods), *Artemia* nauplii and fresh chopped tubifex worms ad libitum. Water changes (50%) were made every other day.

A total of 30 (8°C) or 40 (12°C) fish were acclimated to temperature and laboratory conditions for 4 d. Fish were then starved for 24 h to empty their guts, measured to the nearest mm (TL), blotted with a damp paper towel, and weighed to the nearest 0.0001 g on a Mettler AE 100 electronic balance (accurate to ± 0.0002 g when the same fish was weighed 5 times). Experimental fish were held individually in clear plastic buckets (on black plastic) containing 7 l of filtered seawater with gentle aeration. Diffuse natural light was provided by a large window in the temperature-controlled room (March: 11.5 h light: 12.5 h dark; April: 13 h light: 11 h dark).

At each temperature, 15 fish (chosen at random) were starved and 15 fish were fed ad libitum for 10 d. Ten additional fish at 12°C were fed every other day (approximately 50% maximum rations). Initial fish lengths and weights within each temperature experiment did not differ significantly among ration treatments (*t*-test, 8°C; ANOVA, 12°C). After 10 d, all fish were starved for 24 h to allow their guts to empty, and weighed and measured as above. Daily specific growth rate [$G = \ln(Wt_{10}) - \ln(Wt_1)/t$, where Wt_{10} and Wt_1 are weight on Day 10 and Day 1, respectively, and t is time in days] was calculated for each fish, and was reported as % body weight d⁻¹ [$(e^G - 1) \times 100$]. Mean growth rates at different temperatures and feeding conditions were compared with a 1-way ANOVA ($\alpha = 0.05$).

Immediately after being weighed on Day 10, each experimental fish was frozen on dry ice and freeze-dried at -50°C for 48 h for determination of RNA:DNA. The head, skin and internal organs were removed, and the remaining tissue was powdered for extraction of nucleic acids. RNA and DNA were extracted in ice cold 5% TCA (trichloroacetic acid), and quantified using a slightly modified protocol of Buckley & Bulow (1987) (Malloy & Targett 1994). RNA was quantified by the or-

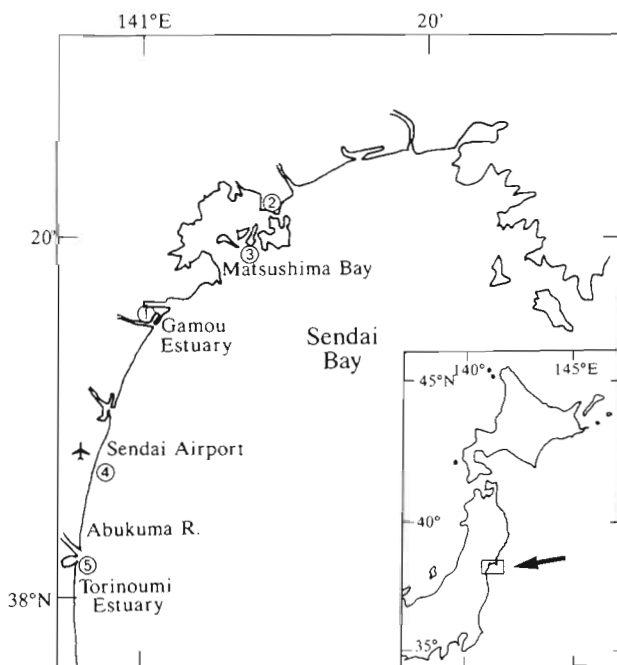


Fig. 1. Location of sampling stations near Sendai Bay, Japan, where juvenile stone flounder were collected for laboratory experiments and determination of *in situ* growth rates. Station numbers and additional site descriptions are in Table 1

cinol reaction, and diphenylamine was used to quantify DNA. Herring sperm DNA and yeast RNA (Boehringer Mannheim) were used as standards for each assay.

The relationship between G and RNA:DNA was determined using multiple linear regression models which initially included Wt_1 , temperature, and all potential interaction terms as independent variables. Only terms which improved the fit of the model (R^2) and which had significance values (p) < 0.05 were included in the final model.

Field collections. Newly settled and early juvenile stone flounder were collected with a push net (79 × 59 cm) and beam trawl (2 m × 30 cm opening, after Kuipers 1975) from 5 different habitats in Sendai Bay, Japan, during March and April 1992 (see Fig. 1, Table 1). These habitats represented 1 estuary (Stn 1), 1 seagrass bed (Stn 2), and 3 deeper inshore settlement areas. Distance of tows was measured using previously measured markers (e.g. buoys, stakes) and/or calibrated flow meters attached to the beam trawl, and/or measured lengths of towing rope. Beam trawls covered $> 1000 \text{ m}^2$ at Stns 4 and 5, and 500 to 1000 m^2 at all other stations. Sampling efficiencies of nets were not considered in calculating fish densities. Water temperature, salinity, and depth were recorded for each sample, and recording temperature probes were left at Stns 1 and 2. Sediment size composition was determined by sieving sediments from the top 2 cm through a series of different sized screens, and size distributions were calculated on a percent dry weight basis.

All fish were collected during daylight hours at least 4 h after dawn to account for possible variability in RNA:DNA caused by diurnal feeding behavior. Some of the juveniles were frozen immediately on dry ice, measured, freeze-dried and analyzed for RNA:DNA ratio as described above. All specimens used for RNA:DNA analysis were post-metamorphic (stages I_3 to J ; Minami 1984, Goto et al. 1989). The remaining juveniles were fixed immediately in 85% ethanol for analysis of gut contents. Gut contents were dissected from the stomach and intestines at least 2 h after ethanol-preserved fish were placed in seawater to stabilize wet weights. Weights of fish (without gut contents) and gut contents were determined to the nearest 0.0001 g, and gut contents were sorted by taxa and the percentage frequency of occurrence was calculated for each prey type. Gut fullness was calculated as (gut content wet weight/fish wet weight) × 100.

Densities of the predatory shrimp *Crangon affinis* at some stations were estimated by averaging numbers of individuals in bottom collections at Stns 1 (push net, March and April), 2 (push net, April),

3, 4 and 5 (push net and beam trawl, March and April) in 1992.

Recent growth rates of field-caught fish were estimated from RNA:DNA ratios and water temperatures at the time of collection using the regression model developed in the laboratory. Mean estimated growth rates were calculated for each site and sampling date, and compared using a 1-way ANOVA ($\alpha = 0.05$) for each month after assumptions of normality and homogeneous variances were confirmed (Zar 1984). Tukey's multiple comparisons test was used to differentiate statistically among different mean growth rates.

This study did not quantify movement of individuals among habitats, or measure immigration rates due to newly settled individuals. Instead, 'retention' ($D_t/D_i \times 100$, where D_i is density in March and D_t is density in April) and ΔD (the instantaneous rate of density decline = $-(\ln(D_t) - \ln(D_i))/t$, where t is time in days) were used to estimate the change in fish density over time at each station.

RESULTS

Effects of temperature and ration on growth rates

Growth rates of juvenile stone flounder in the laboratory were strongly affected by both temperature and feeding rate. Growth rates under ad libitum feeding conditions were significantly higher at 12 than at 8°C (Table 2). Mean specific growth rate of juveniles fed 50% ad libitum at 12°C was not significantly different from that of juveniles feeding unrestricted at 8°C. The mean growth rate of unfed juveniles was -1.32 d^{-1} , and did not differ significantly between 8 and 12°C.

RNA:DNA ratios as predictors of growth rate

RNA:DNA ratios were positively and significantly related to specific growth rates of juvenile stone floun-

Table 2. *Kareius bicoloratus*. Specific growth rates (SGR) of juvenile stone flounder reared in the laboratory at 2 temperatures and a range of ration levels (% ad libitum). Superscripted letters indicate means that are not significantly different as determined by a 1-way ANOVA and Tukey's multiple comparisons tests

Temperature (°C)	Ration (% maximum)	Mean SGR (% body weight d^{-1})	SD	SE	N
8	0	-1.287 ^a	0.428	0.111	15
8	100	4.190 ^b	1.296	0.335	15
12	0	-1.361 ^a	0.385	0.099	15
12	50	4.168 ^b	0.840	0.266	10
12	100	7.077 ^c	1.081	0.279	15

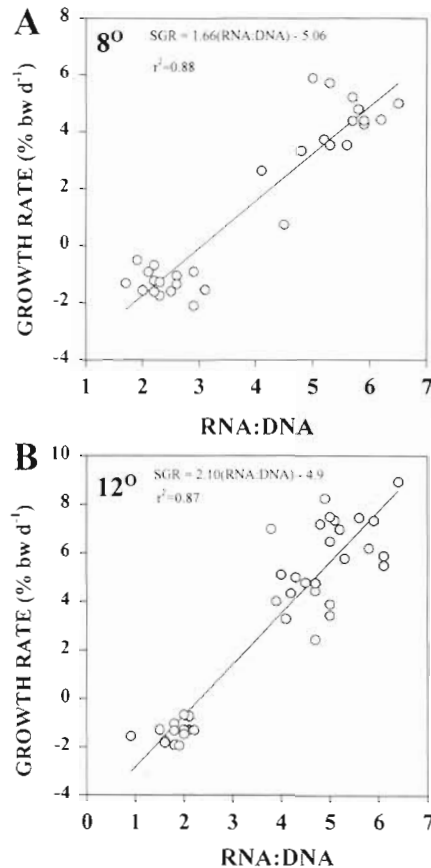


Fig. 2. *Kareius bicoloratus*. Relationship between RNA:DNA ratio and specific growth rate (SGR) for juvenile stone flounder reared in the laboratory at 2 temperatures. Each point represents a single fish. Solid lines are least-squares linear regressions, and dotted lines are 95% confidence intervals of the regression lines

der at both temperatures ($r^2 \geq 0.87$; Fig. 2). However, these relationships (i.e. slopes) were significantly different between fish reared at 8°C and fish reared at 12°C (Fig. 2). Therefore, the best multiple linear regression model predicting growth rate from RNA:DNA ratio included additional independent variables for temperature and for temperature \times RNA:DNA interaction. $G = 0.762(\text{RNA:DNA}) + 0.039(\text{Temp}) + 0.112(\text{RNA:DNA} \times \text{Temp}) - 5.371$ ($R^2 = 0.88$). Fish weight (W_i) (61 to 425 mg) was not a significant covariate in this relationship.

In situ RNA:DNA ratios and growth rates

RNA:DNA ratios of juvenile stone flounder were generally higher in April than in March (Fig. 3). Juveniles from Stns 1 and 4 had the highest mean RNA:DNA ratios during both months. *In situ* growth rates of juvenile stone flounder, calculated from

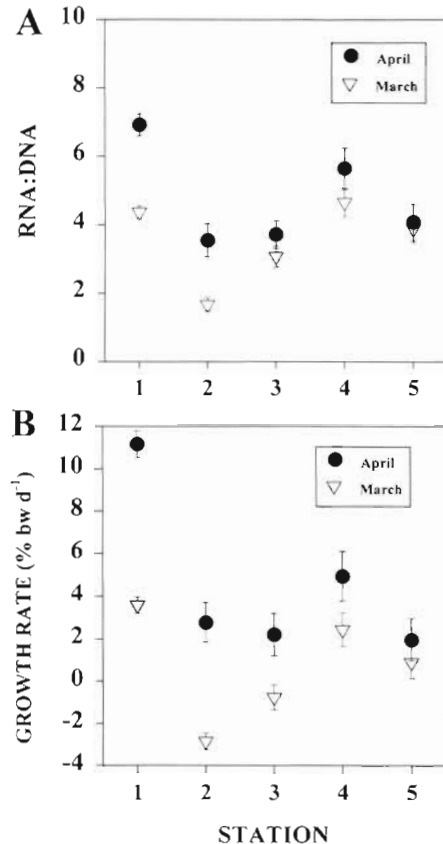


Fig. 3. *Kareius bicoloratus*. RNA:DNA ratios and specific growth rates (SGR) of juvenile stone flounder collected from 5 stations in and around Sendai Bay during March and April 1992. Points represent means ± 1 SE. SGR were determined from RNA:DNA ratios and water temperature using the multiple regression equation described in the text. Sample sizes and collection dates given in Table 1

RNA:DNA ratios and water temperatures, were significantly higher in April than in March at all stations except Stn 5 (Fig. 3). During March, juveniles collected from Stns 1 and 4 had the highest mean growth rates, and those collected from the only vegetated habitat (Stn 2) had the lowest growth rate measured ($1 = 4 > 5 > 3 > 2$). A similar pattern was evident during April, when mean growth rates reached 11.5 and 5.0 d^{-1} at Stns 1 and 4, respectively ($1 > 4 > 2 = 3 = 5$).

To adjust for temperature differences among stations, growth rates were also plotted as the percentage of maximum (calculated from laboratory-derived temperature-growth relationships and water temperatures for each collection) (Fig. 4). In spite of temperature differences of 4 (March) to 8°C (April) among sampling stations, the temperature-corrected pattern of habitat-specific growth rates (Fig. 4) is similar to the pattern for the raw growth rate data (Fig. 3). Growth rates were all less than maximum in March, ranging from 64% of maximum at Stn 1 to negative growth rates at Stns 2

and 3. Growth rates during April were above the expected maximums at Stns 1 and 4, and about half of maximum at Stns 2, 3 and 5.

Densities of juvenile stone flounder ranged from 4.5 1000 m⁻² (Stn 3, April) to 1635.2 1000 m⁻² (Stn 4, March). Retention was highest at Stn 1 (25%), and lowest at Stn 5 (2.3%) [Stn 1 (25%) > Stn 2 (8.7%) > Stn 3 (4.8%) > Stn 4 (3.1%) > Stn 5 (2.3%)], although no statistical evaluation was possible due to the lack of replicates. Instantaneous rates of density decrease at each station, which account for time differences between March and April sampling events, were ordered oppositely, indicating the same trend of increased retention at Stn 1 and lowest retention at Stn 5 (Table 1).

Gut fullness and diet composition

Samples sufficient for gut content analysis were only available from Stns 1, 2 and 4 (Table 3). Juvenile stone flounder gut fullness was highest at Stn 1, intermediate at Stn 4, and lowest at Stn 2 during both months (Table 3). Juveniles from all stations had fuller guts during April than during March. Copepods and polychaetes were the predominant prey items at all stations during both months, although ostracods and mysids were also important components of the diet at Stn 2. All copepods from fish at Stn 2 were small harpacticoids, while those in fish from Stns 1 and 4 were composed of both small harpacticoids and large calanoids. Bivalves were also important prey items of fish in April.

DISCUSSION

Effects of temperature and ration on growth rates

Juvenile stone flounder held in the laboratory grew at relatively fast rates (4 to 7% bw d⁻¹) at the temperatures which characterize field conditions during and immediately following settlement (8 to 12°C). Therefore, growth potential appears to be high during the post-settlement period at ambient temperatures. Growth rates of unfed juveniles were negative, but only moderate in magnitude and not significantly different between 8 and 12°C, suggesting that weight loss due to starvation is relatively slow in fish at these typical sizes and temperatures during and subsequent to settlement. However, fish in the lab were fed ad libitum up until starvation, and lowered initial condition in the field may result in higher mortality and/or starvation rates over similar time periods. Newly settled stone flounder therefore appear to be poised to take advantage of favorable feeding conditions by losing weight slowly during periods of low prey availability, but retaining the metabolic capacity for rapid growth when prey becomes available. This energetic strategy was also observed in summer flounder *Paralichthys dentatus*, another temperate winter-spawned flatfish which relies on inshore estuarine nursery grounds (Malloy & Targett 1994).

Specific growth rates under maximum feeding regimes increased very quickly over a relatively small temperature range (>65% increase over the 4°C range). Consequently, small spatial and temporal variations in temperature during and following settlement

Table 3. *Kareius bicoloratus*. Gut fullness (gut content wet weight/whole body wet weight × 100) and gut contents (% occurrence among all fish) of juvenile stone flounder collected from 3 stations in Sendai Bay, Japan. Station designations are the same as those in Table 1. Values are means (SD); N = 20 fish for all stations except Stn 2 (N = 14 in March, N = 16 in April). Nem: Nematoda; Biv: bivalves or siphons; Poly: polychaeta; Cope: Copepoda; Cuma: Cumacea; Sip: Sipunculoidea; Mys: Mysidacea; Ostra: Ostracoda; Gamma: Gammaridea

Stn		Gut fullness	% Occurrence								
			Nem	Biv	Poly	Cope	Cuma	Sip	Mys	Ostra	Gamma
1	March	1.687 (0.717)	10	5	35	85	0	20	10	35	0
	April	4.917 (1.137)	20	40	100	75	0	35	0	5	15
2	March	1.051 (0.809)	28.6	14.3	14.3	64.3	0	0	0	71.4	0
	April	1.666 (0.925)	18.8	25	12.5	100	0	0	56.3	62.5	18.8
4	March	1.301 (0.642)	10	5	75	30	15	0	0	0	15
	April	2.315 (0.860)	25	30	95	25	15	0	0	0	0

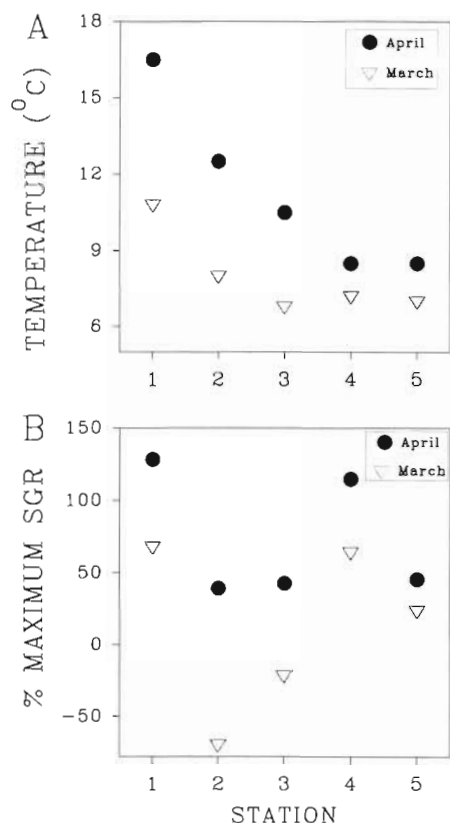


Fig. 4. *Kareius bicoloratus*. Water temperature and growth limitation (% maximum growth rate) of juvenile stone flounder collected from 5 stations in and around Sendai Bay during March and April 1992. Percentage maximum growth rate was calculated as $(SGR_{in situ} / SGR_{max}) \times 100$, where $SGR_{in situ}$ is the mean growth rate for each station and date shown in Fig. 3 and SGR_{max} is the growth rate of juveniles feeding ad libitum calculated from the relationship between maximum growth rate and temperature (derived from laboratory experiments)

could have a dramatic effect on growth rates, and therefore on mortality rates and subsequent recruitment. Specific growth rates generally decrease with increasing body size, suggesting that the observed differences would have been even greater if the fish tested at 12°C had not been larger than those tested at 8°C.

RNA:DNA ratios as predictors of *in situ* growth rates

RNA:DNA ratios provided estimates of *in situ* growth rates within the range expected based on laboratory experiments. Temperatures in the field ranged from 6.8 to 10.8°C during the peak in settlement in 1992 (March), and mean *in situ* growth rates ranged from -2.81 to 3.53% bw d⁻¹. During April, *in situ* growth rates ranged from 1.96 to 11.17 at temperatures between 8.5 and 16.5°C. Although temperature was a

significant covariate, the derived relationship between RNA:DNA ratio and specific growth rate provided reasonable growth rates and low within sample variability over the temperature range used in the lab (8 to 12°C). However, specific growth rates calculated from RNA:DNA ratios and water temperatures exceeded the maximum growth rates predicted in the laboratory at Stns 1 (16.5°C) and 4 (8.5°C) during April. Although the relationship between RNA:DNA ratios and growth rate is frequently less reliable at higher temperatures (i.e. >16°C), as suggested by several previous reports (Buckley et al. 1984, Malloy & Targett 1994), RNA:DNA ratio relationships usually underestimate growth rate at higher temperatures, not overestimate them as was the case in the present study.

In situ water temperatures showed substantial diel variability corresponding to changes in air temperature, especially at shallow sites (e.g. maximum daily temperature changes among all sampling dates were 8.6°C at Stn 1 and 5.2°C at Stn 2). The temperatures used to calculate *in situ* growth rates were averages representative of the sites within 2 to 4 h of collection. Therefore, the 2 unexpectedly high values obtained for *in situ* growth rates might have resulted from overestimates of true mean water temperatures. Additionally, a few collections were made at temperatures outside those used to generate the RNA:DNA-growth rate relationship. Although *in situ* temperatures (7.0 to 16.5°C) varied only a few degrees from those used in the laboratory (8 to 12°C), the apparent temperature-sensitivity of stone flounder growth rates suggest that even small temperature changes could result in significantly different growth responses.

Spatial and temporal patterns of *in situ* growth rates

Juvenile stone flounder grew relatively quickly immediately after settlement in almost all habitats sampled (except during March in the 2 Matsushima Bay habitats: Stns 2 and 3), although growth rates were significantly different among the 5 habitats sampled. Mean growth rates were higher in April than in March at almost all sites, suggesting that higher water temperatures later in the spring were accompanied by consistently high or elevated numbers of prey. This hypothesis is supported by higher gut fullness at all stations during April (even though the fish were larger in April and had higher gut capacities than in March), although gut fullness data are somewhat limited. Higher growth rates and gut fullness in April may also be due to larger juveniles being able to forage on larger and more diverse prey items.

Specific growth rates of field-caught juveniles were highest at Stn 1, the only estuarine habitat sampled,

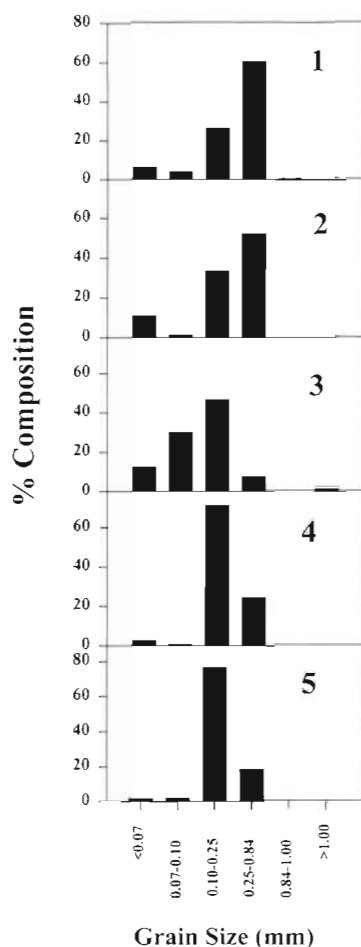


Fig. 5. Grain size composition of bottom sediments at 5 stations in and around Sendai Bay. Compositions were determined by separating sediments into size classes with sieves and calculating the relative proportion (by dry weight) of each fraction. Stations numbers are the same as those in Table 1

during both March (3.6% bw d⁻¹) and April (11.2% bw d⁻¹). This station also had the highest juvenile stone flounder retention (25.6% between March and April), suggesting that emigration and/or mortality was lowest at this site. This station was an estuarine nursery ground characterized by the coarsest sediments, lowest salinity and highest temperatures of any habitat sampled (see Table 1, Fig. 5). High temperatures and adequate prey abundances were probably responsible for much of the dramatically higher growth rates observed at Stn 1. Although quantitative estimates of prey populations are not known, gut fullness was higher at this station than at any of the stations measured in this study. Additionally, this station had the largest sediment grain sizes, which are associated with higher flounder prey densities (Omori & Tsuruta 1988).

Stn 1 also received sufficient freshwater inputs to maintain salinities of ~3 to 16‰, lower than all of the other stations sampled. Several studies have documented a significant metabolic cost of osmoregulation (Moser & Hettler 1989, Abud 1992), and ambient isosmotic conditions may allow a larger scope for growth by reducing the metabolic costs of osmoregulation. Preliminary laboratory studies show that juvenile stone flounder are very tolerant of low salinity (100% survival after 48 h at <2‰; Tsuruta pers. comm.), but the effects of salinity on growth rates have not been investigated.

The remaining stations were influenced primarily by oceanic water in Sendai Bay, including the seagrass bed habitat in Matsushima Bay (Stn 2), and 3 deeper inshore areas (Stns 3 to 5). Growth rates at Stn 4 ranked second behind Stn 1 during both months, and were notably higher than growth rates at the other deep stations. Stn 4 was almost identical to Stn 5, which was also relatively deep and characterized by cooler, high salinity water, but fish at Stn 5 had much lower growth rates. Sediments at Stns 4 and 5 were also similar; somewhat smaller than those at Stn 1 (Fig. 5). However, Stn 4, unlike Stn 5, was in close proximity to a sewage-treatment plant outfall 2 km to the south (Abukuma River Sewage Treatment Plant). Prey densities at Stn 4 may be higher than those at other, similar open water areas in Sendai Bay (Abukuma River Sewage Office 1990–1994). In the present study, the retention at Stn 4 was extremely low (3.1% retention), similar to that at Stn 5 (2.3%), suggesting that emigration to shallower inshore nurseries and/or mortality rates were substantially higher offshore than those at Stn 1. Newly settled stone flounder are known to actively immigrate inshore at night on flood tides (Tsuruta 1991), and the temporal decrease is probably partially a result of emigration to inshore areas.

One surprising result was the consistently low growth rates observed in the only vegetated habitat sampled, Stn 2. *Zostera* spp. beds have been shown repeatedly to be favorable habitats for juveniles of several species, presumably due to higher prey densities and their function as predation refuges (e.g. Sogard & Able 1991). Stn 2 contained very high densities of *Zostera* spp. and primarily (>85%) coarse sediments similar to those at Stn 1 (where growth was highest of all stations sampled) (Fig. 5). However, the sediments in the *Zostera* spp. bed appeared to be stratified, with finer sand covering the coarser-grained sediments, which may account for the apparently low prey densities (inferred from gut fullness data). It is also possible that dense vegetation cover actually reduces the available habitat area of juvenile flounder, which usually prefer to bury in the sediments and adopt a sit-and-wait foraging strategy, or stalk prey in discrete bursts

of swimming followed by settlement and reburial. Sogard (1992) also found that juvenile winter flounder inhabiting *Zostera* spp. beds in New Jersey (USA) estuaries grew less quickly than juveniles in other habitats. She speculated that factors such as temperature and sediment structure might account for the observed lack of enhanced flatfish growth in vegetated habitats.

Sediment characteristics may also be partly responsible for the observed pattern of habitat-specific growth rates in April. Juvenile stone flounder appear to prefer coarser sediments, since they are found predominantly in habitats containing sediments larger than 0.25 mm ϕ (Omori & Tsuruta 1988). We observed a similar correlation between stone flounder densities and sediment grain size (see Table 1, Fig. 5). Other species of flatfish have also been found to display a preference for sediment grain size. For example, Burke (1991) found that juvenile summer flounder *Paralichthys dentatus* in the laboratory preferred coarser-grained sand over finer-grained mud, even if food was available only in less-preferred sediments. Energetics studies have shown that the metabolic rates of flatfishes can increase significantly when the fish are forced to bury in sediments with suboptimal grain sizes or do not have access to sediments at all (Peyraud & Labat 1962, Howell & Canario 1987). Two other western Pacific flatfish species have also shown a preference for sediment types in which they could most easily bury (Tanda 1990). Presumably, selection has resulted in flatfishes with sediment preferences which minimize metabolic costs of burying as well as optimize their ability to forage and hide from predators. Other characteristics of sediments, such as color and organic content, have also been found to be important habitat characteristics for juvenile flatfishes (Burke 1991), but these factors were not addressed in this study.

Juvenile stone flounder growth rates were found to be higher in this study (during April) and others in habitats containing larger-grained sediments (Omori & Tsuruta 1988). Omori & Tsuruta (1988) speculated that this pattern was correlated with the abundance and composition of benthic invertebrates which comprise important infaunal prey items, which decrease with decreasing grain sizes of bottom sediments in Sendai Bay (Omori 1975, Omori & Tsuruta 1988). Omori & Tsuruta (1988) demonstrated that juvenile stone flounder had higher growth rates and stomach fullness when caged on larger sediments (45.5% > 0.25 mm diameter) than when held over smaller sediments (16.4% > 0.25 mm), further suggesting that sediment-specific growth and distribution patterns may reflect patterns of optimal prey distribution.

In general, growth limitation was more severe during March (<0 to 64% predicted maximum growth

rate) than during April (43 to >100% predicted maximum), in spite of the higher temperatures and therefore higher maximum potential growth rates in April. Flounder densities were approximately an order of magnitude lower in April than in March at each station, and gut fullness was higher. Therefore, the relative prey abundance (i.e. prey/flounder ratio) may have been higher in April.

Although the magnitude of growth limitation changed between March and April, spatial patterns in growth limitation among sampling habitats remained very similar during both months. Growth limitation, therefore, seems to be a function of habitat characteristics, but not a function of the temperature or prey population cycles. Sogard (1992) also observed that different habitats seemed to maintain consistent growth potentials for different species and over long time periods (years). Habitat characteristics at Stns 1 and 4 seem to be suited to high growth rates in juvenile stone flounder, although the dramatic decreases in density observed in these habitats between March and April suggests that mortality/emigration in these habitats is substantial.

Potential importance of predation to habitat quality

Although growth rates and gut contents were the only factors used to infer the relative importance of different habitats as stone flounder nursery grounds in the present study, other factors, such as predation rates, are also likely to be important in determining optimal nursery habitats. Predation likely constitutes a large fraction of overall juvenile mortality, and the variables determining optimal predation refuges may differ from those resulting in high growth rates. For example, although juveniles collected from *Zostera* spp. beds had the lowest growth rates observed, they may have lower overall mortality rates due to decreased predation rates. In fact, overall retention at the *Zostera* spp. site (Stn 2) was second highest among the 5 stations in this study. Rapid growth does facilitate lowered mortality rates, but several studies have documented that juvenile fishes will select habitats that offer enhanced protection from predators while sacrificing optimal foraging and/or growth rates (e.g. Werner et al. 1983). Although the predatory crangonid shrimp *Crangon affinis* is less abundant in *Zostera* spp. beds (Table 1), it is unknown if vegetated, inshore habitats afford any protection from piscivorous predators such as gobiid fishes. Sogard (1992) and our data suggest that for flatfish, which rely heavily on burrowing and feeding on benthic invertebrates, vegetated habitats may not represent an optimal predation refuge or foraging habitat.

Low salinity habitats also tend to have lower abundances and generally smaller sizes of *Crangon affinis*. Predation by *C. affinis* has been observed in the laboratory, and juvenile stone flounder otoliths have been found in the guts of *C. affinis* (Yamashita et al. in press), which can reach densities of almost 1 m^{-2} on sandy bottoms at depths $>10\text{ m}$ in Sendai Bay (Table 1). Therefore, inshore nursery areas may not only provide higher temperatures and increased prey availability which facilitate rapid growth, but may be important predation refuges as well. Additional work examining the importance of sediment characteristics (i.e. grain size, color) and the presence of vegetation to predation rates is needed to better understand the value of different nursery areas to this species.

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