Adam Moles[1] and **Ryan Scott,** Auke Bay Laboratory, Alaska Fisheries Science Center, National Marine Fisheries Service, NOAA, 11305 Glacier Highway, Juneau, Alaska 99801-8626

Growth and Behavior of Juvenile Alaskan Flatfishes in the Laboratory

Abstract

Juvenile Pacific halibut reared in the laboratory grew at a faster rate and had a more uniform distribution in behavior tests than juveniles of yellowfin sole and rock sole. Juvenile yellowfin sole and rock sole had similar 90-d growth rates (0.71-0.83% BWD) in the laboratory at 10°C, whereas Pacific halibut grew significantly faster (1.29% BWD). Rock sole grew the same on both mud and sand, despite a known preference for sand substrate in the field. Yellowfin sole and rock sole had an aggregated distribution in the tanks as measured by nearest-neighbor analysis regardless of the presence of other species, whereas Pacific halibut had a uniform distribution. These are the first observations of growth and behavior of juveniles of these species in the laboratory.

Introduction

Understanding interspecific differences in growth and behavior among various species of juvenile flatfishes may increase understanding of such specific differences as time spent in nursery areas, vulnerability to predators, types and sizes of prey items, intraspecific and interspecific competition for food and space, and productivity of the stock. These processes are all influenced to some degree by initial growth (Weatherley and Gill 1987) and social interactions (Olla et al. 1996) of juveniles of many fish species. Growth also hastens or delays recruitment of flatfishes into the fishery: larger age-0 flatfishes have reduced predation and faster maturation than smaller age-0 flatfishes (van der Veer et al. 1994). In addition, behavior can regulate distribution, feeding, and predator avoidance in many fish species (Olla et al. 1996).

Intuitively, different species may have different growth rates and distributions as mechanisms for partitioning the nursery grounds. Juvenile flatfishes reduce competitive interactions between species through differences in habitat, such as depth and substrate (Norcross et al. 1995). These differences in field distribution of similar-sized individuals are probably the result of a complex interaction of a number of variables such as interspecific differences in growth rates and behavioral interactions.

To better understand these initial differences among species of Pacific flatfishes, we assessed growth rates and spatial distribution among newly settled recruits of three commercially important species of flatfishes. We chose young-of-the-year Pacific halibut (*Hippoglossus stenolepis*), yellowfin sole (*Limanda asper*), and rock sole (*Lepidopsetta bilineatus*). The adults of these species constitute over half of the flatfish catch in the northeastern Pacific Ocean, totaling a high of nearly 300,000 metric tons in 1992 (FAO 1995). The combined flatfish fishery is the third largest fishery in the northeastern Pacific Ocean, after walleye pollock (*Theragra chalcogramma*) and Pacific salmon (*Oncorhynchus* spp.) (FAO 1995). Extensive knowledge has been gathered about adults of these flatfish species, but far less is known about the juvenile stage. Because many of the environmental conditions that affect growth and behavior cannot be controlled in the field, our studies were conducted in the laboratory.

Following a planktonic larval stage, the recruits of all three species settle out into subtidal nursery areas (<40 m deep) for the first year or two (Norcross et al. 1995) where they feed largely on crustaceans. Following migration into deeper waters as adults, the Pacific halibut grow to nearly 3 m in length, whereas yellowfin and rock sole are typically <45-55cm. In the study described here, we compared growth rates and spatial distributions among young-of-the-year flatfish juveniles to determine whether differences were evident as early as the first year of life.

---

[1] Author to whom correspondence should be addressed. Email: Adam.Moles@noaa.gov

Methods

Preparation of Animals

Yellowfin sole and rock sole were obtained from Auke Bay, Alaska, by 6-mm-mesh beach seine in June, 1994 and 1995. Pacific halibut were collected in 10–30 m depths in Middle and Kalsin Bays off Kodiak Island by plumb-staff beam trawl (4-mm mesh) in August 1994 and 1995. All specimens (46–109 mm standard length, mean 69 mm ± 15.9) were transported live to the Auke Bay Laboratory and held in flowthrough seawater tanks on a mixed sand and mud substrate. Fish were held for two months to acclimate to laboratory conditions.

Individual fish were identified for repeated size measurements and behavioral observations by marking juveniles on the ventral surface at one or more of four locations along the lateral margin with Alcian Blue dye (65 mg/mL aqueous solution) to give each fish a unique mark. This allowed us to measure the growth rates in each fish rather than following the mean change of a group of fish. This dye marking method (Thedinga and Johnson 1995) is a fast, external, non-invasive procedure that permits mark retention even in rapidly growing juvenile fishes. Unanesthetized flatfish were jet injected with dye using a Panjet held 25 mm above the skin surface. Excess dye was wiped off, and the fish held in a recovery tank for 1 hr to verify dye retention. Alcian Blue dye does not affect juvenile flatfish growth, alter tissue structure, or become incorporated into internal organs (Thedinga et al. 1997).

Growth Tests

Growth tests were conducted in 70-L tanks (30 x 60 x 40 cm) under outdoor translucent panels with a constant 12 hr/d of supplemental full-spectrum lighting. Seawater flow rates were 1.4 L/m at 28‰ salinity and 10°C, such as is commonly observed during late summer in flatfish nursery areas in southeastern and southcentral Alaska. Temperature was controlled by resistive heaters, mercury switches, and associated relays.

Groups of fish were reared on specific substrates. Each species was tested on its preferred sediment, Pacific halibut and rock sole on sand, yellowfin sole on mud (Moles and Norcross 1995). In addition, rock sole was also tested on mud so that growth for that species could be measured on preferred and unpreferred sediments. Mud was gathered intertidally, frozen and thawed three times to kill any organisms present, then sieved to remove particles and macrofaunal prey items organisms larger than 63 µm. Sand (64–249 µm diameter) obtained from a local gravel yard was sieved to yield a mean particle size of 125 µm. Fish were fed to satiation with a daily ration of commercial blood worms (*Tubifex tubifex*) and mysids (*Mysis* spp.) for two weeks before and throughout the test. Crustaceans such as mysids are a common component of juvenile flatfish diets, and the addition of the bloodworms enhanced the feeding response. The fish were fed six times per day to reduce feed waste and size variance due to possible competition for food among fish (Brännäs and Alanärä 1993). All fish ate voraciously when food became available.

There were four treatment groups (Pacific halibut on sand, yellowfin sole on mud, rock sole on sand, and rock sole on mud), each with 20 fish, reared for 90 d. Because it was only possible to obtain 10 distinctive dye marks, it was necessary to spread the 20 test fish of each treatment among two tanks. This gave a final total of 8 tanks of 10 fish each.

Individual lengths (to nearest mm) and weights (in mg) were measured at 0, 30, 60, and 90 d on the same individually-marked fish. Fish were weighed live in a tared beaker of water, then quickly measured for length and returned to the water. Growth rates for length (incremental growth rate) and weight (specific growth rate) were calculated for each fish (Fonds et al. 1995). Daily length increments (dL) were computed from differences in standard length (L) over time as: $dL = (L_{end} - L_{start})/t$, where t is time in days. Specific growth rate was computed as: $G = (\ln W_{end} - \ln W_{start})/t \times 100$, where W is body wet weight. Only the 90 d growth rates are presented here. Differences in growth rates between the four treatment groups were analyzed using a repeated measures one-way ANOVA at day 0 and day 90 followed by Tukey's test of multiple comparisons at $P = 0.05$.

Spatial Behavior Tests

The spatial distribution of flatfishes was determined using a grid painted on the bottom of a 4.8-m diameter 18,000 L circular tank divided into 118 observation areas. Water depth was 1 m, flow rate 20 L/min (to maintain sufficient oxy-

TABLE 1. Mean lengths, weights, and growth rates (±SE) of three species of juvenile flatfishes reared in the laboratory for 90 d. IGR= incremental growth rate as mm increase per day, SGR= specific growth rate as % body weight per day, N= 20. Within IGR or SGR, means with the same superscript are not significantly different (P<0.05).

	Halibut		Yellowfin sole		Rock sole on mud		Rock sole on sand	
	0 d	90 d	0 d	90 d	0 d	90 d	0 d	90
Length (mm)	72 (1.6)	93 (1.4)	71 (4)	89 (4)	66 (4)	79(4)	67 (4.1)	84 (3.9)
Weight (g)	4.10 (0.29)	11.63 (0.62)	4.38 (0.77)	8.89 (1.4)	3.76 (0.79)	6.2 (0.94)	3.96 (0.85)	6.9 (0.92)
IGR		0.24(0.01)^{a}		0.19 (0.01)^{b}		0.15 (0.01)^{b}		0.19 (0.01)^{b}
SGR		1.29 (0.03)^{a}		0.83 (0.04)^{b}		0.71 (0.01) ^{b}		0.82 (0.10)^{b}

gen), salinity 29‰, and temperature 10°C; no sediment was present. Twenty Alcian blue dye-marked juveniles (average length 80 mm) of the test species and 20 unmarked fish of the same or a different species were chosen randomly and introduced to the tank by net. Using the grid, we measured the distance between each marked fish and its marked nearest neighbor at 30 min intervals for 20 hr, allowing us to calculate mean nearest neighbor ratios over 20 hr periods for each group. After 20 hr, the fish were removed and 40 new fish of the next trial were introduced. All 6 combinations (halibut/halibut, yellowfin sole/yellowfin sole, rock sole/rock sole, halibut/yellowfin, halibut/rock sole, and yellowfin sole/rock sole) were tested with five replicate trials each for a total of 100 test individuals per species. No fish was used for more than a single trial to insure independent observations.

The distance-to-nearest neighbor method (Clark and Evans 1954) described the intraspecific spatial distribution of a given species in the presence of other flatfishes, both of the same and of another species. Nearest-neighbor distances were calculated between marked individuals of the same species, but not of different species. The measure of spacing (R) indicates the degree to which the distribution of the sample deviates from a random distribution (R = 1). The smaller the value of R, the more aggregated the distribution; the higher the value, the more uniform the distribution. We examined differences in nearest neighbor ratios using Wilcoxon signed rank tests.

Results

All fish grew substantially over the 90-d test period. Yellowfin sole weights increased 103%, rock sole weights increased 65% (mud) and 74% (sand), and halibut weights increased 184%. Differences in initial size between the species were not significant for length or weight. Initial mean condition factors also did not differ significantly among all tanks and treatment groups.

Pacific halibut grew significantly faster than the other treatment groups (P < 0.001). Daily incremental growth rates (mm/d) were significantly greater (P < 0.05) for Pacific halibut than for rock sole or yellowfin sole (Table 1). By day 90, Pacific halibut had grown 0.24 mm/d, 26% greater than rock sole on sand or yellowfin sole. All of these treatment groups increased in length greater than rock sole on mud. There was a significant difference in incremental growth rate but not in specific growth rate between rock sole on mud and on sand. Other differences in growth rate between species or substrate types were not significant. Yellowfin sole and rock sole grew at nearly identical rates throughout the experiment .

During the behavior tests, halibut distributed themselves uniformly R = 1.83-2.00) in the experimental tanks when other species were not present. In contrast, when all 40 fish in the tank were yellowfin sole, more aggregation occurred (R = 0.86 ± 0.02). Rock sole were significantly (P < 0.001) more aggregated (R = 0.71 ± 0.01) than yellowfin sole. Distribution patterns for each species did not change when a second species was present (Table 2), except for a decrease in uniformity of Pacific halibut when rock sole were present. Both yellowfin sole and rock sole became slightly

TABLE 2. Nearest-neighbor distances (R values ± SE) for experimental groups of marked juvenile flatfishes of the same species (measured species) in the presence of an equal sized group of the same or a different species (influence species). N= 100 fish.

	Influence Species		
Measured Species	Yellowfin sole	Rock sole	Pacific halibut
Yellowfin sole	0.86 (0.01)	0.66 (0.01)	2.00 (0.3)
Rock sole	0.79 (0.01)	0.71 (.01)	1.83 (0.02)
Pacific halibut	0.83 (0.01)	0.73 (0.01)	1.94 (0.02)

more aggregated in the presence of the other species, but this difference was significant only for yellowfin sole ($P < 0.01$). The distribution of Pacific halibut was not significantly different in the presence of yellowfin sole, but was significantly less uniform in the presence of rock sole ($P < 0.01$). Yellowfin sole and rock sole did not alter their spacing in the presence of Pacific halibut.

Discussion

This is the first laboratory estimate of growth rates following settlement for these valuable commercial species, a period that is important to subsequent survival. Flatfishes, as most fishes, grow most rapidly as juveniles (Paul et al. 1994), and any reduction in juvenile growth rate prolongs the juvenile stage. Mortality during this phase and timing of onset of maturation are directly determined by fish size (Zijlstra et al. 1982, Rijnsdorp 1993). As fish size increases, predation on the fish is reduced for some flatfishes, and in such cases survival is related directly to growth (Witting and Able 1993, van der Veer et al. 1994). Fish that are able to grow quickly during the early juvenile stage, such as Pacific halibut, are less susceptible to predation and have a competitive advantage over slower-growing fish.

The differences in growth between Pacific halibut and the other flatfish species examined in this study are interesting, given the unique energy requirements of Pacific halibut. Pacific halibut, which grew more rapidly than yellowfin sole or rock sole in our study, have greater energy expenditures than yellowfin sole. The oxygen requirement for juvenile halibut is four times higher than for yellowfin sole, due to longer migrations and more active feeding (Paul et al. 1994). Juvenile halibut compensate for this high rate by eating relatively larger meals than do adult fish (Paul et al. 1994). In our study, growth rates of Pacific halibut estimated by length were 26% greater than for yellowfin sole, and the specific growth rate of halibut over the 90 d test period was 55% greater than for yellowfin sole. The growth rates of flatfish in our study are similar to estimates of growth in the first year using age and size data from the Gulf of Alaska. Smith et al. (1995) estimated that yellowfin sole in the Gulf of Alaska grow 0.5% BWD in their first year and Paul et al. (1994) estimated that Pacific halibut growth is at least 0.75% BWD. Pacific halibut juveniles grow rapidly, tripling in length by age-1 (Southward 1967). This initial surge of growth is an important factor in determining year class strength in Pacific halibut (Hagen and Quinn 1991).

Despite strong evidence from both laboratory and field studies that rock sole have a preference for sand over mud, the growth rates for rock sole on sand were not significantly greater than rock sole on mud. When given a choice in the laboratory between a variety of substrates, juvenile rock sole select those substrates containing sand nearly every time. When given a choice between mud and sand only, rock sole always selected sand (Moles and Norcross 1995). In the field, juvenile rock sole are often found on sand substrates (Norcross et al. 1995).

Pacific halibut not only differ from the other two species in spatial behavior, but also have different feeding behavior and they engage in territorial partitioning. Pleuronectids rely on visual cues in diurnal feeding (de Groot 1971) in addition to various levels of chemoreception. In our tests, Pacific halibut fed in the water column and relied heavily on visual detection of prey. Adult Pacific halibut maintain home areas spatially distinct from the home areas of other Pacific halibut (Phillip Hooge, U.S. Fish and Wildlife Service, Glacier Bay, Alaska, personal communication). This is similar to the uniform distribution of the juvenile Pacific halibut in our study. In contrast, the demersally feeding yellowfin sole and rock sole may aggregate for the same reason other species school, such as protection from predators.

Female Pacific halibut reach an adult length of 1 m within only 8 yr (Trumble et al. 1993) in contrast to yellowfin sole, which mature at an average length of 28 cm about 9 yr (Wilderbuer et al. 1992). Rock sole are believed to have a similar age and size at 50% maturity as yellowfin sole. This tremendous difference in growth was evident even in the young-of-the-year in our study. Significantly greater growth rates as age-0 juveniles would allow Pacific halibut to move offshore sooner, escape nearshore predators at an earlier age, and mature quickly. Additionally, when Pacific halibut distribute themselves uniformly with respect to each other, competition is reduced and territory is partitioned. Rather than adopt agonistic behavior common to such fish as salmonids (Ryer and Olla 1995) to compete for food, Pacific halibut may establish hunting areas devoid of other

Pacific halibut. The idea that some of the differences in size and distribution of adult flatfishes are the result of differences in growth and behavior of age-0 juveniles deserves additional study in the field.

Acknowledgements

The authors would like to thank Dr. Brenda Norcross of the University of Alaska Fairbanks for her reviews, advice, and encouragement in the execution of this study.

Literature Cited

Brännäs, E., and A. Alanärä. 1993. Monitoring the feeding activity of individual fish with a demand feeding system. Journal of Fish Biology 42:209–215.

Clark, P. J., and F. C. Evans. 1954. Distance to nearest neighbor as a measure of spatial relationships in populations. Ecology 35:445–453.

de Groot, S. J. 1971. On the interrelationships between morphology of the alimentary tract, food and feeding behaviour in flatfishes (Pisces: Pleuronectiformes). Netherlands Journal of Sea Research 5:121–196.

FAO (Food and Agriculture Organization, U.N.). 1995. Review of the state of world fishery resources: marine fisheries. FAO Fisheries Circular 884, Rome, Italy.

Fonds, M., M. Tanaka, and H. W. van der Veer. 1995. Feeding and growth of juvenile Japanese flounder *Paralichthys olivaceus* in relation to temperature and food supply. Netherlands Journal of Sea Research 34:111–118.

Hagen, P. T., and T. J. Quinn II. 1991. Long-term growth dynamics of young Pacific halibut: evidence of temperature-induced variation. Fisheries Research (Amsterdam) 11:283–306.

Moles, A., and B. L. Norcross. 1995. Sediment preference in juvenile Pacific flatfishes. Netherlands Journal of Sea Research 34:177–182.

Norcross, B. L., B. A. Holladay, and F. J. Muter. 1995. Nursery area characteristics of pleuronectids in coastal Alaska, USA. Netherlands Journal of Sea Research 34:161–175.

Olla, B. L., M. W. Davis, C. H. Ryer, and S. M. Sogard. 1996. Behavioural determinants of distribution and survival in early stages of walleye pollock, *Theragra chalcogramma*: a synthesis of experimental studies. Fisheries Oceanography 5 (Suppl. 1):167–178.

Paul, A. J., J. M. Paul, and R. L. Smith. 1994. Energy and ration requirements of juvenile Pacific halibut (Hippoglossus stenolepis) based on energy consumption and growth rates. Journal of Fish Biology 44:1023–1031.

Rijnsdorp, A. D. 1993. Relationship between juvenile growth and the onset of sexual maturity of female North Sea plaice, *Pleuronectes platessa*. Canadian Journal of Fisheries and Aquatic Sciences 50:1617–1631.

Ryer, C. H., and B. L. Olla. 1995. The influence of food distribution upon the development of aggressive and competitive behaviour in juvenile chum salmon, *Oncorhynchus keta*. Journal of Fish Biology 46:264–272.

Smith, R. L., A. J. Paul, and J. M. Paul. 1995. Minimal food requirements for yellowfin sole in Alaska: estimates from laboratory bioenergetics. Pages 285-295 *In* Proceedings of the International Symposium on North Pacific Flatfish. Alaska Sea Grant Program AK-SG-95-04, University of Alaska, Fairbanks, Alaska.

Southward, G. M. 1967. Growth of Pacific halibut. Report 43:1-59. International Pacific Halibut Commission, Seattle, Washington.

Thedinga, J. F., and S. W. Johnson. 1995. Retention of jet-injected marks on juvenile coho and sockeye salmon. Transactions of the American Fisheries Society 124:782–785.

Thedinga, J. F., A. Moles, and J. T. Fujioka. 1997. Mark retention and growth of jet-injected juvenile marine fish. Fishery Bulletin (U.S.) 95: 629–633.

Trumble, R. J., J. D. Neilson, W. R. Bowering, and D. A. McCaughran, D. A. 1993. Atlantic halibut (*Hippoglossus hippoglossus*) and Pacific halibut (*H. stenolepis*) and their North American fisheries. Canadian Bulletin of Fisheries and Aquatic Sciences 227:1-84.

van der Veer, H. W., R. Berghahn, and A. Rijnsdorp. 1994. Impact of juvenile growth on recruitment in flatfish. Netherlands Journal of Sea Research 32:153–173.

Weatherley, A. H. and H. S. Gill. 1987. The Biology of Fish Growth. Academic Press, London, England.

Wilderbuer, T.K., G. E. Walters, and R. G. Bakkala. 1992. Yellowfin sole, *Pleuronectes asper*, of the eastern Bering Sea: biological characteristics, history of exploitation, and management. Marine Fisheries Review 54(4):1–18.

Witting, D. A. and K. W. Able. 1993. Effects of body size on probability of predation for juvenile summer and winter flounder based on laboratory experiments. Fishery Bulletin (U.S.) 91:577–581.

Zijlstra, J. J., R. Dapper, J. IJ Witte. 1982. Settlement, growth and mortality of post-larval plaice (*Pleuronectes platessa*) in the western Wadden Sea. Netherlands Journal of Sea Research 15: 250–272.

Received 9 April 2001
Accepted for publication 28 August 2001