SKEWED SEX RATIO IN CULTURED WINTER FLOUNDER *PSEUDOPLEURONECTES AMERICANUS*: IMPLICATIONS FOR STOCK ENHANCEMENT

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Introduction

Winter flounder *Pseudopleuronectes americanus* is currently being evaluated as a stock enhancement candidate in New Hampshire, USA. Spawning and development through the juvenile stage occurs in estuaries where temperature varies both temporally and spatially. Studies have shown that sexual differentiation, and therefore, the male:female sex ratio in some flatfish species can be influenced by juvenile incubation temperature (e.g. Luckenbach et al. 2003). For flounder used in stock enhancement programs, this temperature-dependent sex differentiation could pose significant problems by altering the expected 1:1 sex ratio and thus, affecting the wild population. In this study we examined a size series of cultured fish to determine the size and age when sexual differentiation occurs in winter flounder, as well as the sex ratio of the cultured population.

Materials and methods

Experimental fish, produced from wild caught broodstock, were cultured in a flow-through seawater system. Incubation temperatures increased from 7-16.7^{o}C during the 323 days of the study. Starting after metamorphosis, a total of 376 cultured fish were sampled from the general hatchery population at approximately 10mm total length (TL) intervals at 110, 124, 160, 231, and 323 days post-hatch (Table 1). On each sampling occasion, at least 30 randomly collected fish were euthanized, fixed in modified Davidson's fixative and stored in 70% alcohol. Tissues were embedding in paraffin, sagittally sectioning (5_m), and stained with hematoxylin and eosin. Slides were numerically coded and examined by three viewers in a blind test to determine gender. Guidelines from other studies, including Luckenbach et al. (2003), were used to determine the sex of winter flounder by looking for structural and cellular gender-identifying characteristics. Sex ratio data were analyzed using Chi-square goodness of fit. In addition, to determine if fish age or size affected the sex ratio, the data were sorted accordingly and reanalyzed.

Results

Of the 376 cultured winter flounder examined, 74 were determined as female, 167 as male, and 61 as unknown (Table I). Gonads from 74 fish were missed during sectioning and therefore these fish were not included in the analyses. It was possible to determine sex for 90-100% of the larger fish size classes. For fish <41mm TL, the proportion of sexually identifiable individuals dramatically decreased such that gender of only 36 and 11% of the fish was determined in the 31-

Table I. Number and disposition of fish processed for sex determination from each size class.

Size Class (mm)	No. Female	No. Male	No. Unknown	No. Missed	Total
< 20	0	0	0	34	34
21-30	3	1	33	2	39
31-40	6	4	18	4	32
41-50	12	32	5	8	57
51-60	17	44	4	5	70
61-70	8	9	1	2	20
71-80	7	17	0	6	30
81-90	4	19	0	6	29
91-100	14	30	0	4	48
101-110	3	11	0	3	17
Total	74	167	61	74	376

40 and 21-30mm TL size classes, respectively. These results indicate that sexual differentiation in winter flounder occurs at some size < 41mm TL. Because gonadal differentiation in the majority of fish < 41mm TL either had not occurred or was not clear to the viewers, these fish were excluded from the sex ratio analyses. A total of 65 females and 162 males were identified from the 41-110mm TL cultured flounder population, yielding a sex ratio that was significantly skewed towards male ($p<0.05$). This trend held true for all five age groups, and all but one size class. The sex ratio of fish 61-70mm TL was not significantly different than 1:1 ($p=0.808$), but because sexual differentiation of the population had already occurred, and was skewed towards males, the most logical explanation for this is the small sample size ($n=17$) in this size category.

Discussion

Winter flounder undergo sexual differentiation at a relatively small size (< 41mm TL) and young age (≤ 110 days post-hatch). In New Hampshire, this corresponds to the time between April and the beginning of August. If, like many other flatfish species, winter flounder exhibit temperature dependent sex determination, incubation temperature during the ontogenetic development of the gonads may affect sex ratio of the population. When the temperatures of the flounder culture tanks were compared to ambient temperatures in the natural environment, it was apparent that fish were reared at temperatures 3-6^{o}C warmer than ambient due to lower flow rate into the rearing tanks. This higher temperature could explain the shift towards a male dominated population. However, because winter flounder can and do spawn throughout the estuary, and some sites further up the estuary have 8-10^{o}C warmer water than the rearing tank temperatures during the critical months, it is possible that our cultured fish experienced a colder temperature regime than they would have in nature if the upper estuary had been their natal area. If true, and if winter flounder exhibit temperature dependent sex determination, then suboptimal incubation temperature could have caused the male-dominated cultured population.

References

Luckenbach, J. A., Godwin, J., Daniels, H. V., and Borski, R. J. 2003. Gonadal differentiation and effects of temperature on sex determination in southern flounder (*Paralichthys lethostigma*). Aquaculture 216: 315-327.