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A genetic linkage map of red drum, *Sciaenops ocellatus*

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Summary

Second-generation, sex-specific genetic linkage maps were generated for the economically important estuarine-dependent marine fish *Sciaenops ocellatus* (red drum). The maps were based on F_1 progeny from each of two single-pair mating families. A total of 237 nuclear-encoded microsatellite markers were mapped to 25 linkage groups. The female map contained 226 markers, with a total length of 1270.9 centiMorgans (cM) and an average inter-marker interval of 6.53 cM; the male map contained 201 markers, with a total length of 1122.9 cM and an average inter-marker interval of 6.03 cM. The overall recombination rate was approximately equal in the two sexes (♀:♂ = 1.03:1). Recombination rates in a number of linkage intervals, however, differed significantly between the same sex in both families and between sexes within families. The former occurred in 2.4% of mapped intervals, while the latter occurred in 51.2% of mapped intervals. Sex-specific recombination rates varied within chromosomes, with regions of both female-biased and male-biased recombination. Original clones from which the microsatellite markers were generated were compared with genome sequence data for the spotted green puffer, *Tetraodon nigroviridis*; a total of 43 matches were located in 17 of 21 chromosomes of *T. nigroviridis*, while seven matches were in unknown portions of the *T. nigroviridis* genome. The map for red drum provides a new, useful tool for aquaculture, population genetics, and comparative genomics of this economically important marine species.

Keywords genetic map, microsatellites, red drum, sex-specific recombination bias.

Introduction

The red drum, *Sciaenops ocellatus*, is an estuarine-dependent, marine fish distributed in the western Atlantic Ocean from Massachusetts to Key West, Florida, and along the coast of the Gulf of Mexico to Tupxan, Mexico. The species represents arguably the most important recreational marine fish in bays and estuaries of the southern US, accounting for nearly one quarter of the directed recreational effort (number of fishers) and contributing significantly in 2006 to the >$10 billion in economic impact to, and >100 000 jobs in, coastal communities from saltwater fishing (U.S. Fish and Wildlife Service 2006; Southwick Associates 2007). Because of declines in abundance during the 1970s and 1980s (Goodyear 1991), several US states now supplement 'wild' red drum stocks with hatchery-raised fish (McEachron *et al.* 1993; Smith *et al.* 1997; Woodward 2000; Tringali *et al.* 2008). The State of Texas, for example, releases 25–30 million red drum fingerlings annually into eight Texas bays and estuaries (Vega *et al.* 2003). There is also a burgeoning aquaculture industry for red drum both in the US and internationally (Lutz 1999; Hong & Zhang 2004).

A central goal of stock-enhancement programmes is to minimize mortality of fingerlings during husbandry, transport, and release into the wild, while private aquaculture seeks to increase the biomass of marketable fish produced per unit time. These goals can be accomplished in part through selective breeding programmes aimed at increasing growth rates and/or decreasing susceptibility to stress, disease and/or temperature change. However, these phenotypic traits are not easily manipulated because they are influenced cumulatively by multiple genes, many with small effects (Liu & Cordes 2004). Traditional selective breeding programmes for improvement of these kinds of traits require generations of breeding and experimentation (Lynch & Walsh 1998), an option that is not economically viable for species like red drum that have long generation times. Instead, it is preferable to employ molecular techniques to develop a large number of DNA markers to identify

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chromosomal regions that harbour genes that have detectable effects on traits of interest, i.e. so-called quantitative trait loci or QTL (see Doerge 2002 for a review). Identifying DNA markers in tight linkage association with QTL then allows marker-assisted selection (MAS) (Ribaut & Hoisington 1998), greatly expediting selective breeding for economically desirable or management-related traits (Lynch & Walsh 1998). The critical initial step in this process is the generation of a moderately dense genetic linkage map (Dekkers & Hospital 2002), an undertaking that requires a large number of markers (Slate 2005) from which patterns of parental descent of defined chromosomal regions can be identified.

Nuclear-encoded microsatellites are excellent DNA markers for generating genetic maps in vertebrate animals. Briefly, microsatellites are abundant short stretches of DNA composed of di-, tri- or tetranucleotide repeat motifs embedded in regions of unique DNA sequence. They are inherited in a Mendelian fashion, found in all eukaryotic species (including fish), and distributed more-or-less evenly throughout euchromatic regions of chromosomes (Weber & May 1989; Vaiman *et al.* 1994). Polymorphism information content (PIC) values for individual microsatellites range as high as 90% and the use of specific polymerase chain reaction (PCR) primer sets ensures the amplification of one single-copy locus, removing problems associated with inferring homology of alleles (Wright & Bentzen 1994). These characteristics have made microsatellites the most commonly used markers for linkage map construction in a variety fishes (Kocher *et al.* 1998; Franch *et al.* 2006; Reid *et al.* 2007; Wang *et al.* 2007).

As a result of their wide distribution along chromosomes, microsatellites are often closely linked to genes affecting adaptive traits, meaning that microsatellite-based linkage maps can be used to identify chromosomal regions under selection. The approach to identify chromosomal regions that are closely linked to genes affecting adaptive traits involves the assay of variation at multiple, selectively neutral loci, such as microsatellites coupled with the search for spatially and temporally stable instances of linkage disequilibrium (Pritchard & Przeworski 2001). Temporally stable linkage disequilibrium is generally hypothesized to be caused by selection, and has proven to be a valuable tool in identifying adaptively important chromosomal segments that often differentiate geographical subpopulations (Przeworski 2002; Schlenke & Begun 2005). Identifying adaptively important chromosomal regions can be extremely important to both stock-structure studies and to conservation and management efforts, because the preservation of genetic resources is paramount to the preservation and protection of biota.

In this article, we present sex-specific genetic linkage maps of the red drum. The maps contain 237 microsatellite markers, including most of the 31 microsatellite markers previously mapped by Karlsson *et al.* (2007). Estimates of map length and the average inter-marker interval were generated for both sexes, and homogeneity in rates of recombination between sexes was tested using contingency G tests. Sequences of 222 of the original microsatellite-containing clones, generated from red drum genomic libraries, were compared with genome sequence data for the spotted green puffer, *Tetraodon nigroviridis*, to screen for ancestral non-coding conserved regions (ANCORs, Aloni & Lancet 2005) between the two species.

Materials and methods

Two full-sib mapping families, each from single-pair crosses, were generated at the Marine Development Center of the Texas Parks and Wildlife Department (TPWD) in Corpus Christi, Texas. The dams and sires were part of the wild-caught brood stock used in the TPWD stock-enhancement programme. Details of spawning procedures, fertilized egg collections and larval rearing were identical to those described in Karlsson *et al.* (2007). F_1 progeny were harvested at a size of 20–30 mm (total length) and preserved in 95% ethanol; fin clips were taken from parents after spawning and also stored in 95% ethanol.

Genomic DNA was extracted from each fish or fin clip, using a modified Chelex extraction protocol (Estoup *et al.* 1996). After 2-min centrifugation at 16 000 × ***g***, 0.3 μl of supernatant was used directly as a template for all PCR amplifications. Genotypes at 269 microsatellites were acquired initially for all four parents and for 103 (Family A) and 104 (Family B) progeny. Primer pairs, PCR amplification conditions and the original references describing these loci maybe found at http:wfsc.tamu.edu/doc under the file name 'PCR primers for red drum (*Scianenops ocellatus*) microsatellites.' PCR amplification products were electrophoresed on 6% polyacrylamide gels and visualized using an ABI Prism 377 sequencer (Applied Biosystems®). Scoring was conducted manually with the aid of GENESCAN V. 3.1.2 (Applied Biosystems®) and GENOTYPER V. 2.5 (Perkin Elmer®). A total of 244 microsatellites were deemed suitable for further analysis based on reliable amplification and suitable levels of polymorphism.

Analyses were initiated using LINKMFEX V. 2.1 (R. Danzmann, University of Guelph, http:// http://www.uoguelph.ca/~rdanzman/software/LINKMFEX). Pairwise recombination fractions (θ) and logarithm of odds (LOD) ratios were computed for each individual in both mapping families. Microsatellite markers were assigned to linkage groups in each individual, using LOD scores of ≥5.9 to exclude microsatellite pairs with $\theta > 0.25$ (Danzmann & Gharbi 2007). A second analysis, using LOD scores ≥4.0, was then undertaken to allow more microsatellites to be incorporated into each linkage group; results of the two assignments were compared for consistency. Finally, all microsatellites that appeared in a given linkage group in any individual in the prior analyses were reanalysed separately without using

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LOD criteria. Construction of the map in this hierarchal manner allowed for inclusion of the maximum number of microsatellite markers per individual and linkage group, while ensuring correct groupings and consistent marker order. Finally, the two female maps and two male maps produced for each linkage group were merged to produce sex-specific linkage maps, using the MERGE module in LINKMFEX. Distances in centiMorgans (cM) were estimated directly from recombination fractions, as recommended by Danzmann & Gharbi (2007).

The degree of conformation to the expectations of Mendelian segregation was tested for each microsatellite, using G tests; significance of multiple tests carried out simultaneously was assessed following sequential Bonferroni correction (Rice 1989). If significant segregation distortion was indicated, gel images were re-examined and samples re-run, when appropriate, to eliminate experimenter error. Recombination rates between adjacent markers were compared using contingency G tests available in the module RECOMDIF (also in LINKMFEX). For each linkage group, recombination rates were compared within sexes between families (i.e. Female A vs. Female B and Male A vs. Male B) and between sexes within families (i.e. Female A vs. Male A and Female B vs. Male B); significance of multiple tests carried out simultaneously was assessed following sequential Bonferroni correction. Finally, overall linkage group recombination rates were compared between families and between sexes, with sequential Bonferroni correction applied for multiple tests.

Similarity between (flanking) sequences of 222 of the original microsatellite-containing clones used were compared with sequences in the spotted green puffer (*Tetraodon nigroviridis*) genome (v.7.0, http://www.genoscope.cns.fr/externe/tetraodon/) to screen for ancestral non-coding conserved regions or ANCORs. *Tetraodon nigroviridis* was selected for these comparisons because, like the red drum, it is an advanced teleost in the series Percomorpha (Nelson 2006). Similarities were assessed using the BLAT algorithm (Kent 2002) available at the ENSEMBL web site (http://www.ensembl.org/Multi/blastview). To minimize increased similarity scores generated by repetitive DNA, all repeated motifs were removed from the red drum microsatellite sequences prior to screening. Pairs of *T. nigroviridis*/*S. ocellatus* sequences were considered similar if they had ≥50 basepairs (bp) of overlap, with ≥80% sequence similarity and generated E-values $\leq e^{-10}$. The highest scoring matches were examined visually to ensure that inferred similarity was not caused by simple, repetitive stretches in the regions flanking microsatellite motifs.

Results

In total, 237 of the 244 (97.1%) microsatellite loci amplified were linked to another microsatellite locus. Of the remaining seven microsatellites, six had the same genotype in both parents in both families and were uninformative; one microsatellite (*Soc540*) remained unlinked. A total of 25 linkage groups were recovered in both sexes (Fig. 1). The two sex-specific maps shared 193 loci. The most inclusive individual map (Female B) contained 201 microsatellites, while the least inclusive map (Female A) contained 190 microsatellites. Gene order in all linkage groups was conserved in all four parents, with one exception. Initial analysis with LINKMFEX indicated a possible difference in gene order in Linkage Group (LG-) 14 in Male B relative to Male A and both females. Maps of both Male A and Male B contained two clustered groups of microsatellites (Fig. 1). In Male B, there appeared to be an inversion of both clustered groups, such that the map order of the markers within each clustered group was concordant with the map order in Male A, but *Soc* 699 and *Soc* 651 were at the terminal ends of the linkage group in Male B. Given the same relative marker order in Male A and both females, and the seemingly low likelihood of two inversions, the discrepancy in Male B was considered to be an artefact caused by the large recombination fraction between the markers in the separate clusters in Male B (>40%). The distance between the two clusters in the male map of LG-14 was therefore not included in the sex-specific map (Fig. 1). Future mapping in males with genetic markers in between the two clustered groups may resolve this discrepancy.

The female map contained 226 microsatellites and was 1270.9 cM in length; the male map contained 204 loci and was 1122.9 cM in length. Female linkage groups ranged from 0.4 to 75.0 cM in length and contained from three to 14 microsatellites; male linkage groups ranged from 0.4 to 66.0 cM in length and contained from two to 13 microsatellites (Fig. 1; Table 1). The female map had an inter-marker interval of 6.53 cM, while the male map had an average inter-marker interval of 6.03 cM. Significant ($P < 0.05$) segregation distortion following Bonferroni correction was detected in 29 of 708 (4.1%) individual segregation tests. Significant distortion was detected for 17 markers in Female A, 11 markers in Male A, and one marker in Male B. No segregation distortion was detected in Female B. Most of the segregation distortion in Female A was clustered in three linkage groups: LG-5 (seven occurrences), LG-15 (three occurrences) and LG-19 (four occurrences); most of the segregation distortion in Male A occurred in two linkage groups: LG-17 (four occurrences) and LG-18 (four occurrences).

Significant differences in recombination rate were found between females and between males in different families and between females and males in each family. Twelve significant differences in recombination rate between the same sex in either family were found across 493 common linkage intervals, while the overall recombination rate between families differed significantly in six of 50 sex-specific linkage groups. Of the latter, the recombination rate in LG-15 was significantly higher in Female A than in Female B, the

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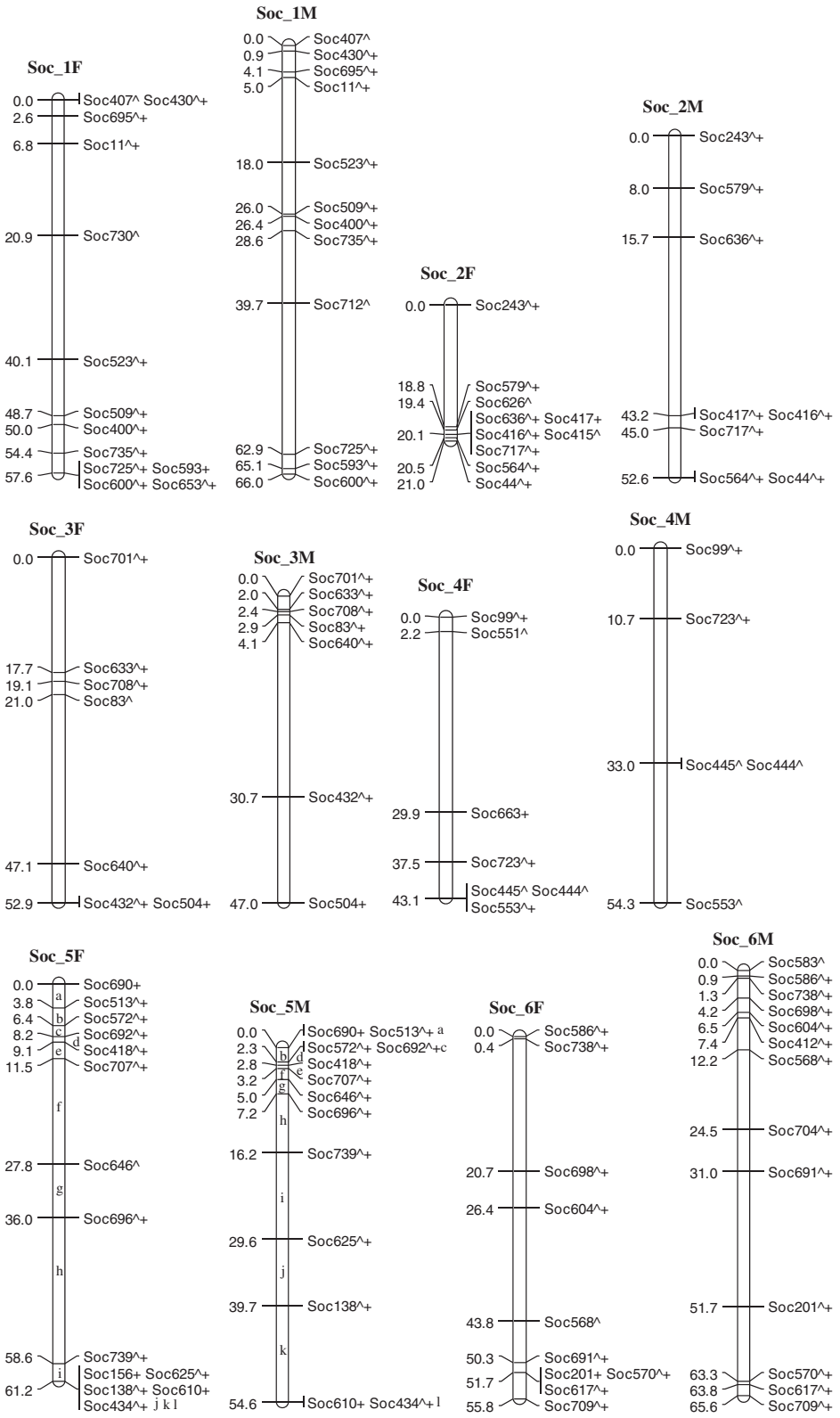

Figure 1 Male (M) and female (F) linkage maps for red drum, *Sciaenops ocellatus*, based on microsatellite markers. Cumulative distances are given in cM to the left of marker positions. ^ indicates marker found in family A and + indicates marker found in family B. Hashed interval in LG-14M indicates distance taken from Male A (see text). Lower case letters on LG-5F and LG-5M correspond to lower case letters in Fig. 3.

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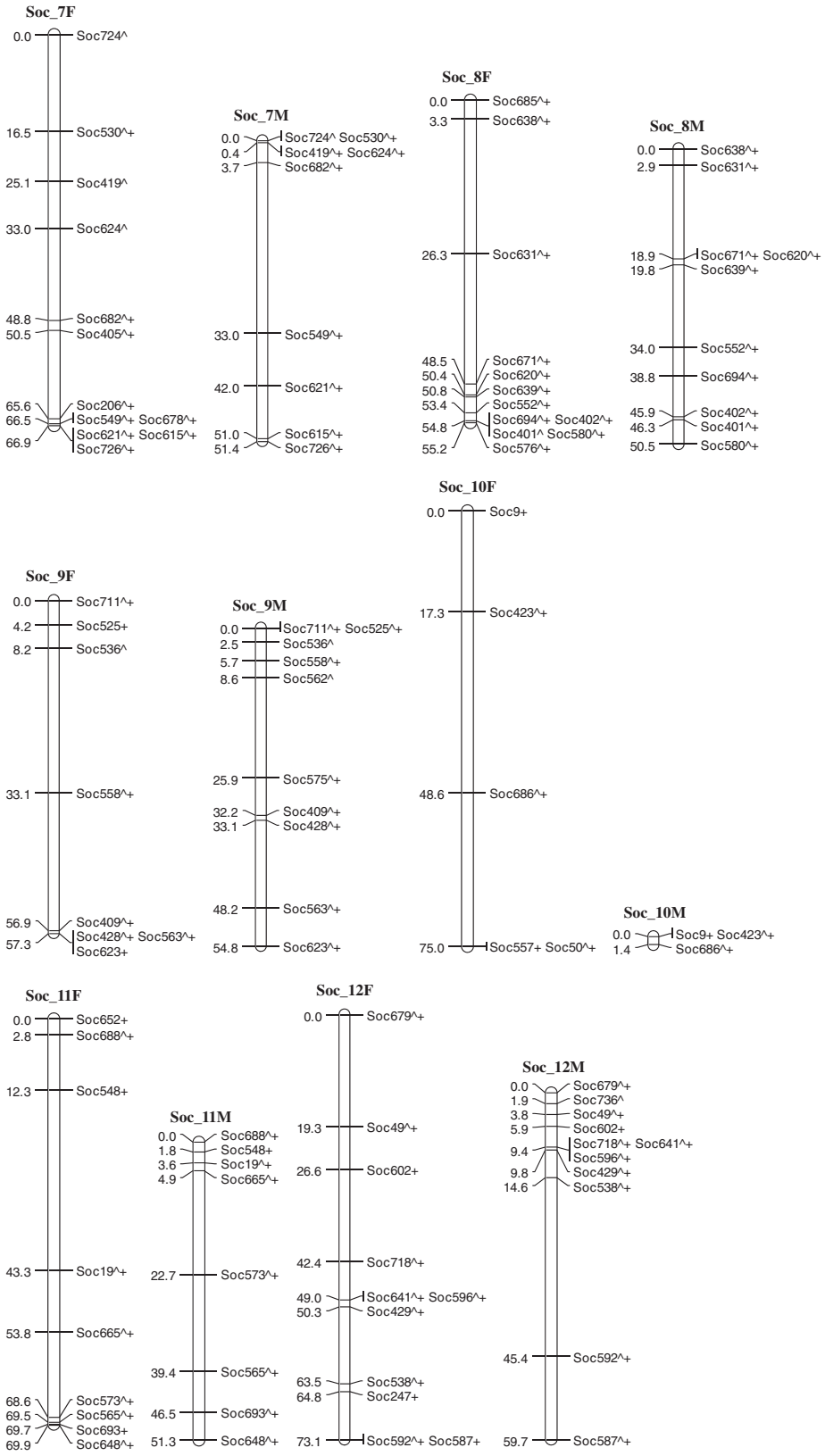

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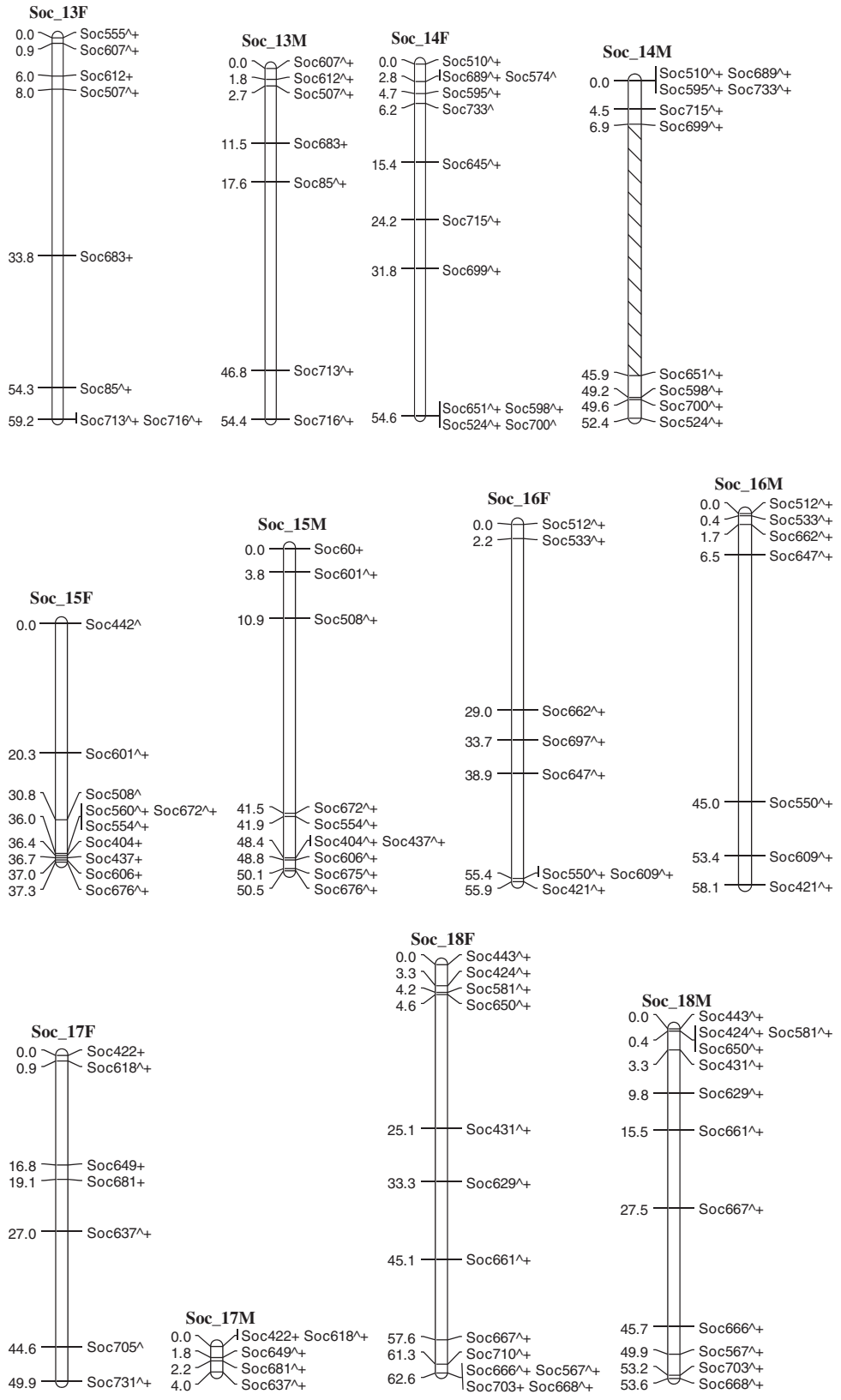

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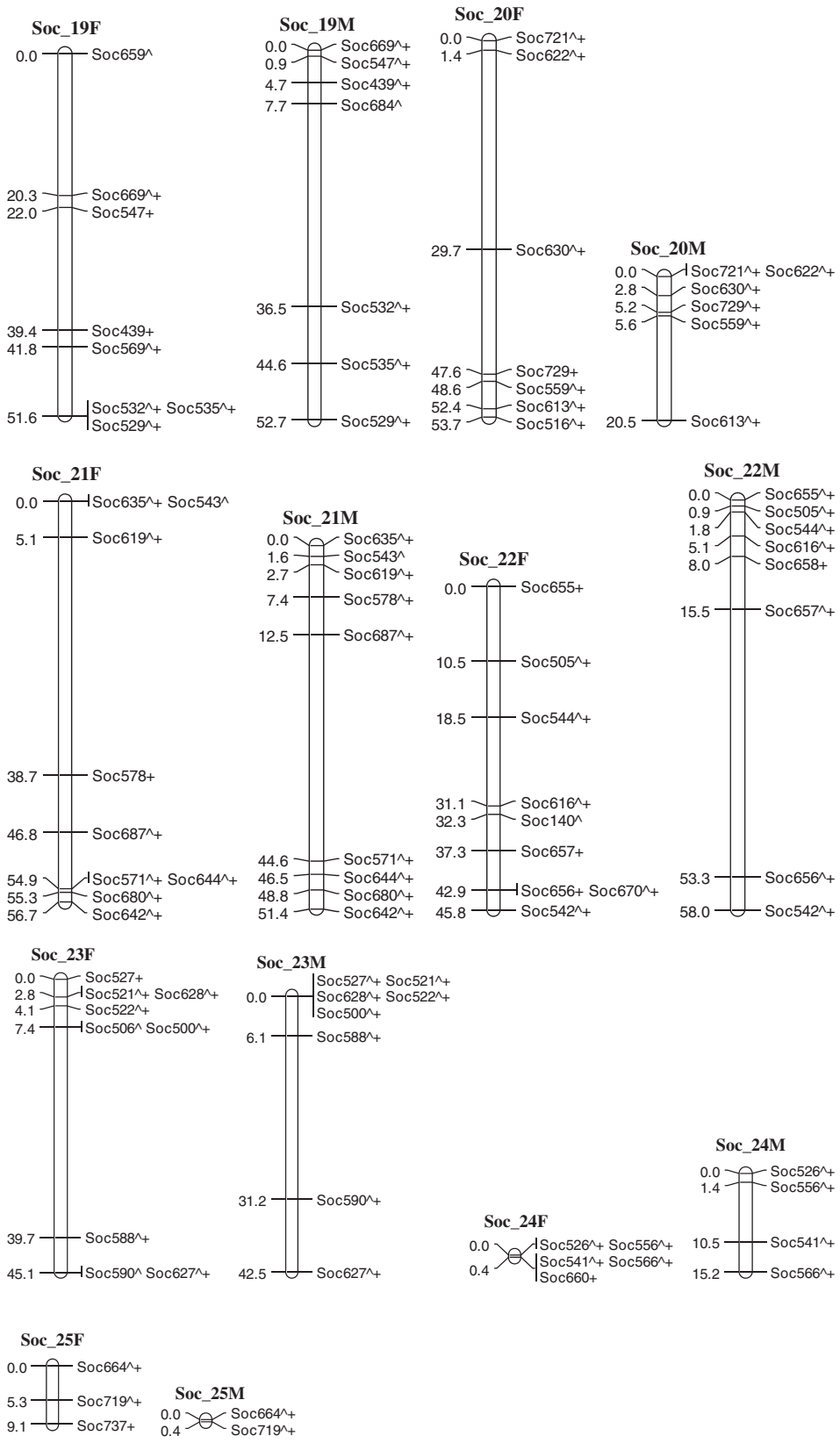

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Table 1 Summary information for sex-specific linkage groups.

	Female			Male			
Linkage group	#	Length	Interval	#	Length	Interval	Rec
LG-1	13	57.6	4.80	12	66.0	6.00	
LG-2	10	21.0	2.33	8	52.6	7.51	♂
LG-3	7	52.9	8.82	7	47.0	7.83	
LG-4	7	43.1	7.18	5	54.3	13.58	
LG-5	14	61.2	4.71	13	54.6	4.55	♂
LG-6	10	55.8	6.20	13	65.6	5.47	
LG-7	12	66.9	6.08	9	51.4	6.45	
LG-8	12	55.2	5.02	10	50.5	5.61	
LG-9	8	57.3	8.19	10	54.8	6.09	
LG-10	5	75.0	18.75	3	1.4	0.70	♀
LG-11	9	69.9	8.74	8	51.3	7.33	
LG-12	11	73.1	7.31	11	59.7	5.97	♀
LG-13	8	59.2	8.46	7	54.4	9.07	
LG-14	12	54.6	4.96	10	52.4	5.82	
LG-15	10	37.3	4.14	10	50.5	5.61	♂
LG-16	8	55.9	6.21	7	58.1	8.30	
LG-17	7	49.9	8.32	5	4.0	1.00	♀
LG-18	13	62.6	5.23	12	53.6	4.87	
LG-19	8	51.6	7.37	7	52.7	8.78	♂
LG-20	7	53.7	8.95	6	20.5	4.10	♀
LG-21	9	56.7	7.09	9	51.4	6.43	
LG-22	9	45.8	5.73	8	58.0	8.29	
LG-23	9	45.1	5.64	8	42.5	6.07	♀
LG-24	5	0.4	0.10	4	15.2	5.07	♂
LG-25	3	9.1	3.03	2	0.4	0.2	♀
Total	226	1270.9	6.53	204	1122.9	6.03	

#, number of microsatellite markers; Length, total length in cM; Interval, average interval between markers (in cM); Rec, sex with significantly larger recombination rate; absence of symbol indicates that there was no significant difference in recombination between the sexes.

recombination rate in LG-1 and LG-23 was significantly higher in Female B than in Female A, and the recombination rate was significantly higher in LG-5, LG-18 and LG-23 in Male A than in Male B. A total of 218 significant differences in recombination rate across 426 common linkage intervals were found between the sexes (both families combined), while the overall recombination rate differed significantly between females and males (both families combined) in 34 of 50 family-specific linkage groups. Recombination rates in both families were significantly higher in females in six linkage groups and significantly higher in males in five linkage groups (Table 1). Finally, the recombination rate was greater in the female map in 80 of 151 common linkage intervals, while the recombination rate was greater in the male map in 66 common linkage intervals; recombination rates were equivalent in the two sexes in five common linkage intervals (Fig. 2). The overall recombination rate was slightly higher in females (♀:♂, 1.13:1); however, when only common intervals were considered, the ♀:♂ recombination rate ratio was 1.03:1.

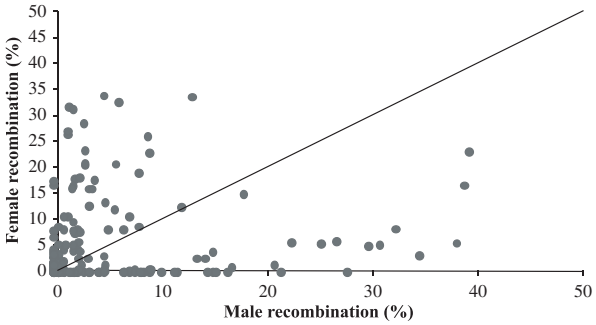

Figure 2 Differences in recombination rates between female and male maps; each point (dot) represents the recombination rate between adjacently paired microsatellite markers common to both maps. The diagonal line represents sex-equal recombination rates.

Sex-specific recombination rates varied regionally within 19 of the linkage groups, generating regions of male-biased and female-biased recombination. In most of these, regions of elevated recombination in either sex were situated at opposite ends of the chromosome (Fig. 1). A particularly noteworthy example of this was evident in LG-5 (Figs 1 & 3). In the four linkage groups (LG-10, LG-17, LG-24 and LG-25) where this pattern was not observed, relatively few markers were mapped for one or both sexes. In the remaining two linkage groups (LG-2 and LG-15), overall map length in females was shorter and no obvious polarity was observed.

A total of 50 clones containing 51 microsatellites isolated from red drum genomic libraries had regions that were highly similar (E-value > e^{-10}) to genomic DNA sequences of *T. nigroviridis*. A total of 43 red drum sequences in 21 of the 25 red drum linkage groups could be placed on 17 of the 21 known *T. nigroviridis* chromosomes. Associations were found between 15 linkage group-chromosome pairs (Fig. 4), although at least one additional microsatellite in three of these pairings also was highly similar to a sequence in an unknown location of the *T nigroviridis* genome. In two

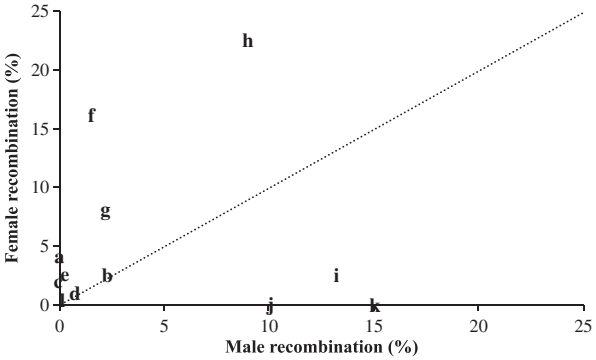

Figure 3 Differences in recombination rates between the female and male maps for LG-5. Lower case letters correspond to intervals with the same letters shown in Fig. 1.

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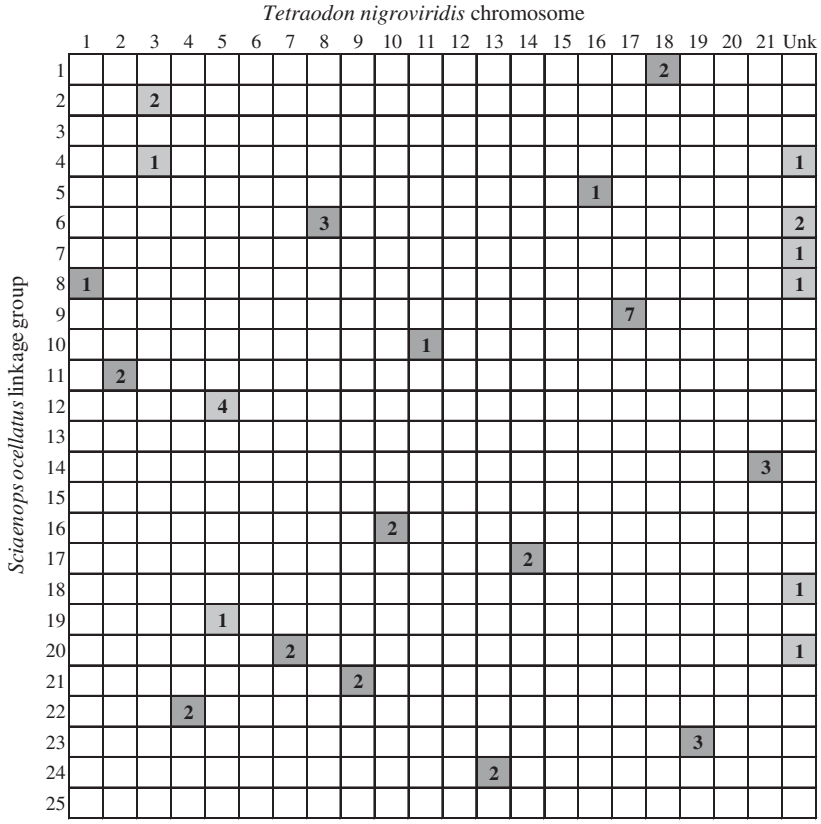

Figure 4 Oxford plot comparing the linkage map of red drum, *Sciaenops ocellatus*, to the genome of *Tetraodon nigroviridis*. Numbers indicate the number of *S. ocellatus* sequences with significant similarity to sequences on a *T. nigroviridis* chromosome.

instances, relationships were found between two red drum linkage groups and one *T. nigroviridis* chromosome. The remaining seven red drum sequences matched sequences in unknown locations of the *T. nigroviridis* genome. Red drum sequences that showed similarity to regions of the *T. nigroviridis* genome ranged in size from 57 to 610 bp, with an average length of 222 bp. Longer regions often were interrupted by short stretches (<200 bp) of dissimilar sequence, and there was one long interruption of 318 bp; the longest uninterrupted region was 230 bp. A total of 25 of the 50 red drum sequences were within 500 bp of putative coding genes in the *T. nigrovirdis* genome; 13 of these were associated with introns and exons, while the remaining 12 were upstream or downstream from a putative coding gene in *T. nigrovirdis*.

Discussion

The length of the sex-average red drum linkage map was 1196.9 cM, with the female map being slightly longer than the male map. The sex-average red drum map is similar in length relative to published maps of other fishes of the series Percomorpha, such as the 1131 cM map in tilapia (Kocher *et al.* 1998) and 1511 cM map in halibut (Reid *et al.* 2007) and it is considerably smaller when compared with map lengths in more primitive teleost fishes, such as the 2178 cM map in platyfish (Walter *et al.* 2004), 2235 cM map in channel catfish (Waldbieser *et al.* 2001) and 2628 cM map in rainbow trout (Young *et al.* 1998). This pattern supports the hypothesis of genome contraction in higher teleosts (Jaillon *et al.* 2004). However, the detection of 25 red drum linkage groups rather than the 24 expected based on karyotyping (Gold *et al.* 1988) indicates that coverage of the current red drum map may not yet be complete. Consistent with this idea, the red drum nucleus contains approximately 1.65 pg of DNA (Gold *et al.* 1988), a quantity which can be converted to an approximate genome size of 1620 Mbp (Dolezel *et al.* 2003).

Six significant instances of family-biased recombination rate were observed in five red drum linkage groups: three between females and three between males. Family-specific differences in recombination rates have been reported in two other fishes, both salmonids (Sakamoto *et al.* 2000; Gharbi *et al.* 2006), and were hypothesized to stem from residual tetrasomy and pseudolinkage, phenomena observed in several salmonid species (May & Johnson 1990). Such phenomena are unlikely in a diploid species (Gold *et al.* 1988) such as red drum. However, variation in recombination rates among individuals within populations is not uncommon (Simchen & Stamberg 1969) and can be attributable to both genetic and/or environmental factors (Rose & Baillie 1979; Dumont *et al.* 2009).

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Significant sex-biased recombination was observed in eleven red drum linkage groups; six were female biased, while five were male biased. In several animal species, females have greater recombination rates than males (Hedrick 2007). It has been suggested that this is because of repressed recombination in the heterogametic sex (Haldane 1922), a pattern that is observed in several mammals where males are heterogametic (Dib *et al.* 1996; Marklund *et al.* 1996; Neff *et al.* 1999). However, in birds, where females are heterogametic, recombination is often still female biased (Hansson *et al.* 2005; Åkesson *et al.* 2007). In fishes, where either females or males can be the heterogametic sex if sex chromosomes occur (Gold 1979), both female and male bias in recombination rates as well as no sex-bias are reported (Sakamoto *et al.* 2000; Singer *et al.* 2002; Coimbra *et al.* 2003; Moen *et al.* 2008; Lee *et al.* 2005; Franch *et al.* 2006). In red drum, the overall ♀:♂ recombination rate when only common intervals are considered was 1.03:1, perhaps consistent with the absence of detectable sex chromosomes in red drum (Gold *et al.* 1988).

At a finer scale, sex-specific recombination rates in red drum varied at opposite ends of most linkage groups. Regional differences in recombination rates within chromosomes have been observed in a number of animal species, including fish, and appear in part to be a consequence of increased female recombination in regions proximal to centromeres and increased male recombination in regions proximal to telomeres (Sakamoto *et al.* 2000; Kong *et al.* 2002; Lynn *et al.* 2005; Reid *et al.* 2007). The observed polarity of recombination bias in many red drum linkage groups is consistent with the report (Gold *et al.* 1988) that all red drum chromosomes are acrocentric and it may indicate relative positions of centromeres and telomeres.

A total of 44 of the 222 (19.8%) red drum sequences that were compared with the *T. nigroviridis* genome could be anchored to a known *T. nigroviridis* chromosome. Most red drum linkage groups were associated with a specific *T. nigroviridis* chromosome. However, in two instances, a pair of red drum linkage groups was associated with a single *T. nigroviridis* chromosome, suggesting occurrence of an interchromosomal rearrangement in a lineage leading to one or the other species. The level of genome similarity observed is comparable with the 11–45% similarity found in comparisons of other percomorph fishes to *T. nigroviridis* (Lee *et al.* 2005; Stemshorn *et al.* 2005; Franch *et al.* 2006; Reid *et al.* 2007; Wang *et al.* 2007).

Ancestral non-coding conserved regions are a common feature of vertebrate genomes and can be found both proximal and distal to known functional genes (Aloni & Lancet 2005). Half (25) of the 50 red drum sequences were within 500 bp of any putative coding gene in the *T. nigroviridis* genome, a proportion less than the 72% of conserved sequences in sea bream (*Sparus aurata*) that were associated with gene regions in *T. nigroviridis* (Franch *et al.* 2006). Whether the 25 conserved red drum sequences that are not situated near putative functional genes in *T. nigroviridis* represent recent shared ancestry remains unknown.

The moderately saturated linkage map for red drum is the first for a species in the percomorph family Sciaenidae. This is a large family of at least 270 species (Nelson 2006), many of which are of considerable economic importance either in commercial or recreational fisheries (Nakamura 1981) or in aquaculture (Lutz 1999; Hong & Zhang 2004). We anticipate that many of the microsatellite primer pairs will amplify microsatellites in other sciaenids, as was recently reported by Renshaw *et al.* (2009), who showed that about half of the microsatellite primers pairs developed for red drum cross amplified microsatellites in the related and economically important sciaenid *Cynoscion nebulosus* (spotted seatrout). In addition, the map will potentially be useful in identifying chromosomal regions that are closely linked to genes affecting adaptive traits. In this way, the map will be of use to stock-structure evaluation of 'wild' red drum populations. The map also will be useful in identifying small-effect genes (QTL), thus permitting MAS for traits of interest to both commercial aquaculture and stock enhancement.

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